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Section A6.1.1 Acute Toxicity

Annex Point IIA VI.6.1.1 6.1.1 Acute oral toxicity in rats (LD_{50} test)

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1988a) Investigation of acute oral toxicity in rats. [redacted], Report No. 17133, 1988-09-08, (unpublished).	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Saltigo GmbH	
1.2.2	Companies with letter of access	–	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes OECD-Guideline 401 (1981) EPA-Guideline Subdivision F, Series 81-1 (1982)	X
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes. – Study was performed on male rats only. – No analytical confirmation of the homogeneity, stability or concentration of the test substance in the administered formulations was reported.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in Section 2 of dossier.	
3.1.1	Lot/Batch number	19001/87	
3.1.2	Specification	As given in Section 2 of dossier.	
3.1.2.1	Description	Yellow clear liquid	
3.1.2.2	Purity	99.4%	
3.1.2.3	Stability	No analytical confirmation of the stability of the test substance was performed.	
3.2	**Test Animals**		
3.2.1	Species	Rat	

Section A6.1.1 Acute Toxicity

Annex Point IIA VI.6.1.1 6.1.1 Acute oral toxicity in rats (LD_{50} test)

3.2.2	Strain	Wistar (Bor: WISW, SPF-Cpb)
3.2.3	Source	Winkelmann, Borchen, Germany
3.2.4	Sex	Males
3.2.5	Age/weight at study initiation	Age: approximately 9 weeks Weight: 213-227 g
3.2.6	Number of animals per group	5
3.2.7	Control animals	No
3.3	**Administration/ Exposure**	Oral
3.3.1	Postexposure period	14 days
3.3.2	Type	Gavage
3.3.3	Concentration	100, 500, 1000, 1600, 2000, 2500 mg/kg bw
3.3.4	Vehicle	The test substance was formulated in demineralized water using Cremophor EL 2% v/v.
3.3.5	Concentration in vehicle	10, 50, 100, 160 , 200, 250 mg/mL
3.3.6	Total volume applied	10 mL/kg bw
3.3.7	Control	–
3.4	**Examinations**	Clinical observations, necropsy, body weights
3.5	**Method of determination of LD_{50}**	Calculation of LD_{50} was carried out according to the method of Bliss as modified by Pauluhn (1983)
3.6	**Further remarks**	None

Section A6.1.1 Acute Toxicity

Annex Point IIA VI.6.1.1 6.1.1 Acute oral toxicity in rats (LD_{50} test)

		4 RESULTS AND DISCUSSION	
4.1	**Clinical signs**	The dose of 100 mg/kg bw was tolerated without any signs by male rats (Table A6_1_1-1). At doses of ≥ 500 mg/kg bw languor, occasional spasm, apathy, reduced motility, laboured breathing and a staggering gait was observed. In addition, rats receiving higher doses showed piloerection, soft stool, salivation, sometimes convulsion and tremor, at times shivering and adoption of a lateral position. In some instances increased urinary excretion, difficulty in breathing, spastic gait occurred. The symptoms occurred shortly after administration and lasted up to the 5th day of the follow-up observation period at the latest. Fatalities occurred at doses ≥ 2000 mg/kg bw on. The animals died beginning 1.5 h after application on the first day of the follow-up observation period.	
4.2	**Pathology**	Animals that died on the study showed the following findings: liver patchy with signs of lobulation, pale spleen, pale kidneys, glandular stomach reddened, and bladder taut with urine. Animals sacrificed at the end of the observation period showed no macroscopically visible damages in organs caused by the test substance.	
4.3	**Other**	There was no effect on body weights during the follow-up observation period.	
4.4	**LD_{50}**	LD_{50} = 2236 mg/kg bw (males)	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	A study for acute oral toxicity in the rat was conducted with the test substance Icaridin. The methods complied with the OECD Guideline 401 and the US-EPA-Guideline, Subdivision F, Series 81-1. The purpose of the study was to enable the product to be classified (labelled), and to assess the potential acute health hazard when handling the test substance.	X

Section A6.1.1 Acute Toxicity

Annex Point IIA VI.6.1.1 6.1.1 Acute oral toxicity in rats (LD_{50} test)

5.2	**Results and discussion**	The clinical signs observed (behavioural, motility and respiratory disturbances, lateral position, languor, spasm, tremor, shivering, shaking, salivation, soft stool and staggering gait) were slight to moderate, occurred shortly after administration and lasted until the 5th day at the latest. There were no delayed effects. Clinical signs were noted at doses ≥ 500 mg/kg. Mortalities occurred from 2000 mg/kg bw on. Animals died at the end of the observation period showed the following findings: liver patchy with signs of lobulation, pale spleen, pale kidneys, glandular stomach reddened, and bladder taut with urine. The necropsy of the sacrificed animals showed no macroscopically visible damages in organs caused by the test substance.
5.3	**Conclusion**	As in this study occurred, the test substance Icaridin proved to be slightly toxic after acute oral administration to male rats.
5.3.1	Reliability	2
5.3.2	Deficiencies	Yes Study was performed on male rats only. The now revoked OECD Guideline 401 (equivalent to EU Method B.1) calls for animals of both sexes to be tested. Acute oral toxicity studies according to the newer EU guidelines B.1bis and B.1tris use testing in a single sex only, but prefer the test be conducted in females. However, toxicity testing in both sexes (acute dermal and inhalation, repeated dose toxicity testing) clearly shows that rats of both sexes display very similar sensitivity towards Icaridin. E.g., the LOAELs in a subacute oral toxicity test (Wahle, 2001a) were 308 and 360 mg/kg/day in males and females, respectively. Similarly, Icaridin was not toxic to either sex of Wistar rats upon acute inhalation exposure to the limit dose (Pauluhn, 1990). It can therefore be concluded that no appreciable sex difference exists with regard to the acute oral toxicity of Icaridin to Wistar rats. The use of only one sex should therefore be sufficient for classification of Icaridin for its acute oral toxicity.

Section A6.1.1 Acute Toxicity

Annex Point IIA VI.6.1.1 6.1.1 Acute oral toxicity in rats (LD_{50} test)

	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 November 2006
Materials and Methods	Applicant's version is adopted.
Results and discussion	Applicant's version is adopted.
Conclusion	Other conclusions: Applicant's version is adopted.
Reliability	2
Acceptability	Acceptable
Remarks	*A comment on the fact that the OECD guideline 401 is not valid since 20 December 2002 should have been given under heading 2 Guidelines and Quality Assurance – not in 5.1 Applicant's Summary and conclusion*

Table A6_1_1-1. Table for acute oral toxicity

Dose [mg/kg bw]	Toxicological results*	Duration of clinical signs	Time of death	Mortality (%)
Males				
100	0/0/5	---	---	0
500	0/5/5	2 min – 3.5 h	---	0
1000	0/5/5	2 min – 2 d	---	0
1600	0/5/5	3 min – 5 d	---	0
2000	1/5/5	1 min – 4 d	5 h	20
2500	4/5/5	2 min – 2 d	1.5h – 1 d	80
LD_{50} = 2236 mg/kg bw				

* first number = number of dead animals
second number = number of animals with signs of toxicity
third number = number of animals used

Section A6.1.2 Acute Toxicity

Annex Point IIA VI.6.1.2 6.1.2 Acute dermal toxicity in rats (Limit Test)

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1991) Acute Dermal Toxicity Study with Technical Grade KBR3023 in Rats. [redacted] Study No. 90-022-GD, 1991-08-27 (unpublished).	X
1.2	**Data protection**	Yes	
1.2.1	Data owner	Saltigo GmbH	
1.2.2	Companies with letter of access	–	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes OECD Guideline 402 (1987) US-EPA Guideline 81-2 (1984) Japanese MAFF 59 NohSan No. 4200 (1985)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in Section 2 of dossier.	
Lot/Batch number		19010/89	
Specification		As given in Section 2 of dossier.	
3.1.1.1	Description	Clear, viscous liquid	
3.1.1.2	Purity	98.5 %	
3.1.1.3	Stability	Not tested	
3.2	**Test Animals**		
3.2.1	Species	Rat	
Strain		Sprague-Dawley	
Source		Sasco Inc., Houston, Texas, USA	
Sex		Males and females	
Age/weight at study initiation		Males: 8 weeks, 248-274 g; females: 10 weeks, 193-211 g	
Number of animals per group		5 per sex	
3.2.2	Control animals	No	

Section A6.1.2 Acute Toxicity

Annex Point IIA VI.6.1.2 6.1.2 Acute dermal toxicity in rats (Limit Test)

3.3	**Administration/ Exposure**	Dermal	
3.3.1	Postexposure period	14 days	
3.3.2	Area covered	Approximately 16 cm²	
3.3.3	Occlusion	Occlusive patch	X
3.3.4	Vehicle	Undiluted	
3.3.5	Total volume applied	Varied with body weight	
3.3.6	Duration of exposure	24 h	
3.3.7	Removal of test substance	Yes, with tap water	
3.3.8	Controls	No	
3.4	**Examinations**	Clinical observations, necropsy, gross pathology, body weights	
3.5	**Method of determination of LD_{50}**	Not applicable; no mortalities occurred.	
3.6	**Further remarks**	–	
		4 RESULTS AND DISCUSSION	
4.1	**Clinical signs**	No treatment-related clinical signs occurred.	
4.2	**Pathology**	No treatment-related findings were detected.	
4.3	**Other**	Clinical signs consisting of red nasal stain, red lachrymal stain, perianal stain, and urine stain were attributed to wrapping, since they began on day 0 and ceased within 24 h after unwrapping.	
4.4	**LD_{50}**	LD_{50} > 2000 mg/kg bw	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The acute dermal toxicity of technical grade Icaridin was tested in young adult male and female Sprague-Dawley rats (5/sex) using the dermal limit dose of 2000 mg/kg bw according to OECD Guideline 402.	
5.2	**Results and discussion**	No deaths resulted from the treatment at the dermal limit dose, therefore, additional dose levels were not tested and LD_{50} values were not determined. Treatment-related clinical signs were not observed and body weight gain was not affected. No treatment-related gross lesions were observed. The dermal LD_{50} for Icaridin was > 2000 mg/kg bw.	
5.3	**Conclusion**		
5.3.1	Reliability	1	
5.3.2	Deficiencies	No	

Section A6.1.2 Acute Toxicity

Annex Point IIA VI.6.1.2 6.1.2 Acute dermal toxicity in rats (Limit Test)

	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 November 2006
Materials and Methods	Applicant's version is adopted.
Results and discussion	Applicant's version is adopted.
Conclusion	Other conclusions: Applicant's version is adopted.
Reliability	1
Acceptability	Acceptable
Remarks	A spelling mistake in the family name of the second author: Philips. (3.3.3). The dressing on the application site should have been non-occlusive instead of occlusive. This does not change the acceptability of the study in this case since occlusion would represent worst case.

Section A6.1.2 Acute Toxicity

Annex Point IIA VI.6.1.2 6.1.2 Acute dermal toxicity in rats (Limit Test)

Table A6_1-1. Table for acute dermal toxicity

Dose [mg/kg bw]	Number of dead / number of investigated	Time of death (range)	Observations
Males			
2000	0/5	---	No treatment related clinical signs occurred.
Females			
2000	0/5	---	No treatment related clinical signs occurred.
LD_{50} value	> 2000 mg/kg bw (males and females)		

Section A6.1.3 Acute Toxicity

Annex Point IIA VI.6.1.3 6.1.3 Acute inhalation toxicity to the rat (Limit Test)

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1990) Study for Acute Inhalation Toxicity in the Rat to OECD Guideline No.403. [redacted] Report No. 19220, 1990-07-04 (unpublished)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Saltigo GmbH	
1.2.2	Companies with letter of access	–	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes OECD-Guideline No. 403 (1981) EC Guideline B.2 (1993)	
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes, only two concentrations were tested.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in Section 2 of dossier.	
3.1.1	Lot/Batch number	19009/89	
3.1.2	Specification	As given in Section 2 of dossier.	
3.1.2.1	Description	Viscous, colourless, clear liquid	
3.1.2.2	Purity	99.1 %	
3.1.2.3	Stability	Was ensured for the period of the study	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Wistar (Bor:WISW) SPF-Cpb	
3.2.3	Source	Winkelmann, Borchen, Germany	
3.2.4	Sex	Males and females (1:1)	
3.2.5	Age/weight at study initiation	Age: 2-3 months Weight: 170-190 g	
3.2.6	Number of animals per group	5 per sex per dose	
3.2.7	Control animals	Yes; 10 per sex	

Section A6.1.3 Acute Toxicity

Annex Point IIA VI.6.1.3 6.1.3 Acute inhalation toxicity to the rat (Limit Test)

3.3	**Administration/ Exposure**	Inhalation
3.3.1	Postexposure period	14 days
3.3.2	Concentrations	Nominal concentrations: 0, 15,000, 37,500 mg/m³. Analytical concentrations: 0, 2153, 4364 mg/m³
3.3.3	Particle size	MMAD (mass median aerodynamic diameter) ± GSD (geometric standard deviation): 2153 mg/m³ air: MMAD = 1.62 ± 1.44 µm 4364 mg/m³ air: MMAD = 1.80 ± 1.49 µm
3.3.4	Type or preparation of particles	To produce an aerosol of the test compound, a PEG400-ethanol-mix (50:50 (v/v)) was used as vehicle. The vehicle/test compound solution was sprayed into a baffle section by the means of a binary jet and compressed air and then released into the inhalation chamber.
3.3.5	Type of exposure	Nose/head only
3.3.6	Vehicle	Polyethyleneglycol 400/ethanol 50:50 (v/v)
3.3.7	Concentration in vehicle	25% (v/v)
3.3.8	Duration of exposure	4 h
3.3.9	Controls	Controls were exposed to vehicle under the same conditions as the treatment groups.
3.4	**Examinations**	Clinical observations, necropsy, body weight.
3.5	**Method of determination of LC_{50}**	LC_{50} calculation is performed according to the method of Rosiello *et al.* (1977), as modified by Pauluhn (1983). This procedure is based on the "maximum likelihood" method of Bliss (1938).
		4 RESULTS AND DISCUSSION
4.1	**Clinical signs**	After treatment with 2153 and 4364 mg/m³ air, no animal showed any symptoms (see Table A6_1-1.3).
4.2	**Pathology**	Gross pathological examinations of the 2153 mg/m³ air and the 4364 mg/m³ air group rats sacrificed at the end of the observation period showed no findings.
4.3	**Other**	In the 2153 mg/m³ group no relevant effects on the body weight occurred. In the 4364 mg/m³ group only a slight reduction in body weight gains was detected.
4.4	**LC_{50}**	LC_{50} > 4364 mg/m³ air for males and females.

Section A6.1.3 Acute Toxicity

Annex Point IIA VI.6.1.3 6.1.3 Acute inhalation toxicity to the rat (Limit Test)

		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The method used to perform the study complied with the OECD-Guideline No. 403 and with the EC Guideline B.2. A study for acute inhalation toxicity in the rat was conducted with the test substance Icaridin. The purpose of the study was to enable the product to be classified (labelling), and to assess the potential acute health hazard when handling the substance.	
5.2	**Results and discussion**	Icaridin as an aerosol exhibited no inhalation toxicity to the rat up to the maximal technically producible concentration of 4364 mg/m^3 air.	
5.3	**Conclusion**	The results of this study demonstrate that Icaridin has no acute hazard potential for humans if the product is handled under proper conditions of use. LC_{50} >4364 mg/m³ air for males and females.	X
5.3.1	Reliability	1	
5.3.2	Deficiencies	No Although less than three concentrations were tested, the test fulfils the requirements for a Limit Test. The highest concentration tested was also the highest technically attainable and no mortalities occurred at this concentration.	

Section A6.1.3 Acute Toxicity

Annex Point IIA VI.6.1.3 6.1.3 Acute inhalation toxicity to the rat (Limit Test)

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 November 2006
Materials and Methods	Applicant's version is adopted.
Results and discussion	Applicant's version is adopted.
Conclusion	Other conclusion: The results of this study demonstrate that Icaridin as an aerosol does not exhibit inhalation toxicity up to the maximum technical producible concentration of 4364 mg/m^3 air. LC_{50} >4364 mg/m³ air for male and female rats.
Reliability	1 – when evaluated as a limit test applying the highest technically obtainable respirable concentration of the a.i.
Acceptability	Acceptable
Remarks	(5.3) The conclusion include areas not tested for in the study.

Section A6.1.3 Acute Toxicity

Annex Point IIA VI.6.1.3 6.1.3 Acute inhalation toxicity to the rat (Limit Test)

Table A6_1-1.3 Table for acute inhalation toxicity

Dose [mg/m³ air]	Toxicological results*	Duration of clinical signs	Time of death	Mortality (%)	Particles ≤ 5 µm (%)
Males					
0	0/0/10	—	—	—	—
2153	0/0/5	—	—	—	100
4364	0/0/5	—	—	—	100
LC_{50} > 4364 mg/m³ air					
Females					
0	0/0/10	—	—	—	—
2153	0/0/5	—	—	—	100
4364	0/0/5	—	—	—	100
LC_{50} > 4364 mg/m³ air					

*first number = number of dead animals

second number = number of animals with signs

third number = number of animals used

Section A6.1.4 Acute Dermal Irritation

Annex Point IIA VI.6.1.4 6.1.4 Acute dermal irritation

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1997a). Primary Dermal Irritation Study in Rabbits with Technical Grade KBR 3023. [redacted] Report No.: 107638, 1997-02-02, amended: 1997-04-28 (unpublished).	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Saltigo GmbH	
1.2.2	Company with letter of access	–	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes	
		US-EPA-FIFRA Guideline 81-5 (1982) (≅ OECD 404, 1992)	X
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes	
		The first observation was done 30 min after patch removal instead of 60 min after exposure.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in Section 2 of dossier.	
3.1.1	Lot/Batch number	030693	
3.1.2	Specification	As given in Section 2 of dossier.	
3.1.2.1	Description	Clear, viscous liquid	
3.1.2.2	Purity	96.7%	X
3.1.2.3	Stability	Assured by the study sponsor	
3.2	**Test Animals**		
3.2.1	Species	Rabbit	
3.2.2	Strain	New Zealand White, Hra:(NZW)SPF	
3.2.3	Source	HRP Inc., Denver, Pennsylvania	
3.2.4	Sex	Males and females	
3.2.5	Age/weight at study initiation	Age: adult Body weights: 2137-2260 g (males), 2139-2268 g (females)	
3.2.6	Number of animals per group	3 per sex	
3.2.7	Control animals	No	

Section A6.1.4 Acute Dermal Irritation

Annex Point IIA VI.6.1.4 6.1.4 Acute dermal irritation

3.3	**Administration/ Exposure**	
3.3.1	Application	Dermal
3.3.1.1	Preparation of test substance	Test substance was used as delivered.
3.3.1.2	Test site and preparation of test site	The test substance was tested on intact, clipped skin On the day, prior to application the back and if necessary the flanks of the animals were clipped free of hair.
3.3.2	Occlusion	Semi-occlusive
3.3.3	Vehicle	None
3.3.4	Concentration in vehicle	n.a.
3.3.5	Total volume applied	0.5 mL
3.3.6	Removal of test substance	Yes, with tap water.
3.3.7	Duration of exposure	4 h
3.3.8	Postexposure period	72 h
3.3.9	Controls	Untreated clipped skin areas of the test animals served as control
3.4	**Examinations**	
3.4.1	Clinical signs	Yes, at least once daily
3.4.2	Dermal examination	Yes
3.4.2.1	Scoring system	According to Draize scoring system. Erythema 0-4: 0: No erythema, 1: very slight erythema (barely perceptible), 2: well-defined erythema, 3 moderate to severe erythema, 4: severe erythema (beet redness) to slight eschar formation (injuries in depth) Oedema 0-4: 0: No oedema, 1: very slight oedema (barely perceptible), 2: well-defined oedema (edges of area well-defined by definite raising), 3: moderate to severe oedema (raised approximately 1mm), 4: severe oedema (raised more than 1 mm extending beyond the area of exposure)
3.4.2.2	Examination time points	4 h, 24 h, 48 h, 72 h
3.4.3	Other examinations	None
3.5	**Further remarks**	None

Section A6.1.4 Acute Dermal Irritation

Annex Point IIA VI.6.1.4 6.1.4 Acute dermal irritation

		4 RESULTS AND DISCUSSION	
4.1	**Average score**		
4.1.1	Erythema	0.0 for all examination time points	
4.1.2	Oedema	0.0 for all examination time points	
4.2	**Reversibility**	n.a.	
4.3	**Other examinations**	none	
4.4	**Overall result**	The application of the test substance onto the skin of rabbits caused no dermal irritation.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The dermal irritation potential of Technical Grade Icaridin was tested in adult male and female white albino rabbits. The methods used in this study are in accordance with the guidelines of the US EPA Title 40 CRF Part 160 (1983) and OECD-Guideline 404.	X
5.2	**Results and discussion**	There were no remarkable signs of dermal irritation after any of the observation periods.	X
5.3	**Conclusion**	The test substance, Technical Grade Icaridin, is considered not to be a dermal irritant of the rabbit skin.	
5.3.1	Reliability	1	
5.3.2	Deficiencies	–	

Section A6.1.4 Acute Dermal Irritation

Annex Point IIA VI.6.1.4 6.1.4 Acute dermal irritation

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 November 2006
Materials and Methods	Applicant's version is adopted
Results and discussion	Applicant's version is adopted with one amendment. The average scores (24h, 48h. 72h) for erythema and oedema are 0 and 0, respectively and should be stated as a result under this section since this is the classification criteria.
Conclusion	Applicant's version is adopted
Reliability	1
Acceptability	Acceptable
Remarks	Recent guidelines have been changed for irritation studies in order to reduce the number and potential suffering of test animals. 3.1.2.2 Purity: < than the specified minimal purity of 97.0%

Section A6.1.4 Acute Dermal Irritation

Annex Point IIA VI.6.1.4 6.1.4 Acute dermal irritation

Table A6_1-4S-1. Table for skin irritation study

Score (average animals investigated)	Time	Erythema	Oedema
Average score Draize scores (0 to maximum 4)	30 min	0	0
	24 h	0	0
	48 h	0	0
	72 h	0	0
Other times		---	---
Average score	24h, 48h, 72h	0.0	0.0
Reversibility: *		–	–
Average time for reversibility		–	–

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

Section A6.1.4 Acute Eye Irritation

Annex Point IIA VI.6.1.4 6.1.4 Acute eye irritation

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1997b), Primary Eye Irritation Study in Rabbits with Technical Grade KBR 3023. [redacted] Report No.: 107637, 1997-04-24 (unpublished).	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Saltigo GmbH	
1.2.2	Company with letter of access	–	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes OECD Guideline 405 (1987) US-EPA-FIFRA Guideline 81-4 (1984) Japanese MAFF 59 NohSan 4200 (1984)	
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes The first observation was done at 30 min instead of 60 min after exposure.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in Section 2 of dossier.	
3.1.1	Lot/Batch number	030693	
3.1.2	Specification	As given in Section 2 of dossier.	
3.1.3	Description	Clear, viscous liquid	
3.1.4	Purity	96.7%	X
3.1.5	Stability	Assured by the study sponsor	
3.2	**Test Animals**		
3.2.1	Species	Rabbit	
3.2.2	Strain	New Zealand White, Hra:(NZW)SPF	
3.2.3	Source	HRP, Inc., Denver, Pennsylvania	
3.2.4	Sex	♂ + ♀	
3.2.5	Age/weight at study initiation	Age: adult Body weights: 2349-2391 g (♂), 2273-2330 g (♀)	
3.2.6	Number of animals per group	per sex	
3.2.7	Control animals	No; the untreated eye served as control.	

Section A6.1.4 Acute Eye Irritation

Annex Point IIA VI.6.1.4 6.1.4 Acute eye irritation

3.3	**Administration/ Exposure**	Ocular instillation	
3.3.1	Preparation of test substance	Test substance was used as delivered.	
3.3.2	Amount of active substance instilled	0.1 g	
3.3.3	Exposure period	24 h	
3.3.4	Postexposure period	14 days	
3.4	**Examinations**		
3.4.1	Ophthalmoscopic examination	Yes	
3.4.2	Scoring system	Grades of ocular lesions: Cornea 0 – 4 (0 = no finding, 1 = slight, disperse, diffuse opacity, 2 = extensive, diffuse opacity, iris blurred, 3 = mother-of-pearl-like opacity, iris and pupil hardly recognisable, 4 = complete opacity, ulceration) Iris 0 – 2 (0 = no finding, 1 = swelling, reddening, positive light reaction, 2 = severe reddening and swelling, no light reaction) Conjunctivae Redness 0 – 3 (0 = blood vessels normal, 1 = vessels abnormally filled, 2 = diffuse reddening, 3 = diffuse deep reddening) Swelling 0 – 4 (0 = no swelling, 1 = slight swelling, 2 =severe swelling, lids everted, 3 = lids cover one half of eye, 4 = lids cover more than half eye, necroses and ulcers on the conjunctivas)	
3.4.3	Examination time points	30 min, 24 h, 48 h, 72 h, 7 d, 14 d	X
3.4.4	Other examinations	None	
3.5	**Further remarks**	None	
		4 RESULTS AND DISCUSSION	
4.1	**Clinical signs**	One animal vocalised during substance instillation but appeared normal afterwards.	
4.2	**Average score**		
4.2.1	Cornea	24h: 1.0 48h: 1.3 72h: 1.0 Average per animal: 1.0, 1.3, 1.0, 1.0, 1.0, 1.3	
4.2.2	Iris	24h: 0.5 48h: 0.5 72h: 0.2 Average per animal: 1.0, 0.0, 0.7, 0.0, 0.7, 0.0	

Section A6.1.4 Acute Eye Irritation

Annex Point IIA VI.6.1.4 6.1.4 Acute eye irritation

4.2.3	Conjunctiva		
4.2.4	Redness	24h: 2.0 48h: 1.8 72h: 2.0 Average per animal: 2.0, 2.0, 2.0, 2.0, 2.0, 1.7	
4.2.5	Chemosis	24h: 1.3 48h: 1.0 72h: 0.8 Average per animal: 1.0, 1.3, 1.0, 1.3, 0.7, 1.0	
4.3	**Reversibility**	Yes. Iris lesions had reversed by 96 h, corneal opacity and conjunctival swelling by 7 days, and conjunctival redness by day 14.	
4.4	**Other**	None	
4.5	**Overall result**	The test substance is slightly irritating to the eye.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The eye irritation potential of technical grade Icaridin was tested in adult male and female white albino rabbits. The methods used in this study are in accordance with OECD Guideline 405.	
5.2	**Results and discussion**	Icaridin produced ocular irritation characterised by corneal and iridial involvement and conjunctival irritation. Positive irritation reactions were observed in all animals beginning one hour post instillation. All treated eyes were clear of positive reactions by day 7, although mild conjunctival irritation was still present in four animals. On day 14, all signs of irritation had resolved in all animals.	X
5.3	**Conclusion**	Technical grade Icaridin is slightly irritating to the eyes of rabbits. Corneal opacity (completely reversible) was grade ≥ 1.0 in all of the tested rabbits. The criteria of Regulation 1272/2008 for classification as **Eye Irrit. 2, H319,** are met.	
5.3.1	Reliability	1	
5.3.2	Deficiencies	–	

Section A6.1.4 Acute Eye Irritation

Annex Point IIA VI.6.1.4 6.1.4 Acute eye irritation

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	22 November 2006
Materials and Methods	Applicant's version is adopted
Results and discussion	Applicant's version is adopted with one amendment. The average scores (24h, 48h, 72h) for cornea and iris are 1,1 and 0,4, respectively. For redness and chemosis in the conjunctiva average scores of 1,9 and 1,0 were obtained respectively. This should be stated as a result under this section since this serves as basis for the classification.
Conclusion	Applicant's version is adopted
Reliability	1
Acceptability	Acceptable
Remarks	Recent guidelines have been changed for irritation studies in order to reduce the number and potential suffering of test animals. The use of the high number of rabbits in this study does not compromise the validity of the study. 3.1.2.2 Purity: < than the specified minimal purity of 97.0% 3.4.1.2 Examination time points and Table A6_1_4E-1: The first reading point was not 30 min. but 1 hour after dose application.

Section A6.1.4 Acute Eye Irritation

Annex Point IIA VI.6.1.4 6.1.4 Acute eye irritation

Table A6_1_4E-1. Results of eye irritation study

	Cornea	Iris	Conjunctiva	
			Redness	Chemosis
Score (average of animals investigated)	0 to 4	0 to 2	0 to 3	0 to 4
1 h	0.0	0.3	2.0	2.6
24 h	1.0	0.5	2.0	1.3
48 h	1.3	0.5	1.8	1.0
72 h	1.0	0.2	2.0	0.8
Average 24 h, 48 h, 72 h	1.1	0.4	1.9	1.0
Reversibility*	c	c	c	c
Average time for reversion	7 d	48 h	14 d	96 h
* *c :* *completely reversible* *n c :* *not completely reversible* *n :* *not reversible*				

Section 6.1.4 Acute Eye Irritation

Annex Point IIA VI.6.1.4 6.1.4 Acute eye irritation in rabbits

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1988): KBR 3023 – Studies on the local irritant / corrosive effect on skin and eyes (rabbits) in accordance with OECD Guideline No. 404 and 405. [redacted] Report No. 17019, 1988-08-10 (unpublished)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Saltigo GmbH	
1.2.2	Company with letter of access	–	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes OECD Guideline 405 (1981)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.3	**Test material**	Icaridin	
3.1.1	Lot/Batch number	19001/87*e*	
3.1.2	Specification	As given in Section 2	
3.3.2.1	Description	Clear liquid	
3.3.2.2	Purity	99.40 %	
3.3.2.3	Stability	Guaranteed during the study period	
3.4	**Test Animals**		
3.1.3	Species	Rabbit	
3.1.4	Strain	HC –NZW	
3.1.5	Source	Interfauna UK Ltd.	
3.1.6	Sex	Males	
3.1.7	Age/weight at study initiation	Adult; 2.9 to 3.1 kg	
3.1.8	Number of animals per group	3	
3.1.9	Control animals	No. The untreated eye of each animal was used as control.	

Section 6.1.4 Acute Eye Irritation

Annex Point IIA VI.6.1.4 6.1.4 Acute eye irritation in rabbits

3.5	**Administration/ Exposure**	
3.1.10	Preparation of test substance	Test substance was used as delivered
3.1.11	Amount of active substance instilled	100 µL
3.1.12	Exposure period	24 h
3.1.13	Postexposure period	21 days
3.6	**Examinations**	State of the cornea, iris, conjunctivae, lacrimation and aqueous humour
3.1.14	Ophthalmoscopic examination	Yes
3.6.2.1	Scoring system	To assess the state of the cornea, iris, conjunctivae and lacrimation the *Draize* scales according to OECD Guideline 405 were used.
3.6.2.2	Examination time points	60 min, 24 h, 48 h, 72 h, 7 d, 14 d and 21 d
3.1.15	Other investigations	None
3.7	**Further remarks**	
		4 RESULTS AND DISCUSSION
4.1	**Clinical signs**	–
4.2	**Average score**	
4.2.1	Cornea	Individual results animal 1 / animal 2/ animal 3 24h: 1 / 0 / 1 48h: 1 / 0 / 1 72h: 1 / 0 / 1
4.2.2	Iris	Individual results animal 1 / animal 2/ animal 3 24h: 0 / 0 / 0 48h: 0 / 0 / 0 72h: 0 / 0 / 0
4.2.3	Conjunctiva	

Section 6.1.4 Acute Eye Irritation

Annex Point IIA VI.6.1.4 6.1.4 Acute eye irritation in rabbits

4.2.3.1	Redness	Individual results animal 1 / animal 2/ animal 3 24h: 1 / 1 / 2 48h: 1 / 0 / 1 72h: 0 / 0 / 1	
4.2.3.2	Chemosis	Individual results animal 1 / animal 2/ animal 3 24h: 1 / 0 / 1 48h: 0 / 0 / 1 72h: 0 / 0 / 0	
4.3	**Reversibility**	Yes minor corneal (clouding) and conjunctival reactions (redness and chemosis) All effects were totally reversible within the first days after application of the product.	
4.4	**Other**	None	
4.5	**Overall result**	The test substance is slightly irritating to the eye.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	One-hundred µL of the test substance were administered into the conjunctival sac of one eyelid of each test animal. After administration the eyelid was gently closed for approximately 1 sec. The second eye of each animal left untreated to serve as control. After 24 h the treated eye was washed with physiological saline. Findings were recorded 1 h, 24 h, 48 h, 72 h, 7 d, 14 d and 21 d after administration. The methods used in this study were also comparable with the OECD-guideline 405	
5.2	**Results and discussion**	There were only minor corneal and conjunctival reactions which were totally reversible within the first days after treatment. Regarding the high dose per eye and the way of administration the test substance can be considered as slightly irritant to the eye.	X
5.3	**Conclusion**	The test substance is slightly irritating to the eye. Corneal opacity (completely reversible) was grade ≥ 1.0 in 2 out of 3 tested rabbits. The criteria of Regulation 1272/2008 for classification as **Eye Irrit. 2, H319,** are met.	
5.3.1	Reliability	1	
5.3.2	Deficiencies	No	

Section 6.1.4 Acute Eye Irritation

Annex Point IIA VI.6.1.4 6.1.4 Acute eye irritation in rabbits

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 November 2006
Materials and Methods	Applicant's version is adopted.
Results and discussion	Applicant's version is adopted with one amendment. The average scores (24h, 48h, 72h) for cornea and iris are 0,7 and 0, respectively. For redness and chemosis in the conjunctiva average scores of 0,8 and 0,3 were obtained respectively. This should be stated as a result under this section since this serves as basis for the classification. In table A6-1-4E-1 under cornea the column "area" should be deleted as it is not used in Draize scoring system.
Conclusion	Applicant's version is adopted.
Reliability	1
Acceptability	Acceptable
Remarks	The present OECD guideline405 (dated April 2002) use a preferred sequential testing strategy in order to avoid excessive use of test animals and excessive suffering in the animals used. The use of the former test guideline does not compromise the validity of the test result.

Section 6.1.4 Acute Eye Irritation

Annex Point IIA VI.6.1.4 6.1.4 Acute eye irritation in rabbits

Table A6_1_4E-1. Results of eye irritation study

	Cornea		Iris	Conjunctiva	
	Opacity	Area		Redness	Chemosis
Score (average of animals investigated)	0 to 4	0 to 4	0 to 2	0 to 3	0 to4
60 min	0.7	2.7	0.0	1.7	1.7
24 h	0.7	2.7	0.0	1.3	0.7
48 h	0.7	2.7	0.0	0.7	0.3
72 h	0.7	2.7	0.0	0.3	0.0
Average 24 h, 48 h, 72 h	0.7	2.7	0.0	0.8	0.3
Reversibility*	c	c	n.a.	c	c
Average time for reversion	≤ 7d	≤ 7d	n.a.	≤ 7d	≤ 7d
* *c :* *completely reversible* *n c :* *not completely reversible* *n :* *not reversible*					

Section A6.1.5 Skin sensitisation

Annex Point IIA VI.6.1.5 6.1.5 Skin sensitisation test in guinea pigs (BUEHLER Test)

Official use only

1 REFERENCE

1.1	**Reference**	[redacted] (1991) Study for skin-sensitising effect on guinea pigs. [redacted], Report No.: 20623, 1991-09-05 (unpublished), amended by Report No.: 20623A; 1999-08-30
1.2	**Data protection**	Yes
1.2.1	Data owner	Saltigo GmbH AG
1.2.2	Companies with letter of access	–
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes OECD Guideline No. 406 (1981) EC Method B.6 (1984) EPA-FIFRA 81-6 (1984)
2.2	**GLP**	Yes
2.3	**Deviations**	Yes – Only 12 animals were used in the treatment group. – Induction and challenge were done on the same skin area. – Examinations of skin reactions were done 24 h, 48 h and 72 h instead of 30 h and 54 h after start of challenge exposure. – The sensitivity and reliability of the experimental technique was not demonstrated in time intervals according to OECD Guideline 406.

3 MATERIALS AND METHODS

3.1	**Test material**	As given in Section 2
3.1.1	Lot/Batch number	19009/89
3.1.2	Specification	As given in Section 2
3.1.2.1	Description	Colourless, clear viscous liquid
3.1.2.2	Purity	99.4%
3.1.2.3	Stability	Until 1991-08-27
3.1.2.4	Preparation of test substance for application	a) Induction: used undiluted b) Challenge: used undiluted
3.1.2.5	Pretest performed on irritant effects	Yes
3.2	**Test Animals**	

Section A6.1.5 Skin sensitisation

Annex Point IIA VI.6.1.5 6.1.5 Skin sensitisation test in guinea pigs (BUEHLER Test)

3.2.1	Species	Guinea pigs
3.2.2	Strain	Bor:DHPW
3.2.3	Source	Versuchstierzucht Winkelmann, Borchen, Germany
3.2.4	Sex	Males
3.2.5	Age/weight at study initiation	5-8 weeks, 319-401 g
3.2.6	Number of animals per group	12
3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	BUEHLER Test
3.3.1	Induction schedule	Day 0 – day 6-8 – day 13-15 (see Table A6_1_5-1)
3.3.2	Duration of induction exposures	6 h
3.3.3	Way of induction	Topical, occlusive
3.3.4	Concentrations used for induction	0.5 mL test substance (100%)
3.3.5	Challenge schedule	Day 27-29 (see Table A6_1_5-1)
3.3.6	Duration of challenge exposure	6 h
3.3.7	Concentrations used for challenge	0.5 mL test substance (100%)
3.3.8	Rechallenge	No
3.3.9	Scoring schedule	24 h, 48 h and 72 h after challenge exposure
3.3.10	Removal of the test substance	Induction: After 6 h of exposure the test substance was removed with sterile physiological saline Challenge: After 6 h of exposure the treated areas were chemically depilated.
3.3.11	Positive control substance	Not done; see 2.3
3.4	**Examinations**	Skin irritation of treated skin areas at the following time points: Induction: 24 h Challenge: 24 h, 48 h, 72 h Clinical symptoms (every day during the complete study) Body weight (Day 0 and at the end of study)
3.4.1	Pilot study	Skin irritation
3.5	**Further remarks**	–
		4 RESULTS AND DISCUSSION
4.1	**Results of pilot studies**	The test substance was applied in concentrations of 12.5%, 25%, 50% and 100% on different skin areas of 5 guinea pigs for 6 h under occlusive conditions.

Section A6.1.5 Skin sensitisation

Annex Point IIA VI.6.1.5 6.1.5 Skin sensitisation test in guinea pigs (BUEHLER Test)

		After the exposure the treated skin was chemically depilated and skin reactions were assessed after 24 h, 48 h and 72 h. Only in the 100% group one animal showed slight patchy erythema after 24 h and eschar formations in places and the treatment areas after 48 h and 72 h. Therefore the induction and challenge concentration for the main study was set to 100%.		
4.2	**Results of test**			
4.2.1	24 h after challenge	Test compound group 0/12 (Number of animals with signs of allergic reactions/number of animals)	Control group 0/12	
4.2.2	48 h after challenge	Test compound group 1/12 (Number of animals with signs of allergic reactions/number of animals)	Control group 1/12	
4.2.3	Other findings	None		
4.3	**Overall result**	Icaridin was not sensitizing to the skin of guinea pigs.		
		5 APPLICANT'S SUMMARY AND CONCLUSION		
5.1	**Materials and methods**	The test was done according to the OECD Guideline 406 (BUEHLER test) but with the deviations listed under "2.3" (see above). A study for skin sensitisation in guinea pigs was conducted with the test substance Icaridin. The purpose of the study was to enable the product to be classified (labelled), and to assess the potential acute health hazard when handling the test substance		
5.2	**Results and discussion**	After the challenge only one animal in the control and test compound group showed slight redness in places 48 h and 72 h after start of treatment. The study was performed during the updating of OECD Guideline 406. In the former version no precise requirements for positive controls were given. Therefore a positive control of the BUEHLER test was carried out more than 6 months before and after the completion of this study.		
5.3	**Conclusion**	Despite the fact that no positive control test was accomplished in the required time frame, the results of this study show that Icaridin has no skin-sensitising potential under the conditions of the Buehler test.		X
5.3.1	Reliability	2		X
5.3.2	Deficiencies	Yes. The study was performed with a less-than-required number of animals and without an appropriate reliability check. However, the performing laboratory has a record of reliably performing BUEHLER tests in the DHPW strain of guinea pigs as documented in an amendment to the study report. The amendment also reports MAGNUSSON-KLIGMAN tests that were performed in this strain within 6 months of the Icaridin study and reliably detected sensitisation by the strong sensitizer DNCB. DNCB was on the list of suggested positive controls in the versions of		

Section A6.1.5 Skin sensitisation

Annex Point IIA VI.6.1.5 6.1.5 Skin sensitisation test in guinea pigs (BUEHLER Test)

OECD 406 or EC Method B.6 that were in force at the time the study was conducted. The use of moderate-to-mild sensitizers in reliability checks was not yet required. In addition, the number of animals to be used in BUEHLER tests (treatment group) is given as "10-20".

The test was therefore conducted in accordance with the applicable guidelines in their versions at the time of the study. The deficiencies do not disqualify the conclusion that Icaridin is not sensitising to skin.

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	02 October 2006
Materials and Methods	Applicant's version is adopted
Results and discussion	Applicant's version is adopted
Conclusion	The study is not accepted due to several deficiencies and therefore no reliable information concerning icaridins sensitising properties are currently available. Furthermore the guinea-pig Maximisation test is the preferred test and no scientific justification has been provided for using another test (Buehler). A new skin sensitisation test (Maximisation test) are requested before the annex I entry.
Reliability	3
Acceptability	Not acceptable
Remarks	Due to several deficiencies (e.g 12 animals tested instead of 20 which compromises the study considerable because the risk for false negative is very high check med FDIR) in this test in relation to current guidelines a second study of the skin sensitising potential has been requested. The amended report on sensitivity of the Guinea pig strain to a moderate skin sensitiser has no relevance as with respect to Buehler testing as no maximisation is used in that test system.

Section A6.1.5 Skin sensitisation

Annex Point IIA VI.6.1.5 6.1.5 Skin sensitisation test in guinea pigs (BUEHLER Test)

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

Inductions	**BUEHLER test**	**Observations/Remarks**
	Day of treatment or time point	
Induction 1	day 0	no findings
Induction 2	day 6-8	no findings
Induction 3	day 13-15	no findings
Challenge	day 27-29	see under "scoring"
Scoring 1	24 h after end of challenge	no findings
Scoring 2	48 h after start of challenge	only slight patchy erythema
Scoring 3	72 h after start of challenge	only slight patchy erythema

Table A6_1_5-2. Result of skin sensitisation test

	Number of animals with signs of allergic reactions / number of animals in group		
	Negative control	**Test group**	**Positive control**
Scored after 24 h	0 / 12	0 / 12	–
Scored after 48 h	1 / 12	1 / 12	–
Scored after 72 h	1 / 12	1 / 12	–

Section A6.1.5 Skin sensitisation

Annex Point IIA VI.6.1.5 6.1.5 Skin sensitisation test in guinea pigs (Maximisation Test)

Official use only

		1 REFERENCE
1.1	**Reference**	[redacted] (2007) Icaridin – Study for Skin-Sensitising Effect in Guinea Pigs. [redacted] Report No. AT04065, 2007-08-31 (unpublished)
1.2	**Data protection**	Yes
1.2.1	Data owner	Saltigo GmbH
1.2.2	Companies with letter of access	–
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes, OECD Guideline No. 406 (1992), EC Method B.6 (1996) OPPTS 870.2600 (2003)
2.2	**GLP**	Yes
2.3	**Deviations**	Animals were not treated with sodium lauryl sulphate prior to topical induction although the test substance *per se* is not irritating.
		3 MATERIALS AND METHODS
3.1	**Test material**	As given in Section 2
3.1.1	Lot/Batch number	CHCAEC0060
3.1.2	Specification	As given in Section 2
3.1.2.1	Description	Clear, colourless liquid
3.1.2.2	Purity	98.9%
3.1.2.3	Stability	Until 2008-06-21 Stability in vehicle (PEG 400) was verified for up to 2 h.
3.1.2.4	Preparation of test substance for application	c) Intradermal induction: solution in polyethylene glycol 400 d) Topical induction + Challenge: used undiluted
3.1.2.5	Pretest performed on irritant effects	Yes
3.2	**Test Animals**	
3.2.1	Species	Guinea pigs
3.2.2	Strain	Crl: HA (SPF)
3.2.3	Source	Charles River Laboratory, Kißlegg, Germany.
3.2.4	Sex	Females
3.2.5	Age/weight at study initiation	5-6 weeks, 336 - 414 g

Section A6.1.5 Skin sensitisation

Annex Point IIA VI.6.1.5 6.1.5 Skin sensitisation test in guinea pigs (Maximisation Test)

3.2.6	Number of animals per group	Range finding test: 2 animals Main test: 20 (test substance group) / 10 (controls)
3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	MAGNUSSON-KLIGMAN Test
3.3.1	Induction schedule	Day 0: intradermal induction Day 7: topical induction
3.3.2	Duration of induction exposures	48 h
3.3.3	Way of induction	Intradermal + topical (occlusive)
3.3.4	Concentrations used for induction	Intradermal: 5% (= 20 mg test item/animal) Topical: 100% (= 500 mg test item/animal)
3.3.5	Challenge schedule	Day 21
3.3.6	Duration of challenge exposure	24 h
3.3.7	Concentrations used for challenge	100% (= 500 mg test item/animal)
3.3.8	Rechallenge	No
3.3.9	Scoring schedule	24 h and 48 h after end of challenge exposure
3.3.10	Removal of the test substance	The test substance was removed with sterile physiological saline.
3.3.11	Positive control substance	Alpha-hexyl cinnamic aldehyde (HCA) in PEG 400 (intradermal induction: 5%, topical induction: 25%, challenge: 12% and 6%) Bayer AG Report No. PH-34921, May 14, 2007
3.4	**Examinations**	The animals were observed for clinical signs at least once daily throughout the entire study period. The body weights of the animals were recorded on day 1 before the first induction and at the end of the study. Skin reactions were assessed using the 0-3 scoring system of Magnusson and Kligman.
3.4.1	Pilot study	Skin irritation
3.5	**Further remarks**	–
		4 RESULTS AND DISCUSSION
4.1	**Results of pilot studies**	Intradermal injection of 5% test substance in adjuvant mix caused a white wheal with red surrounding after 24 hours; the injection site was incrusted after 48 hours. No skin reactions were provoked by topical occlusive 24- or 48-h exposures to the undiluted test substance.

Section A6.1.5 Skin sensitisation

Annex Point IIA VI.6.1.5 6.1.5 Skin sensitisation test in guinea pigs (Maximisation Test)

4.2	**Results of test**	*see Table A6_1_5-1*	
4.2.1	24 h after challenge	Test compound group: 0/20; Control group: 0/10 (Number of animals with signs of allergic reactions/number of animals)	
4.2.2	48 h after challenge	Test compound group: 0/20; Control group: 0/10 (Number of animals with signs of allergic reactions/number of animals)	
4.2.3	Other findings	Reliability check: after the challenge with a 12% and 6% HCA formulation in both concentrations 100% of the HCA-induced animals exhibited dermal reactions in the challenge treatment. There was no reddening of the skin observed on control group animals.	
4.3	**Overall result**	Icaridin was not sensitizing to the skin of guinea pigs.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	A Guinea Pig Maximization Test (GPMT) was performed with Icaridin on 30 female guinea pigs (20 animals for the test item group and 10 control animals) to determine potential skin-sensitizing properties. Additional two animals were used for dose finding. The study was conducted according to OECD Guideline No. 406 (1992) with the following test item concentrations: Intradermal induction: 5%, topical induction: 100%, challenge: 100% The test item was formulated in polyethylene glycol 400 to yield a solution.	X
5.2	**Results and discussion**	The challenge with the 100% test item concentration led to no skin effects in the animals of the test item group and to no skin effects in the animals of the control group. A reliability check, performed with alpha-hexyl cinnamic aldehyde within a 6-month interval to the current study, demonstrated the reliability of the employed test system.	
5.3	**Conclusion**	**Icaridin is not a skin sensitizer.**	X
5.3.1	Reliability	1	
5.3.2	Deficiencies	The pre-treatment of guinea pigs with SLS before topical induction was not conducted. The need for this pre-treatment has been discussed in a review by Steiling *et al.* [*Food Chem. Toxicol.* **39** (2001), 293-301]. It is considered by the authors that such treatment is unnecessary and, in the interests of both good scientific practice and animal welfare considerations, should be discontinued for the following reasons: – In the guinea pig maximisation test FCA is given by intracutaneous injection to enhance immune responses. Even in the absence of SLS pre-treatment the skin is markedly inflamed. Further irritation resulting from exposure to SLS may compromise performance of the test while adding significantly to the trauma to which guinea pigs are subjected. – The rationale for the use of SLS is to increase the bioavailability of the test material. However, the critical event in this context is the ability of the test chemical to gain access to the viable epidermis where interaction with Langerhans cells takes place and the production of	

Section A6.1.5 Skin sensitisation

Annex Point IIA VI.6.1.5 6.1.5 Skin sensitisation test in guinea pigs (Maximisation Test)

relevant skin cytokines is induced or increased. The available data suggest that topical treatment with SLS can enhance the percutaneous penetration of some chemicals without increasing the amount of material found within the viable epidermis (dermal absorption). In some instances the concentration of test chemical within the epidermis has been shown to be reduced following pre-treatment with SLS. Further, although the notion is that SLS pre-treatment may be effective in enhancing skin reactions in guinea pigs to weak contact allergens, the basis for such observations is not clear, and not consistent with the experience of other investigators.

- Pre-treatment with SLS may compromise the scientific integrity of the test since it may result in hyperirritable skin, a decrease in the irritation threshold and an associated risk of "false-positive" reactions. In humans also, treatment with SLS has been associated with false positive skin reactions. Moreover, there is some evidence that SLS may have the potential to suppress skin reactivity with the risk of "false-negative" results. A non-specific hyperreactivity induced by pre-treatment with SLS may cause lowered or "false-negative" responses in cases where a reduction in irritation threshold has influenced the challenge concentration based on range-finding studies conducted with SLS-treated guinea pigs.
- There is limited evidence to suggest that, in some circumstances, SLS may itself act as an allergen

On these grounds, the omission of SLS pre-treatment from the test protocol does not represent a deficiency of the present study.

Evaluation by Competent Authorities

Use separate "evaluation boxes" to provide transparency as to the comments and views submitted

Evaluation by Rapporteur Member State

Date	*09.03.2009*
Materials and Methods	*Applicant's version is adopted*.
Results and discussion	*Applicant's version is adopted*.
Conclusion	Applicant's version is adopted; however it is reformulated as follows. *5.1 … the induction concentration was based on the results of a pilot study.* 5.3 *Icaridin is not considered to be a potential skin sensitizer based upon the results of this study.*
Reliability	*1*
Acceptability	acceptable
Remarks	2.2 GLP. It is remarkable that the GLP compliance statement was signed 21 July 2005, which was in fact two years before the study was performed. *2.3 The deviation from guideline by not pre-treating the skin with SLS is accepted.*

Section A6.1.5 Skin sensitisation

Annex Point IIA VI.6.1.5 6.1.5 Skin sensitisation test in guinea pigs (Maximisation Test)

Table A6_1_5-1. Result of skin sensitisation test

	Number of animals with signs of allergic reactions / number of animals in group		
	Negative control	**Test group**	**6% HCA (pos control)**
Scored after 24 h	0/10	0/20	20/20
Scored after 48 h	0/10	0/20	20/20

Section 6.2 Annex Point IIA VI.6.2	Metabolism studies in mammals ADME study in rats using oral administration	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	The absorption, distribution, metabolism and excretion of Icaridin following oral administration to rats have not been studied. Instead, an ADME study in rats was performed using dermal and intravenous application of ^{14}C-Icaridin ([redacted], 1997). Icaridin is used in products intended for dermal application and dermal penetration will certainly be the dominant route of uptake. Consequently, dermal exposure has been chosen as the method of administration in most studies on the various toxicological end points. Elucidation of the metabolic behaviour after dermal exposure is therefore most relevant for the assessment of potential health effects in humans. The AOEL will be derived from a dermal NOAEL and data on dermal, but not gastro-intestinal, absorption is necessary to calculate this value. It can also be reasonably assumed that data on the metabolic fate of Icaridin following dermal exposure is representative for the overall metabolic and toxicokinetic behaviour of the compound. The reasoning behind this assumption is the following:	X
	The metabolic pathways following i.v. or dermal dosage do not differ significantly, i.e., there were no metabolites that occurred after i.v. dosing with Icaridin that have not also been found in tissues or excreta from rats dosed dermally ([redacted], 1997). This suggests that there is no considerable effect of the route of entry on the metabolic fate of Icaridin.	X
	Icaridin was non-toxic after both acute oral and acute dermal dosage to rats ([redacted], 1990; [redacted], 1991). Furthermore, the dermal and dietary subchronic studies on rats revealed analogous effects ([redacted], 1995; [redacted], 2001b). In both studies, hypertrophy of liver and increased kidney weights were observed in the highest dose groups. This implies that Icaridin does not provoke route-specific toxicity after acute or subchronic exposure and that the metabolic fate of the parent compound is essentially independent from the route of exposure. Considering the existing data and taking animal welfare into account, submission of an oral metabolism study in rats is not justified. <u>References:</u> [redacted] (1997) Rat metabolism study after intravenous injection and after dermal application [Hydroxymethyl-1-^{14}C]. Report No. PF-4178 [redacted]. (1990) Study on the acute oral toxicity to rats. Report No. PH-19295 [redacted]. (1995) A repeated dose 90-day dermal toxicity study with technical grade Icaridin in rats. Report No. 90-122-HC	

Section 6.2 Annex Point IIA VI.6.2	Metabolism studies in mammals **ADME study in rats using oral administration**
	[redacted] (1991) Acute dermal toxicity study with technical grade Icaridin in rats. Study No. 90-022-GD [redacted] (2001b) Technical grade Icaridin: A subchronic toxicity study in the rat (14-week interval). Report No. 110223
Undertaking of intended data submission [ ]	
	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	10 November 2006
Evaluation of applicant's justification	In the rats the identical NOAELs values in the dermal and oral subchronic studies could also be a result of poor oral absorption. Since no oral ADME study is available this is actually unknown. The argument regarding essentially identical metabolites in rats following i.v or dermal application does not give any information about what happens after oral absorption and a route specific response. I.v and dermal are more alike as no first pass metabolism effect in the liver will occur and the substance will enter the blood system right after passing the skin (were metabolism could also occur). It is therefore more relevant to compare with i.v metabolism than oral metabolism due to the intended use of the substance on the skin.
Conclusion	The applicant's justification is acceptable with the above mentioned comments.
Remarks	As it is assumed that oral uptake is 100%, it can be accepted that there is no oral ADME study. The oral absorption will as default be set to 100% although this is not the worst case situation.

Section A6.2 Metabolism

Annex Point IIA VI.6.2 6.2 Metabolism of [^{14}C]-Icaridin in human volunteers

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1 REFERENCE

1.1 Reference [redacted] (1997), [Hydroxyethyl-1-^{14}C]KBR 3023: Human Volunteer Metabolism Study After Dermal Application. [redacted] Report No. PF 4187, 1997-01-07 (unpublished).

1.2 Data protection Yes

1.2.1 Data owner: Saltigo GmbH

1.2.2 Companies with letter of access: –

1.2.3 Criteria for data protection: Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study Yes

US-EPA Guideline 85-1 (1982) (≅ OECD Guideline 417 (1984))

2.2 GLP Yes

2.3 Deviations GLP deviation: Radioassay and pooling of urine samples were started on June 23, 1994, whereas the study protocol was signed by the study director on June 29, 1994.

3 MATERIALS AND METHODS

3.1 Test material Mixture of unlabelled and ^{14}C-labelled Icaridin

3.2 Non-labelled parent compound

3.2.1 Lot/Batch number: 921103ELB04

3.2.2 Specification

3.2.2.1 Description: Clear, colourless liquid

3.2.2.2 Purity: 98.3%

3.2.2.3 Stability: Expiry date: December 1994

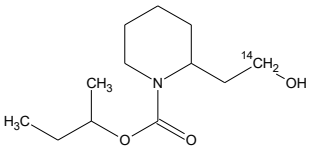
3.3 Labelled parent compound [2-(2-Hydroxyethyl-2-^{14}C)]-Icaridin

3.3.1 Lot/Batch number: KML 2159

3.3.2 Specification

Section A6.2 Metabolism

Annex Point IIA VI.6.2 6.2 Metabolism of [^{14}C]-Icaridin in human volunteers

3.3.2.1	Description	–
3.3.2.2	Purity	>98% radiochemical purity, >99% chemical purity 96 µCi/mg (=3.55 MBq/mg)
3.3.2.3	Stability	–
3.3.2.4	Radiolabelling	
3.4	**Test Subjects**	
3.4.1	Species	Human
3.4.2	Source	Volunteers
3.4.3	Sex	Males
3.4.4	Age/weight at study initiation	18-28 years; 62-72.9 kg
3.4.5	Number of subjects	6 per group
3.4.6	Control subjects	No
3.5	**Administration/ Exposure**	Dermal
3.5.1	Duration of treatment	8 hours
3.5.2	Post-exposure period	6 days
3.5.3	Specific activity of test substance	2.5 µCi/mg (=93 kBq/mg)
3.6	**Dermal application**	
3.6.1	Type	Non-occlusive, on the volar aspect of the forearm
3.6.2	Concentration of test substance	15%; undiluted, both
3.6.3	Vehicle	Ethanol
3.6.4	Concentration in vehicle	–
3.6.5	Volume applied	15% formulation: 100 µL (0.625 mg Icaridin/cm²) Undiluted formulation: 15 µL (0.625 mg Icaridin/cm²)
3.7	**Examinations**	
3.7.1	Biokinetic parameters	Metabolism
3.7.2	Samples	Urine, stool, blood
3.7.3	Sampling time (0 h = start of	Blood: 0, 2, 4, 6, 8, 10, 12, 16, 24, 36, 48, 72, 96, 120 h Urine: pre-dose, 0-4, 4-8, 8-12, 12-24, 24-36, 36-48, 48-60, 60-72, 72-

Section A6.2 Metabolism

Annex Point IIA VI.6.2 6.2 Metabolism of [^{14}C]-Icaridin in human volunteers

	application)	84, 84-96, 96-108, 108-120, 120-128 h Faeces: pre-study, individually throughout the study period up to 128 h Tape stripping of surface skin layers: 1, 23 and 45 h after end of exposure.
		4 RESULTS AND DISCUSSION
4.1	**Toxic effects, clinical signs**	15% formulation group: one person: mild impaired concentration on days 1-2 one person: mild headache on days 1-6 Undiluted formulation group: one person: mild dizziness on day 1
4.2	**Recovery of labelled compound**	15% formulation: 98.13% Undiluted formulation: 97.00%
4.3	**Metabolism**	Glucuronic acid conjugates of the parent compound or phase-I metabolites were the main metabolites identified in urine (M15, M5, M14, Table A6_2-1, Figure A6_2-1). Hydroxylation of the isobutyl moiety and oxidation of the hydroxyethyl group were the main phase-I reactions that occurred.
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	This report contains the metabolism part of a study investigating percutaneous absorption, metabolism and excretion of ^{14}C-Icaridin in male volunteers. Details of the "in-life" part of this study (Selim, 1994, Report-No. P1092004) are summarised elsewhere in this dossier. The test compound Icaridin was administered either as a 15% (w/w) solution in ethanol or as the undiluted technical grade product. After 8 h of exposure the protective wrappings were removed and the skin area was thoroughly cleaned, to remove remaining test substance. Blood, urine, faeces and tape strippings of the skin were collected at the times described under "3.4.3" (see above). Only urine contained an appreciable fraction of the radioactivity. Urine of the volunteers was analysed for parent test compound and metabolites by HPLC. Metabolites identified in a previous rat metabolism study with Icaridin (Ecker & Weber, 1997, Report No. PF 4178) were utilised for identification of radioactive metabolites.
5.2	**Results and discussion**	Icaridin after dermal administration mainly underwent phase-II metabolism resulting in glucuronic acid conjugates of the parent compound as main metabolites. Unchanged parent compound was not found in human urine. Since Icaridin contains two chiral centres, two separated peaks of glucuronide conjugates were found (metabolites M14 and M15; 15.8% and 27.3%). The identity of the conjugates was verified by treatment with glucuronidase. The resulting aglycon was identified as unchanged parent compound by co-chromatography with authentic Icaridin. Besides the two glucuronide conjugates, metabolites hydroxylated in the piperidine ring and in the 2-methylpropyl ester side chain and those with the alcohol group oxidised to a carboxyl group were found in minor amounts. One metabolite (M5), the structure of which was not

Section A6.2 Metabolism

Annex Point IIA VI.6.2 6.2 Metabolism of [^{14}C]-Icaridin in human volunteers

		elucidated, made up approx. 17% of the metabolites. It was also found to a minor extent in the rat metabolism study. From its chromatographic behaviour it was tentatively identified to be isomeric with M6. The absolute amount of M5 in the human study was too little for structure elucidation by spectroscopic means. The most prominent rat metabolites (M7, M8, M9, and M10) amounted to approx. 12% of urinary radioactivity. Including the tentatively identified metabolite M5, about 93% of urinary radioactivity was identified.	
5.3	**Conclusion**	After dermal administration Icaridin mainly underwent phase-II metabolism resulting in glucuronic acid conjugates of the parent compound as main metabolites. Unchanged parent compound was not found in human urine.	X
5.3.1	Reliability	1	
5.3.2	Deficiencies	None	

Section A6.2 Metabolism

Annex Point IIA VI.6.2 6.2 Metabolism of [^{14}C]-Icaridin in human volunteers

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	27 October 2006
Materials and Methods	Applicant's version is adopted
Results and discussion	Applicant's version is adopted
Conclusion	After dermal administration Icaridin mainly underwent phase-II metabolism resulting in glucuronic acid conjugates (metabolites M14 and M15; 15.8% and 27.3%) of the parent compound as main metabolites. Besides the two glucuronide conjugates, metabolites hydroxylated in the piperidine ring and in the 2-methylpropyl ester side chain and those with the alcohol group oxidised to a carboxyl group were found in minor amounts. Unchanged parent compound was not found in human urine.
Reliability	1
Acceptability	Acceptable
Remarks	

Section A6.2 Metabolism

Annex Point IIA VI.6.2 6.2 Metabolism of [^{14}C]-Icaridin in human volunteers

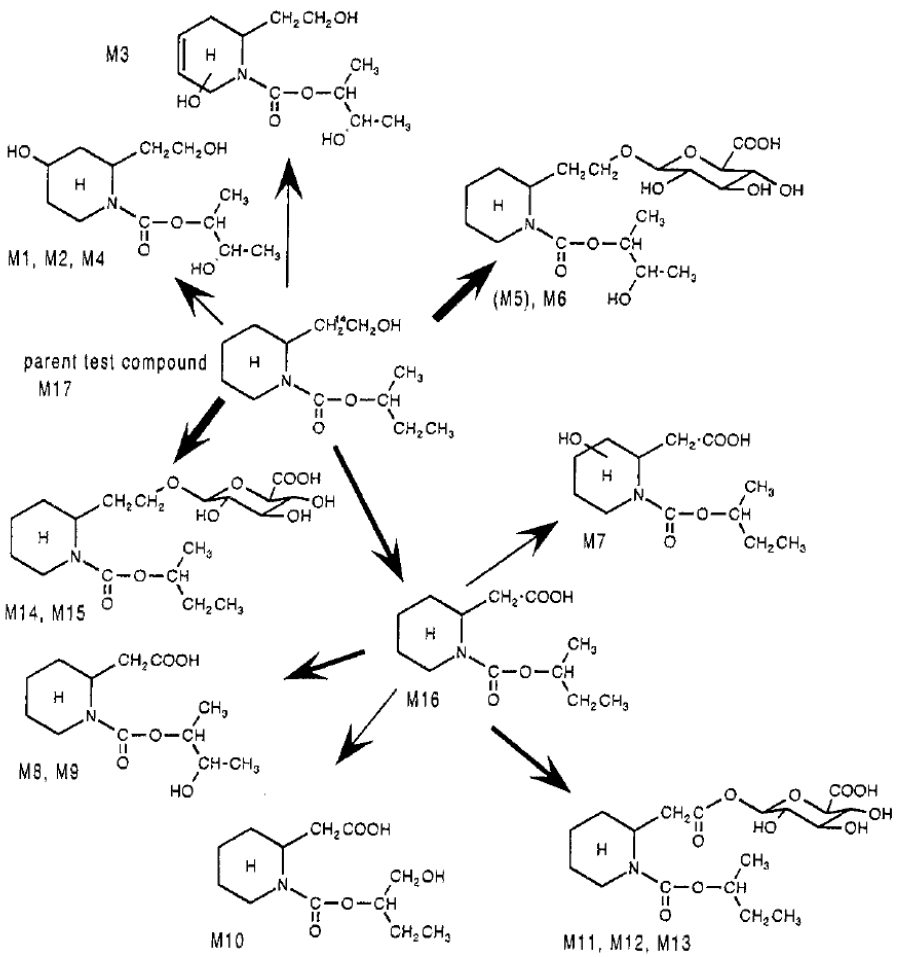
Table A6_2-1 Quantification of metabolites in human urine following dermal application (8 h exposure, 6 days post-exposure) of [^{14}C]-Icaridin in 15% ethanol

Compound	% of radioactivity in urine
M1 + M4	3.1
M5	17.4
M6	2.3
M7	0.9
M8	6.2
M9	2.6
M10	2.4
M11 + M13	6.9
M14	15.8
M15	27.3
M16	8.5
Sum identified	93.5
Sum unidentified	6.5
Total	100.0

Section A6.2 Metabolism

Annex Point IIA VI.6.2 6.2 Metabolism of [^{14}C]-Icaridin in human volunteers

Figure A6_2-1 Proposed metabolic pathway of [^{14}C]-Icaridin in human volunteers. Width of the arrows indicates the quantity of the metabolite in urine.



Section A6.2 Absorption, distribution, metabolism and excretion

Annex Point IIA VI.6.2 6.2 ADME of [^{14}C]-Icaridin in the rat

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1 REFERENCE

1.1	**Reference**	[redacted] (1997): [Hydroxyethyl-1-^{14}C]KBR 3023: Rat Metabolism Study After Intravenous Injection and After Dermal Application. [redacted] Report No. PF 4178, 1997-02-27 (unpublished).
1.2	**Data protection**	Yes
1.2.1	Data owner	Saltigo GmbH
1.2.2	Companies with letter of access	–
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I

2 GUIDELINES AND QUALITY ASSURANCE

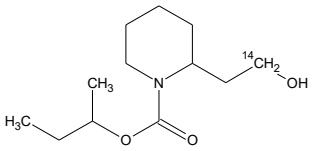
3.3	**Guideline study**	Yes US-EPA Guideline 85-1 (1982)(≅ OECD Guideline 417 (1984))
3.4	**GLP**	Yes
3.5	**Deviations**	None

3 MATERIALS AND METHODS

3.1	**Test material**	Mixture of unlabelled and ^{14}C-labelled Icaridin
3.2	**Non-labelled parent compound**	
3.2.1	Lot/Batch number	Intravenous studies: 890814ELB01 Dermal studies: 921103ELB04
3.2.2	Specification	
3.2.2.1	Description	Clear, colourless liquid
3.2.2.2	Purity	Batch # 890814ELB01: 99.1% Batch # 921103ELB04: 98.3%
3.2.2.3	Stability	Batch # 890814ELB01: Expiry date: June 1993 Batch # 921103ELB04: Expiry date: December 1994
3.3	**Labelled parent compound**	[2-(2-Hydroxyethyl-2-^{14}C)]-Icaridin
3.3.1	Lot/Batch number	Intravenous studies: KML2061 Dermal studies: KML2169
3.3.2	Specification	

Section A6.2 Absorption, distribution, metabolism and excretion

Annex Point IIA VI.6.2 6.2 ADME of [^{14}C]-Icaridin in the rat

3.3.2.1	Description	–	
3.3.2.2	Purity	Batch # KML2061: 99.55% radiochemical purity, 3.92 MBq/mg Batch # KML2169: >98% radiochemical purity, 3.55 MBq/mg	
3.3.2.3	Stability	–	
3.3.2.4	Radiolabelling		
3.4	**Test animals**		
3.4.1	Species	*Rattus norvegicus*	
3.4.2	Strain	Sprague-Dawley RAI/BOE (SPF) = Crl:CD BR (SPF)	
3.4.3	Source	Lippische Versuchstierzucht, Hagemann GmbH, Extertal, Germany Charles River Wiga GmbH, Sulzfeld, Germany	
3.4.4	Sex	Males + females	
3.4.5	Age/weight at study initiation	Males: 9-10 weeks, females: 18-21 weeks Body weight: about 250 g at time of application	
3.4.6	Number of animals	5 per sex and group (total: 50)	
3.4.7	Control animals	No	
3.5	**Administration/ Exposure**	Dermal or intravenous	
3.5.1	Application	**Intravenous**	
3.5.1.1	Type	Groups 1 + 2: 20 mg/kg bw i.v., tail vein Groups 3 + 4: 20 mg/kg bw i.v, femoral vein	
3.5.1.2	Duration of treatment	–	X
3.5.1.3	Post-exposure period	2 days	
3.5.1.4	Specific activity of test substance	392 kBq/mg	
3.5.1.5	Vehicle	Physiological saline	
3.5.1.6	Concentration in vehicle	5 mg/mL	
3.5.1.7	Volume applied	4 mL/kg bw	
3.5.2	Application	**Dermal**	

Section A6.2 Absorption, distribution, metabolism and excretion

Annex Point IIA VI.6.2 6.2 ADME of [^{14}C]-Icaridin in the rat

3.5.2.1	Type	Non-occlusive, on the shaved dorsal skin	
		Groups 5 + 6: 1 × 5 mg/rat (~20 mg/kg bw)	
		Groups 7 + 8: 15 × 5 mg/rat (~20 mg/kg bw), 14 non-radioactive doses followed by one radioactive dose after 24 h	X
		Groups 9 + 10: 1 × 50 mg/rat (~200 mg/kg bw)	
3.5.2.2	Duration of treatment	7 days	
3.5.2.3	Post-exposure period	–	
3.5.3	Specific activity of test substance	355 kBq/mg (20 mg/kg bw groups) or 35 kBq/mg (200 mg/kg bw groups)	
3.5.3.1	Vehicle	Ethanol	
3.5.3.2	Concentration in vehicle	20 mg/kg bw groups: 0.9 mg/µL 200 mg/kg bw groups: 1.0 mg/µL	
3.5.3.3	Volume applied	20 mg/kg bw groups: 5 µL (0.45 mg Icaridin/cm²) 200 mg/kg bw groups: 50 µL (5.0 mg Icaridin/cm²)	
3.6	**Examinations**		
3.6.1	Biokinetic parameters	Dermal absorption, distribution, metabolism, excretion	
3.6.2	Samples	Urine, faeces, expired air (tail i.v. test only), blood, skin, perirenal fat, uterus, ovaries, other tissues, application material, cage wash, skin wash	
3.6.3	Sampling time (0 h = start of application)	Blood: dermal tests: 1, 1.5, 2, 3, 4, 6, 8, 24, 32, and 48 h after treatment, then in 24-h intervals until sacrifice i.v. tests: 1, 1.5, 2, 3, 4, 6, 8, 24, 32, and 48 h after treatment	
		Urine: dermal tests: in 24-h intervals until sacrifice i.v. tests: 8, 24, 32, and 48 h after dosage	
		Faeces: in 24-h intervals until sacrifice	
		Expired air: 8, 24, 32, and 48 h after injection into the tail vein	
		Sacrifice: dermal tests: 7 days after application i.v. tests: 2 days after dosage	
		4 RESULTS AND DISCUSSION	
4.1	**Toxic effects, clinical signs**	Not reported	X
4.2	**Recovery of labelled compound**	I.v. tests: 93.8 ± 2.64%	
		Dermal tests: 66.9 – 81.2%	
4.3	**Dermal absorption**	See Table A6_2-1 in appendix.	
		Absorbed dose: 40% (high-dose ♀) – 66% (pre-treatment ♂) More than 90% of the radioactivity absorbed during the application	X

Section A6.2 Absorption, distribution, metabolism and excretion

Annex Point IIA VI.6.2 6.2 ADME of [^{14}C]-Icaridin in the rat

		period was absorbed within the first 24 h.	
4.4	**Excretion**	See Table A6_2-2 in appendix.	X
		The recovery achieved in the dermal tests is significantly below 100%. This was attributed to evaporation of the volatile test material during the 7 days of dermal non-occlusive exposure.	
4.5	**Distribution**	See Table A6_2-3 in appendix.	X
4.6	**Toxicokinetics**	See Table A6_2-4 in appendix.	
4.7	**Metabolism**	Eighteen metabolites, including parent test compound, were isolated and identified. Isolation and identification was performed preferentially with urine from the intravenously dosed animals because of the much higher urinary metabolite concentration.	
		The vast majority of radioactivity was excreted in urine. A list of the metabolites that could be identified is given in Table A6_2-5 in the appendix. Only their abundance in urine is presented here; the only faecal metabolites accounting for >1% of the applied dose were M8 and M9. Two metabolites, M18 and M19, were found exclusively in faeces.	
		A metabolic pathway could be proposed that is depicted in Figure A6_2-1.	X
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The absorption, distribution, excretion and metabolism of [^{14}C]-Icaridin was investigated in young adult male and female Sprague-Dawley rats.	
		The test compound was administered either intravenously as a single dose of 20 mg/kg bw or dermally as single doses of 20 or 200 mg/kg bw. In addition, [^{14}C]-Icaridin was applied dermally to rats at a dose of 20 mg/kg bw following 14 daily dermal exposures to unlabelled Icaridin.	
		Rats in the i.v. study were sacrificed 2 days after injection, whereas rats in the p.c. study were sacrificed immediately after the 7-day-exposure period.	
		Radioactivity was measured in plasma and in the excreta in intervals and in the body and individual tissues at sacrifice. Excreta were analysed –after extraction, if necessary- for parent compound and metabolites by HPLC followed by structure elucidation using MS and/or NMR.	
5.2	**Results and discussion**	**Dermal application**:	
		- Due to the volatilising nature of Icaridin, 20-30% of the amount applied was not recovered at sacrifice.	X
		- The absorption was incomplete depending on the dose level but independent of sex: the percentage values decreased from low dose groups to high dose groups indicating a saturation effect at the high dose level.	
		- The vast majority of radioactivity absorbed by the skin actually entered the body leaving only little potential for accumulation of Icaridin in the skin.	
		- Dermal absorption was fast: more than 90% of the fraction absorbed during the whole test duration of 7 days was absorbed	

Section A6.2 Absorption, distribution, metabolism and excretion

Annex Point IIA VI.6.2 6.2 ADME of [^{14}C]-Icaridin in the rat

		within the first 24 h after application with absorption half-lives varying from ca. 50 min to 3 h. - Plasma kinetics were sex- and dose-dependent. The plasma levels in the high dose groups were slightly higher than expected from a strict dose-proportionality. Females exhibit higher renal elimination than males. - The percentage excretion was dose- and sex-dependent. - The renal excretion process was fast: $T_{50\%}$ = 21-35 h; $T_{90\%}$ = 82-92 h (males), $T_{90\%}$ = 64-80 h (females) **Intravenous application**: - The distribution and elimination kinetics were very fast and sex dependent. - Renal excretion was higher in females than in males - The majority of the metabolites were phase-I biotransformation products. The formation of the metabolites was independent of dose-level, sex and route of administration.	
5.3	**Conclusion**	The vast majority of radioactivity absorbed by the skin actually entered the body leaving only little potential for accumulation of Icaridin in the skin. The dermal absorption was fast: more than 90% of the fraction absorbed during the whole test duration of 7 days was absorbed within the first 24 hours after application. A dermal absorption fraction (systemic exposure) of 58.2% to 62.9% for the low and pre-treated dose groups and 39.9% to 54.6% for the high dose groups was observed in this study. The majority of the metabolites were phase-I biotransformation products. The formation of the metabolites were independent of dose-level, sex and route of administration.	X
5.3.1	Reliability	1	X
5.3.2	Deficiencies	None	X

Section A6.2 Absorption, distribution, metabolism and excretion

Annex Point IIA VI.6.2 6.2 ADME of [^{14}C]-Icaridin in the rat

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	27 October 2006
Materials and Methods	Applicant's version is adopted with the following corrections: Part 3.4.2.1 Labelled test compound was applied dermally at a target dose of 20 mg/kg bw 24 hours following 14 doses (one dose per day, intervals of 24 hours) of the non-labelled test compound. Part 3.4.1.2 Duration of treatment: single iv dose Part 3.4.2.1 Type: A rubber ring with an inner area of 10 cm² was glued onto the shaved part of the dorsal skin using contact glue. The liquid test compound was distributed within the rubber ring. A filter paper was glued to the rubber ring and additional a protective tube-gauze dressing was covering the filter paper.
Results and discussion	Applicants version is adopted with the following corrections: Part 4.1 Toxic effects, clinical signs: After iv injections no toxicological effects were observed. Part 4.3 Dermal absorption: The absorption was depending on the dose level but independent of sex: the percentage values decreased from low dose groups (approximately 60.5% - 66.1%) to high dose groups (approximately 40.5% and 54.9%) during the 7 days of exposure. The amount of Icaridin recovered in the urine and faeces decreases significant during the first 168 hours of the exposure period (7 days) (not shown in table) indicating that the amount of substances recovered in the skin is not becoming systemically available over time. This gives a dermal absorption fraction, calculated as the sum of the amounts measured on/in the skin after washing, all tissues and organs prepared, excreta and cage wash (systemic exposure) of 58.2% to 62.9% for the low and pre-treated dose groups and 39.9% and 54.6% for the high dose groups after 7 days exposure. Part 4.4 Excretion: The amount of substance evaporating from the skin during the 7 days of treatment was not investigated in this study. However, in the study by Warren, D.L. and Sturdivant, D.W. (1997) the evaporation behaviour of Icaridin was investigated in an *in vitro* test. That test showed a maximum evaporation of Icaridin of 2% during an 8 hours exposure period. Therefore it can be argued that the low recovery (67-81%) found in this dermal study can be explained by evaporation. This study had an exposure period of 7 days and no occlusion was used which may result in an evaporation of Icaridin corresponding to maximum 40% of the applied dose. Part 4.5 Distribution: The Radiolabelled residue also depended on sex and dose level with the lower percentage values in the female groups and in the high dose groups: the residue in the body excluding the gastrointestinal tract and the skin at the application site at sacrifice ranged from 0.07% of the applied dose (high dose, females) to 0.25 (low dose, males).

Section A6.2 Absorption, distribution, metabolism and excretion

Annex Point IIA VI.6.2	6.2 ADME of [^{14}C]-Icaridin in the rat
Conclusion	5.3. The vast majority of radioactivity absorbed by the skin actually entered the body leaving only little potential for accumulation of Icaridin in the skin. The dermal absorption was fast: more than 90% of the fraction absorbed during the whole test duration of 7 days was absorbed within the first 24 hours after application. A dermal absorption fraction of 58.2% to 62.9% for the low and repeated dose groups (15x) and 39.9% to 54.6% for the high dose groups was observed in this study. These results indicate that single low dose (20 mg/kg bw) administration compared to a repeated dose administration (pre-treated dose group) has no impact on the absorption fraction. Also, the dermal absorption at the high dose (200 mg/kg bw) is lower than at the low dose. Only minor amounts of Icaridin was recovered in different organs (the highest levels in liver, kidney and fat) indicating that Icaridin is not accumulated in the body. The amount recovered in the high dose is approximately 6 times higher than in the low dose group. No differences were observed comparing single dermal administration to dermal repeated dose administration (pre-treated dose group) The majority of the metabolites were phase-I biotransformation products. The formation of the metabolites were independent of dose-level, sex and route of administration. The most prominent metabolites in all dose groups (both i.v. and dermal administration) were compounds containing the 2-acetylpiperidin moiety with the carbamoyl group linked to either 1-methylpropyl (M16), 1-methyl-2-hydroxypropyl (M8 and M9) or 1-hydroxymethylpropyl (M10) alcohol. These metabolites accounted together for 53.4 to 63.8% of the totally excreted radioactivity in the dermal treated animals and 51.9 to 77.0% in the intravenously dosed animals. Compounds containing the 2-(2-hydroxyethyl)-piperidin part linked to these alcohols (M1, M2, M4) and the unsaturated analogue M3 were minor metabolites as were the glucuronic acid conjugates af these metabolites (M6, M11 to M13) including that of parent test compound (M14 and M15). The excretion was sex- and dose dependent. The excretion ratio renal/faecal distinctly changed in the female groups (dermal administration) in favour of the renal fraction: a portion of 46.1% and 42.6% was excreted with urine and 12.3% and 14.9% with the faeces of the male low dose and pre-treated animal group. In the respective female group, the renal excretion amounted to 55.1% and 55.7%, and the faecel excretion to 7.1% and 6.5%. The total fraction of radioactivity excreted by the high dose groups was comparatively lower: a portion of 25.7% and 35.7% was excreted via urine, and a fraction of 6.9% and 3.6% was eliminated with the faeces in males and females, respectively. The renal excretion process was fast after both intravenously and dermal administration. After dermal administration 50% of the total renal fraction was excreted within $T_{50\%}$ = 21 to 35 hours after application in all animal groups of either sex.
Reliability	2
Acceptability	Acceptable
Remarks	5.3.2 Deficiencies; Evaporation not measured to confirm theory of evaporation as explanation for low recovery of dermal applied radioactivity.
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.2 Absorption, distribution, metabolism and excretion

Annex Point IIA VI.6.2 6.2 ADME of [^{14}C]-Icaridin in the rat

Table A6_2-1 Characteristics of absorption following dermal application of [^{14}C]-Icaridin [% of applied dose] after 7 days of exposure

Test specification	20 mg/kg bw, single		20 mg/kg bw, 15x		200 mg/kg bw, single	
Sex	♂	♀	♂	♀	♂	♀
% absorbed[1]	60.51	64.71	66.10	64.44	54.89	40.48
% on/in skin at the application site	1.20	1.75	7.88	1.55	0.29	0.60
% entering the body	59.31	62.96	58.22	62.89	54.60	39.88

[1] Absorbed dose: Sum of amounts on/in the skin after washing,in all tissues and organs prepared, in excreta and in cage wash

Table A6_2-2 Characteristics of excretion following application of [^{14}C]-Icaridin [% of applied dose] after 7 days of exposure

Route	I. V (tail vein)		I.V (femoral vein)		Dermal					
Group	20 mg/kg bw, single		20 mg/kg bw, single		20 mg/kg bw, single		20 mg/kg bw, 15x		200 mg/kg bw, single	
Sex	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Matrix										
Sum of non-biological material involved (cage wash thereof)	–	–	–	–	13.41 (0.49)	9.92 (0.57)	11.54 (0.27)	17.28 (0.49)	45.75 (21.57)	26.85 (0.38)
$^{14}CO_2$	0.02	0.02	–	–	–	–	–	–	–	–
Urine	74.39	90.36	82.28	86.82	46.09	55.10	42.63	55.65	25.69	35.72
Faeces	16.51	6.23	12.34	4.80	12.27	7.09	14.85	6.52	6.90	3.59
Skin, application site	–	–	–	–	1.20	1.75	7.88	1.55	0.29	0.60
Body excl. GIT	0.20	0.17	0.20	0.16	0.25	0.13	0.22	0.17	0.13	0.07
GIT plus contents	0.32	0.19	0.23	0.09	0.21	0.07	0.25	0.06	0.31	0.12
Recovery	91.44	96.97	95.05	91.87	73.36	74.04	77.26	81.22	78.96	66.89

Section A6.2 Absorption, distribution, metabolism and excretion

Annex Point IIA VI.6.2 6.2 ADME of [^{14}C]-Icaridin in the rat

Table A6_2-3 Equivalent concentration C [µg/g] in tissues and organs

Route	I.V (femoral vein)		Dermal					
Group	20 mg/kg bw, single		20 mg/kg bw, single		20 mg/kg bw, 15x		200 mg/kg bw, single	
Sex	♂	♀	♂	♀	♂	♀	♂	♀
Matrix								
Skin, application site	–	–	16.489	23.863	110.497	19.664	46.911	95.253
Skin	0.027	0.034	0.122	0.079	0.079	0.109	0.583	0.264
Carcass	0.039	0.029	0.010	0.011	0.030	0.010	0.120	0.134
Erythrocytes	0.042	0.034	0.012	0.013	0.019	0.015	0.131	0.130
Plasma	0.020	0.018	0.008	0.005	0.010	0.005	0.131	0.060
Bone (femur)	0.020	0.015	0.009	0.007	0.014	0.008	0.105	0.038
Brain	0.010	0.006	0.003	0.002	0.006	0.004	0.034	0.086
Heart	0.032	0.023	0.010	0.010	0.013	0.010	0.135	0.086
Lung	–	–	0.025	0.032	0.036	0.036	0.240	0.286
Liver	0.208	0.096	0.042	0.029	0.063	0.036	0.580	0.181
Spleen	0.031	0.032	0.009	0.008	0.016	0.009	0.102	0.058
Kidneys	0.122	0.115	0.037	0.046	0.051	0.054	0.477	0.291
Fat (perirenal)	0.095	0.111	0.065	0.086	0.066	0.024	0.717	0.503
Muscle (femur)	0.022	0.015	0.007	0.005	0.010	0.007	0.088	0.040
Testes	0.029	–	0.005	–	0.008	–	0.071	–
Uterus	–	0.070	–	0.024	–	0.013	–	0.122
Ovaries	–	0.040	–	0.039	–	0.023	–	0.325
Body excl. GIT	0.043	0.033	0.045	0.026	0.044	0.033	0.290	0.156
Total body	0.081	0.048	0.260	0.346	1.478	0.306	1.430	1.541

Section A6.2 Absorption, distribution, metabolism and excretion

Annex Point IIA VI.6.2 6.2 ADME of [^{14}C]-Icaridin in the rat

Table A6_2-4 Toxicokinetic parameters derived from plasma curve analysis.

Route	I.v.		Dermal					
Group	20 mg/kg bw, single		20 mg/kg bw, single		20 mg/kg bw, 15x		200 mg/kg bw, single	
Sex	♂	♀	♂	♀	♂	♀	♂	♀
Lag-time (t_{lag}) [h]	–	–	0.9	0.9	0.9	0.8	0.3	0.7
Absorption half-life ($t_{½\,a}$) [h]	–	–	1.5	0.8	1.9	1.2	1.9	3.4
Time of max. exp. (T_{max}) [h]	–	–	6.00	6	8	8	6	8
Max. conc. exp. (C_{max}) [µg/mL]	–	–	0.57	1.6	0.45	0.8	4.48	11.7
1st elimination half-life ($t_{½(1)}$) [h]	0.9	0.7	35.7	23.9	41.8	28.9	10.9	9.1
2nd elimination half-life ($t_{½(2)}$) [h]	5.2	2.8	–	–	–	–	144	105
3rd elimination half-life ($t_{½(3)}$) [h]	45.5	73.0	–	–	–	–	–	–
Area under curve (AUC) [µg/(mL×h)]	60.2	101	32.3	58.1	29.2	37.1	198	360
Mean residence time (MRT) [h]	10.1	11.7	53.7	36.6	63.1	44.2	122	56.5
Total clearance (CL) [mL/(min×kg)]	5.2*)	3.2*)	5.1#)	3.2#)	6.4#)	5.0#)	8.8#)	3.7#)
Urine $T_{50\%}$ [h] +)	5.3	5.1	28.8	21.2	33.6	35.1	23.7	20.9
Urine $T_{90\%}$ [h] +)	18.8	17.7	81.8	64.2	82.8	80.3	91.7	67.6

*) Calculated for the actual dose level administered

#) Calculated for the actual dose level applied and the fraction entering the body

+) Calculated from the time course of renal excretion by linear interpolation

Section A6.2 Absorption, distribution, metabolism and excretion

Annex Point IIA VI.6.2 6.2 ADME of [^{14}C]-Icaridin in the rat

Table A6_2-5 Quantification of [^{14}C]-Icaridin metabolites in rat urine [% of applied dose]

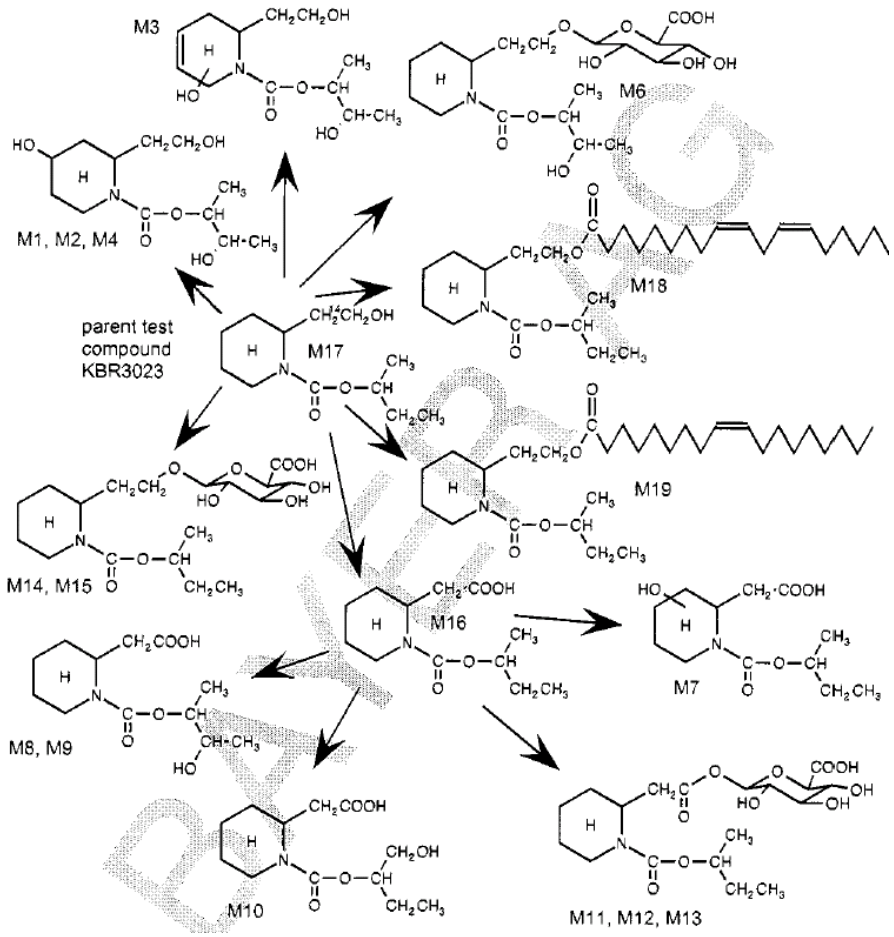
Route	I.V (tail vein*)		Dermal					
Group	20 mg/kg bw, single		20 mg/kg bw, single		20 mg/kg bw, 15x		200 mg/kg bw, single	
Compound	♂	♀	♂	♀	♂	♀	♂	♀
M1	0.96	1.37	0.81	1.05	0.95	1.26	0.46	0.54
M2	3.74	2.72	2.18	1.94	2.93	2.27	1.28	1.01
M3	1.41	1.94	0.92	1.07	0.67	1.30	0.53	0.67
M4	4.95	3.58	2.06	1.73	3.33	1.81	1.28	0.68
M5	2.60	1.39	0.62	0.67	–	0.88	–	–
M6	-	2.13	0.88	1.83	0.66	1.73	0.40	0.86
M7	-	1.74	1.18	1.31	0.90	1.14	0.67	0.73
M8	18.49	12.86	9.27	7.77	9.61	7.11	5.09	4.49
M9	17.51	21.12	9.83	12.39	8.42	11.64	4.67	7.27
M10	7.95	3.69	4.00	2.71	4.36	2.64	2.28	1.59
M11	-	-	–	–	–	–	–	–
M12 + M13	-	1.45	–	0.76	–	–	–	1.17
M14	-	-	0.62	0.78	0.43	0.78	0.34	0.46
M15	-	2.75	0.89	1.46	0.44	1.31	0.54	0.94
M16	3.34	16.16	3.87	12.77	2.68	11.54	2.07	10.24
M17	-	-	0.46	0.38	0.17	0.59	0.06	0.31
M18	-	-	Faeces only	Faeces only	Faeces only	Faeces only	Faeces only	Faeces only
M19	-	-	Faeces only	Faeces only	Faeces only	Faeces only	Faeces only	Faeces only
Sum identified	60.94	72.91	37.60	48.62	35.54	46.02	19.69	30.96
Sum unidentified	0.43	11.22	4.78	4.92	5.38	5.10	3.29	3.27
Total analysed	70.91	84.13	42.38	53.54	40.92	51.12	22.98	34.23
Difference to pool urine	0.08	3.20	0.84	-0.19	-0.85	1.67	0.39	-0.21
Rest of urine (96-148 h)	3.40	3.04	2.87	1.75	2.56	2.86	2.32	1.70
Total faeces	16.51	6.27	11.07	6.10	13.00	6.00	6.26	3.38
Total excreta	90.90	96.64	57.16	61.20	55.63	61.65	31.95	39.10
Carcass	0.55	0.38	1.66	1.95	8.35	1.78	0.73	0.79
Non-biological material	-	-	13.41	9.92	11.54	17.28	45.75	26.85
Total	91.45	97.02	72.23	73.07	75.52	80.71	78.43	66.74
Loss (% of recovered dose)	-	-	1.20	0.99	1.85	0.52	0.64	0.21
Loss (% of applied dose)	8.55	2.98	27.77	26.93	24.48	19.29	21.57	33.26
Recovery	91.44	96.97	73.43	74.06	77.37	81.23	79.07	66.95

Section A6.2 Absorption, distribution, metabolism and excretion

Annex Point IIA VI.6.2 6.2 ADME of [^{14}C]-Icaridin in the rat

* Values given for the i.v. groups refer to the total percentage of metabolites in all excreta + carcass

Figure A6_2-1 Proposed metabolic pathway of [^{14}C]-Icaridin in rats.



Section A6.2 Percutaneous absorption (*in-vivo* test)

Annex Point IIA VI.6.2 6.2 Dermal Absorption and Excretion in Human Volunteers

Official use only

1 REFERENCE

1.1	**Reference**	[redacted] (1994): A Single Dose Open Label Study to Investigate the Absorption and Excretion of a ^{14}C-Labelled Insect Repellent (KBR 3023) from two Different Formulations after Dermal Application to Healthy Volunteers. Clinical part: [redacted] Analytical part: [redacted] Report-No. P1092004, 1994-06-20 (unpublished)
1.2	**Data protection**	Yes
1.2.1	Data owner	Saltigo GmbH
1.2.2	Company with letter of access	–
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	No No particular Guideline exists for tests of dermal absorption in humans.
2.2	**GLP / GCP**	Yes
2.3	**Deviations**	Yes Certificates of analysis for the test compounds are missing.

3 MATERIALS AND METHODS

3.1	**Test material**	Mixture of unlabelled and ^{14}C-labelled Icaridin
3.1.1	Non-labelled parent compound	
3.1.2	Lot/Batch number	921103ELB04
3.1.3	Specification	
3.1.3.1	Description	Clear, colourless liquid
3.1.3.2	Purity	98.3%
3.1.3.3	Stability	Expiry date: December 1994
3.1.4	Labelled parent compound	[2-(2-Hydroxyethyl-2-^{14}C)] Icaridin
3.1.5	Lot/Batch number	KML 2159
3.1.6	Specification	

Section A6.2 Percutaneous absorption (*in-vivo* test)

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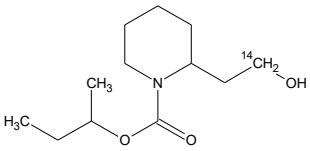
3.1.6.1	Description	–	
3.1.6.2	Purity	>98% radiochemical purity, >99% chemical purity 96 µCi/mg (=3.55 MBq/mg)	
3.1.6.3	Stability	–	
3.1.6.4	Radiolabelling		
3.2	**Test Subjects**		
3.2.1	Species	Human	
3.2.2	Source	Volunteers	
3.2.3	Sex	Males	
3.2.4	Age/weight at study initiation	18-28 years; 62-72.9 kg	
3.2.5	Number of subjects per group	6	
3.2.6	Control subjects	No	
3.3	**Administration/ Exposure**	**Dermal**	
3.3.1	Preparation of test site	The application site was surrounded by an adhesive template and outlined with ink.	
3.3.2	Concentration of test substance	Undiluted or 15% in ethanol	
3.3.3	Specific activity of test substance	2.5 µCi/mg (=93 kBq/mg)	
3.3.4	Volume applied	15% formulation: 100 µL (0.625 mg Icaridin/cm²) Undiluted formulation: 15 µL (0.625 mg Icaridin/cm²)	
3.3.5	Size of test site	24 cm²	
3.3.6	Exposure period	8 hours	
3.3.7	Sampling time	Blood: 0, 2, 4, 6, 8, 10, 12, 16, 24, 36, 48, 72, 96, 120 h Urine: pre-dose, 0-4, 4-8, 8-12, 12-24, 24-36, 36-48, 48-60, 60-72, 72-84, 84-96, 96-108, 108-120, 120-128 h Faeces: pre-study, individually throughout the study period up to 128 h Tape stripping of surface skin layers: 1, 23 and 45 h after end of exposure. Please specify in details number of tape strips per time interval (0 h = start of application)	X
3.3.8	Samples	Urine, faeces, tape strippings, liquid and swabs used for washing the skin, protective appliances, gloves	

Section A6.2 Percutaneous absorption (*in-vivo* test)

Annex Point IIA VI.6.2 6.2 Dermal Absorption and Excretion in Human Volunteers

		4 RESULTS AND DISCUSSION	
4.1	**Toxic effects, clinical signs**	15% formulation group: one person: mild impaired concentration on days 1-2 one person: mild headache on days 1-6 Undiluted formulation group: one person: mild dizziness on day 1	
4.2	**Dermal irritation**	No effects occurred in both treatment groups.	
4.3	**Recovery of labelled compound**	15% formulation: 98.13% Undiluted formulation: 97.00%	
4.4	**Percutaneous absorption**	Arithmetic mean of all volunteers, 120 h post exposure: 15% formulation: 3.80% Undiluted formulation: 1.68% See Table A6_2-2 for dermal absorption in individual volunteers.	X
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The test compound Icaridin was administered either as a 15% (w/w) solution in ethanol or as the undiluted technical grade product. Six healthy male volunteers were treated with each of the formulations. The test substance was applied on 4 × 6 cm² of intact, non-shaven area of the volar aspect of the forearm. The application the site was covered with a non-occlusive dome which allowed free movement of air but protects from accidentally loss of radioactivity due to physical contact. After 8 h of exposure, the protective wrappings were removed and the skin area was thoroughly cleaned, to remove remaining test substance. Blood, urine, faeces and tape strippings of the skin were collected at the times described under "3.3.7" (see above). All collected samples were analysed for total radioactivity.	
5.2	**Results and discussion**	A single dermal application of 0.1 mL of an ethanol solution containing 15% of [^{14}C] Icaridin was well tolerated by 6 healthy male volunteers. A single dermal application of 15 µL neat [^{14}C] Icaridin was well tolerated by the test subjects. Less than 4% of the dermal applied dose of Icaridin, either as a 15% formulation or the neat technical grade substance, was absorbed through the skin during an 8-h exposure period and 120 h thereafter. The absorbed part of Icaridin was rapidly excreted via the urine. More than 93% of the absorbed dose was excreted within the first 24 h after exposure had begun. There was no evidence that dermal applied Icaridin accumulated in the skin.	
5.3	**Conclusion**	Dermal absorption of Icaridin as a 15% solution as well as the neat substance is less than 4%.	X
5.3.1	Reliability	1	
5.3.2	Deficiencies	No	

Section A6.2 Percutaneous absorption (*in-vivo* test)

Annex Point IIA VI.6.2 6.2 Dermal Absorption and Excretion in Human Volunteers

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	12 October 2006
Materials and Methods	Applicant's version is adopted with the following comments to the methods: Tape stripping of surface skin layers: 1, 23 and 45 h after end of exposure. Strips of adhesive cellophane tape (3M Company Scotch Magic, 9 mm) were applied evenly to the same area of skin and stripped off in a few seconds in a slow even fashion. Sixteen strips were applied for each time point.
Results and discussion	Applicant's version is adopted
Conclusion	Applicant's version is modified. . Mean dermal absorption of 15% (w/w) Icaridin in an ethanol solution as well as the neat substance is less than 4%. Large individual variation was noted and a maximum uptake of 7 % was noted from the 15% ethanol solution. The representative product applied for Autan Pump Spray 20% contains 20% Icaridin and less than 40% ethanol. The tested formulation containing 15% icaridin diluted in ethanol therefore represents worst case both with respect to the amount of a.i. and the solvent ethanol since dermal absorption are inverse related to the concentration and ethanol is recognized as enhancing dermal absorption in general.
Reliability	1
Acceptability	Acceptable
Remarks	Part 4.4: Table 6_2-1 shows the summary of the dermal absorption parameters for the volunteers; including total absorption and recovery. Table 6_2-1 is revised to include urine data for all sampling points

Section A6.2 Percutaneous absorption (*in-vivo* test)

Annex Point IIA VI.6.2 6.2 Dermal Absorption and Excretion in Human Volunteers

Table A6_2-1. Table for percutaneous absorption (*in vivo* test) after 24 hours exposure time and subsequently observations period up till 128 hours

	Labelled compound	
	15%	**100%**
Compound applied	0.203 mg/kg	0.207 mg/kg
	0.625 mg/cm²	0.625 mg/cm²
Compartments with compound detected	**% of dose**	
1. Protective coverings, swabs, skin rinsates	94.16	95.23
2. Tape strippings (stratum corneum)*	0.03	0.02
3. Blood	< LOQ*	< LOQ*
4. Urine - total	3.76	1.66
Control	0.01	0.01
0 – 4 hr	0.23	0.11
4 – 8 hr	1.37	0.54
8 – 12 hr	1.30	0.63
12 – 24 hr	0.64	0.26
24 – 36 hr	0.10	0.06
36 – 48 hr	0.03	0.02
48 – 60 hr	0.02	0.01
60 – 72 hr	0.01	0.00
72 – 84 hr	0.01	0.01
84 – 96 hr	0.01	0.00
96 – 108 hr	0.01	0.01
108 – 120 hr	0.03	0.00
120 – 128 hr	0.00	0.00
5. Faeces	0.01	0.00
6. Gloves	0.16	0.08
Sum of #2 – 5: stratum corneum, blood, excreta (= absorption)	3.80	1.68
Sum of all detected labelled compound (#1 – 6) (=recovery)	**98.13**	**97.00**

*LOQ = 2× background level (4 dpm/500 µL)

* Sixteen strips were applied for each time point (1, 23 and 45 hours after removal of the dose).

Table A6_2-2. Individual dermal absorption values

#	Initials	Body weight [kg]	Formulation	Dermal absorption*
1	J.J.	70.1	15%	2.81
2	W.V.	84.3	15%	3.22
3	M.B.	66.4	15%	4.93
4	J.P.	81.0	15%	7.01
5	H.K.	74.8	15%	2.21
6	A.A.	69.4	15%	2.62
Median (CV)		72.5	15%	3.02 (1.12)
Geometric mean		74.1	15%	3.49
Arithmetic mean ± SD		74.3	15%	3.80 ± 1.83
7	M.M.	64.7	undiluted	2.19
8	M.N.	68.2	undiluted	1.76
9	C.K.	76.3	undiluted	0.74
10	J.B.	76.0	undiluted	1.67
11	T.B.	62.2	undiluted	2.31
12	J.H.	82.7	undiluted	1.41
Median (CV)		72.1 (0.87)	undiluted	1.72 (0.19)
Geometric mean		71.3	undiluted	1.58
Arithmetic mean ± SD		71.7 ± 7.90	undiluted	1.68 ± 0.57

* Combined % of applied dose in urine, faeces and tape strippings (Sixteen strips for each time point 1, 23 and 45 hours after removal of the dose)

Section A6.2 Percutaneous absorption (*in vivo* test)

Annex Point IIA VI.6.2 6.2 Study for percutaneous absorption in rats

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1 REFERENCE

1.1	**Reference**	[redacted] (1997): Dermal Absorption of Technical KBR 3023. [redacted] Report-No.: 107488, 1997-01-28, (unpublished)
1.2	**Data protection**	Yes
1.2.1	Data owner	Saltigo GmbH
1.2.2	Company with letter of access	–
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I

2 GUIDELINES AND QUALITY ASSURANCE

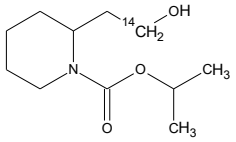
2.1	**Guideline study**	Yes EPA Pesticide Assessment Guidelines Subdivision F, Series 85-3 (1982) ≅ OECD Guideline 427
2.2	**GLP**	Yes
2.3	**Deviations**	Yes, the following deviations from OECD Guideline 427 were noted: - Each group of animals was exposed to the test substance for exactly 8 h - Absorption of the test compound was assessed after 8 h, 24 h and at 3-7 days after application

3 MATERIALS AND METHODS

3.1	**Test material**	2-(2-hydroxyethyl-2-^{14}C)] Icaridin
3.1.1	Lot/Batch number	Non-labelled test compound: PT.030693 Labelled test compound: KML 2169
3.1.2	Specification	Deviating from specification given in Section 2 as follows: Icaridin was fortified with ^{14}C-labelled Icaridin

Section A6.2 Percutaneous absorption (*in vivo* test)

Annex Point IIA VI.6.2 6.2 Study for percutaneous absorption in rats

3.1.2.1	Description	Clear colourless liquid
3.1.2.2	Purity	Non-labelled test compound: 98.2% Labelled test compound: > 99%
3.1.2.3	Stability	No data
3.1.2.4	Radiolabelling	
3.2	**Test Animals**	
3.2.1	Species	Rat
3.2.2	Strain	Sprague-Dawley
3.2.3	Source	SASCO Inc., St. Louis, MO, USA
3.2.4	Sex	♂ + ♀
3.2.5	Age/weight at study initiation	Males: adults 209-250 g Females: adults 202-245 g
3.2.6	Number of animals per group	12 per sex
3.2.7	Control animals	Yes. One male and one female rat were treated with water.
3.3	**Administration/ Exposure**	Dermal
3.3.1	Preparation of test site	24 h prior to dosing an area of 15 cm² on the back of each animal was clipped free of hair and wiped with an acetone-wetted gauze pad.
3.3.2	Concentration of test substance	Undiluted Icaridin was applied as follows: 8 mg/kg bw , 40 mg/kg bw, 200 mg/kg bw 0.133 mg/cm² 0.67 mg/cm² 3.33 mg/cm²
3.3.3	Specific activity of test substance	3.56 MBq / mg (96 µCi / mg)
3.3.4	Volume applied	8 mg/kg bw group: 2 µL 40 mg/kg bw group: 10 µL 200 mg/kg bw group: 50 µL
3.3.5	Size of test site	15 cm²
3.3.6	Exposure period	8 h
3.3.7	Sampling time	8 h, 24 h after application and at a time between post-treatment days 3-7 after application when urine counts from all animals of a specific group were indistinguishable from background levels of radioactivity.
3.3.8	Samples	Urine, faeces, blood, organs, carcass, skin with substance not removable, liquid used for washing the skin, protective appliances, cages washings

Section A6.2 Percutaneous absorption (*in vivo* test)

Annex Point IIA VI.6.2 6.2 Study for percutaneous absorption in rats

3.4 Volatility study

Volatility of the test compound under simulated test conditions was assessed by applying ^{14}C-icaridin onto artificial skin (DuoDERM™) attached to a dish heated to 36°C. The application site was prepared with a rubber ring providing 15 cm² of enclosed skin area. Covers were prepared to fit on top of the ring with PTFE and carbon filter paper. Each dish was placed onto a warming tray, set to approximately 36°C, for a minimum of 30 minutes before test material application or use. An aliquot of approximately 2 or 50 µL of prepared radiolabeled icaridin was applied to each dish. After an 8-hour duration on the warming tray and complete disassemblage of treatment dish, an assessment of radiolabel available from individual components was conducted.

4 RESULTS AND DISCUSSION

4.1 Volatility study

4.2 Toxic effects, clinical signs

None

(The observed signs of nasal stain and lacrimation were considered as incidental to the experimental procedures and not as a response to treatment with technical Icaridin.)

4.3 Dermal irritation

None

4.4 Recovery of labelled compound

	Males	Females
8 mg/kg bw group:	91.0%	92.8
40 mg/kg bw group:	108.3%	101.3
200 mg/kg bw group:	90.6%	87.0

X

4.5 Percutaneous absorption

Absorption values ranged from 17% to 23% of the applied doses. Average absorption values per dosage are given under 5.3.

About 1.64% and 0.39% of the applied dose were volatile when volumes of 2 and 50 µL, respectively, were pipetted directly onto the artificial skin substrate.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The animals were divided into three groups with area concentrations of 0.133 mg/cm², 0.67 mg/cm² and 3.33 mg/cm², respectively. Each group consists of 12 males and 12 females. In each group a specific amount of the labelled test compound was applied to a dorsal skin area which was previously shaved. After 8 h of application the test compound was wiped from the skin. Groups of 4 males and 4 females were sacrificed from each treatment group at 3 time points: immediately after the 8 h-time point, 24 h after application, and at a time between days 3-7 post-treatment when urine counts from all animals of a specific group had reached background levels of radioactivity. Each of these durations refers to time zero being actual application of the test material.

Samples were collected at the following time points: Urine and faeces were sampled at termination for the animals sacrificed after 8 h and 24 h and at daily intervals for those animals maintained for 7 days. Blood, organs, and skin were sampled at sacrifice.

All samples were evaluated in duplicate, with the exception of those too small to divide and applicator pipettes.

Section A6.2 Percutaneous absorption (*in vivo* test)

Annex Point IIA VI.6.2 6.2 Study for percutaneous absorption in rats

5.2	**Results and discussion**	Total recovery of radiolabel from all groups was high and averaged 95.2% of the applied dose. The test material was not completely absorbed and the absorption patterns were similar between males and females as well as between all dosage groups. Average absorption values for both sexes treated with 0.133, 0.67, or 3.33 mg/cm² Icaridin were 23.3%, 18.9% or 17.0%, respectively.	X X
		A concentration-dependent limit for the extent of Icaridin absorption over 8 h was not found within the range of dosages used. In all treatment groups the primary route of excretion was via the urine. The majority of radioactivity was excreted within 2 days after application. Data also suggested that the radiotracer did not bind to the application site of the skin.	
5.3	**Conclusion**	The average (both sexes) absorption values for applied Icaridin doses of 0.133, 0.67 and 3.33 mg/cm² were 23.3%, 18.9%, or 17.0%.	
5.3.1	Reliability	1	
5.3.2	Deficiencies	No	

Section A6.2 Percutaneous absorption (*in vivo* test)

Annex Point IIA VI.6.2 6.2 Study for percutaneous absorption in rats

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	15 October 2006
Materials and Methods	Applicant's version is adopted
Results and discussion	Applicant's version is adopted except the following text regarding the recovery of the applied dose: The extent of absorption at 8-hours from all dose groups ranged from 13.7 – 37.4%, while the extent of absorption after 24 hours ranged from 10.8 – 27.6%, and the extent of absorption after 7 days ranged from 15.4 – 32.3% (Direct values – inclusive of both sexes) (see table A6_2-1 and 2). These data do not suggest a greater extent of absorption form animals that are maintained for up to 7 days. Therefore the absorption fraction is calculated as the average of the absorption values obtained after 8, 24 and 168 hours. Since the amount of substance observed in urine and faeces increases with time after the 8 hours exposure (24 and 168 hour sampling time) the skin residues must be considered available for systemic absorption. The applicant has evaluated that '*The test material was not completely absorbed and the absorption patterns were similar between males and females as well as between all dosage groups'.* The data in table A6_2-1 and A6_2-2 indicates some differences between sexes with the females having the largest absorption fraction. In the low dose group (0.133 mg/cm²) an average amount of 15.1% of the applied dose was found in the urine from female rats compared to 9.1% in male rats.
Conclusion	Applicant's version is adopted. The average dermal absorption values (both sexes) for applied Icaridin doses of 8, 40 and 200 mg/kg bw/day were 23.3% (28% in females and 18.8% in males), 18.9% (20.5% in females and 17.5% in males), and 17.0% (20.8% in females and 13.3% in males).
Reliability	1
Acceptability	Acceptable
Remarks	4.3 Total recovery of radiolabel from all groups was generally high and averaged 95.2% of the applied dose. However, in one dose group (200 mg/kg bw – females) the recovery rate was just below the one accepted in the guideline was observed (87%). RMS has revised table A6_2-1 and A6_2-2 to include more specific data on the elimination through urine, faeces, by cage wash, and the amount recovered in the skin.

Section A6.2 Percutaneous absorption (*in vivo* test)

Annex Point IIA VI.6.2 6.2 Study for percutaneous absorption in rats

Table A6_2-1.Table for percutaneous absorption (in vivo test) after 8 hours exposure time and a subsequently observation period up till 168 hours – first evaluation of samples

Male rats			
	Absolute amount [mg / cm²]		
	0.133	**0.67**	**3.33**
Compound applied	8 mg/kg	40 mg/kg	200 mg/kg
Compartments with compound detected	**% of dose**		
1. Protective appliances	21.4	17.9	9.9
2. Skin wash	50.7	73.0	65.0
3. Skin (with substance not removable) (average)	3.2	3.9	4.7
8 hours	5.5	7.6	9.2
24 hours	2.1	3.0	4.0
168 hours	1.9	1.0	0.8
4. Blood	0.2	0.1	0.1
5. Urine (average)	9.1	7.8	3.7
8 hours	4.7	2.8	1.5
24 hours	9.2	8.1	3.3
168 hours	13.5	12.4	6.4
6. Faeces (average)	2.2	1.9	1.2
8 hours	0.2	0.1	0.1
24 hours	2.1	1.5	0.6
168 hours	4.4	4.1	3.0
7. Remaining carcass	3.6	3.2	2.5
8. Cage wash (average)	0.5	0.6	1.1
8 hours	0.5	0.1	0.3
24 hours	0.7	0.6	0.3
168 hours	0.4	1.1	2.6
9. Applicator pipettes	11.5	11.1	6.8
Average Sum of #3 – 8: blood, excreta, removed organs, remaining carcass (= absorption)	18.8	17.5	13.3

Section A6.2 Percutaneous absorption (*in vivo* test)

Annex Point IIA VI.6.2 6.2 Study for percutaneous absorption in rats

After 8 hours (Sum of #3 – 8: blood, excreta, removed organs, remaining carcass	16.9	14.2	13.7
After 24 hours (Sum of #3 – 8: blood, excreta, removed organs, remaining carcass	17.9	16.5	10.8
After 7 days (Sum of #3 – 8: blood, excreta, removed organs, remaining carcass	24.0	21.9	15.4
Sum of all detected labelled compound (#1 – 9) (=recovery)	91.0	108.3	90.6

Section A6.2 Percutaneous absorption (*in vivo* test)

Annex Point IIA VI.6.2 6.2 Study for percutaneous absorption in rats

Table A6_2-2.Table for percutaneous absorption (in vivo test) after 8 hours exposure time and a subsequently observation period up till 168 hours – second evaluation of samples

Female rats			
	Absolute amount [mg / cm²]		
	0.133	**0.67**	**3.33**
Compound applied	8 mg/kg	40 mg/kg	200 mg/kg
Compartments with compound detected	**% of dose**		
1. Protective appliances	18.0	16.4	7.1
2. Skin wash	47.2	64.4	60.0
3. Skin (with substance not removable) (average)	3.0	4.4	7.0
8 hours	4.8	8.8	13.7
24 hours	2.1	3.4	6.2
168 hours	2.2	1.0	1.0
4. Blood	0.4	0.2	0.2
5. Urine (average)	15.1	9.9	7.3
8 hours	9.7	4.5	2.0
24 hours	16.3	13.8	7.3
168 hours	19.3	11.7	12.7
6. Faeces (average)	1.9	1.0	1.4
8 hours	0.7	0.1	0.1
24 hours	1.4	1.1	0.6
168 hours	3.7	1.8	3.5
7. Remaining carcass	5.6	3.7	3.0
8. Cage wash (average)	2.0	1.3	1.9
8 hours	3.0	1.2	0.4
24 hours	1.8	1.2	1.0
168 hours	1.1	1.5	4.3
9. Applicator pipettes	11.5	7.8	7.3
Average Sum of #3 – 8: blood, excreta, removed organs, remaining carcass (= absorption)	28.0	20.5	20.8

Section A6.2 Percutaneous absorption (*in vivo* test)

Annex Point IIA VI.6.2 6.2 Study for percutaneous absorption in rats

After 8 hours (Sum of #3 – 8: blood, excreta, removed organs, remaining carcass	24.2	18.5	37.4
After 24 hours (Sum of #3 – 8: blood, excreta, removed organs, remaining carcass	27.6	23.4	18.3
After 7 days (Sum of #3 – 8: blood, excreta, removed organs, remaining carcass	32.3	19.9	24.7
Sum of all detected labelled compound (#1 – 9) (=revovery)	92.8	101.3	87.0

Section 6.3.1 Repeated dose toxicity

Annex Point IIA VI.6.3 6.3.1 Five-week dietary toxicity study in rats

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1 REFERENCE

1.1	**Reference**	[redacted] (2001a), Technical Grade KBR 3023: A Subchronic Toxicity Testing Study in the Rat (5-Week Interval). [redacted] Report No. 110222, 2001-04-05 (unpublished)
1.2	**Data protection**	Yes
1.2.1	Data owner	Saltigo GmbH
1.2.2	Company with letter of access	–
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I

2 GUIDELINES AND QUALITY ASSURANCE

2.1.1	Guideline study	Yes OPPTS Guideline No. 870.3050 (≅ OECD Guideline 407)
2.1.2	GLP	Yes
2.1.3	Deviations	Yes, deviations from OECD 407 included: – Exposure duration was 35-38 days instead of 28 days. – Clinical observations were performed on a weekly basis and not daily. – Animals were checked only once daily for morbidity and mortality instead of twice daily. – Functional observations were not performed.

3 MATERIALS AND METHODS

3.1	**Test material**	As given in Section 2 of dossier.
3.1.1	Lot/Batch number	Lot# Pt 030693
3.1.2	Specification	As given in Section 2 of dossier.
3.1.2.1	Description	Clear liquid
3.1.2.2	Purity	97.1%
3.1.2.3	Stability	> 6 months when stored at room temperature Stability and homogeneity in feed: confirmed
3.2	**Test Animals**	
3.2.1	Species	Rat
3.2.2	Strain	Sprague-Dawley (Crl:CD®(SD)IGS BR)
3.2.3	Source	Charles River Laboratories, Inc., Raleigh, NC, USA
3.2.4	Sex	♂ + ♀
3.2.5	Age/weight at study initiation	8 weeks; ♂: ~260 g, ♀: ~170 g

Section 6.3.1 Repeated dose toxicity

Annex Point IIA VI.6.3 6.3.1 Five-week dietary toxicity study in rats

3.2.6	Number of animals per group	10 per sex and group
3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	Oral
3.3.1	Duration of treatment	5 weeks
3.3.2	Frequency of exposure	Daily
3.3.3	Post-exposure period	–
3.4	**Oral**	
3.4.1	Type	In food
3.4.2	Concentration	Nominal doses: 100, 150, 300, 1000 mg/kg bw Actual doses: 1281, 1934, 3938, 13'196 ppm ♂: 99, 152, 308, 1034 mg/kg bw; ♀: 121, 189, 360, 1141 mg/kg bw ad libitum
3.4.3	Controls	Plain diet
3.5	**Examinations**	
3.5.1	Observations	
3.5.1.1	Clinical signs	Yes, once weekly.
3.5.1.2	Mortality	Yes, once daily.
3.5.2	Body weight	Yes, once weekly.
3.5.3	Food consumption	Yes, once weekly.
3.5.4	Water consumption	No
3.5.5	Ophthalmoscopic examination	No
3.5.6	Haematology	Yes, all surviving animals after 4 weeks on study Parameters: haematocrit, haemoglobin concentration, erythrocyte count, total and differential leukocyte count, platelet count, MCV, MCH, MCHC, reticulocyte and Heinz body counts, erythrocyte morphology
3.5.7	Clinical chemistry	Yes, all surviving animals after 4 weeks on study Parameters: sodium, potassium, chloride, calcium, phosphorus, fasting glucose, total cholesterol, total bilirubin, triglyceride, urea, uric acid, blood urea nitrogen, total bilirubin, creatinine, total protein, albumin, globulin, creatine kinase, alanine aminotransferase, aspartate aminotransferase (AST), alkaline phosphatase, gamma-glutamyl transpeptidase, lactate dehydrogenase
3.5.8	Urinalysis	Yes, all surviving animals after 3 weeks on study Parameters: appearance, volume, clarity, colour, osmolality, specific gravity, pH, protein, glucose, ketones, blood, urobilinogen, leukocytes, nitrite, microscopic observation of solids

Section 6.3.1 Repeated dose toxicity

Annex Point IIA VI.6.3 6.3.1 Five-week dietary toxicity study in rats

3.6	**Sacrifice and pathology**	
3.6.1	Organ weights	Yes Organs: liver, kidneys, adrenals, testes, epididymides, uterus, ovaries, thymus, spleen, brain, heart, lungs, thyroid
3.6.2	Gross and histopathology	Yes. Gross pathology: all dose groups; Organs: adrenals, aorta, bone, bone marrow, brain, cervix, clitoral gland, epididymides, eyes, exorbital lachrymal gland, gonads, gross lesions, Harderian glands, heart, kidneys, liver, lungs, lymph nodes, mammary gland, muscle, oesophagus, pancreas, parathyroid, peripheral nerve, pituitary, preputial gland, prostate, rectum, salivary glands, seminal vesicles, skin, small and large intestines, spinal cord, spleen, stomach, thymus, thyroid, trachea, urinary bladder, uterus, vagina, Zymbal's gland Histopathology: control and high dose group, tissues with effects were examined at lower dose levels to establish NOELs Organs: as above, except: exorbital lachrymal gland, preputial and clitoral gland, vagina
3.6.3	Other examinations	–
3.6.4	Statistics	Continuous data: Evaluation of homogeneity of variance was done with Bartlett's test. Group means were analysed by one-way ANOVA followed by Dunnett's test. In the event of unequal variances, data were analysed by Kruskal-Wallis ANOVA followed by the Mann-Whitney-U test. Frequency data: chi-square and Fisher exact test.
		4 RESULTS AND DISCUSSION
4.1	**Observations**	
4.1.1	Clinical signs	No effects
4.1.2	Mortality	No treatment-related effect on mortality
4.2	**Body weight gain**	Reduced body weight gain in both sexes of the high-dose group (see Table A6_3-2).
4.3	**Food consumption and compound intake**	No effects
4.4	**Ophtalmoscopic examination**	Not performed
4.5	**Blood analysis**	
4.5.1	Haematology	At 1000 mg/kg: increased platelet counts (♂)
4.5.2	Clinical chemistry	At 1000 mg/kg: increased serum cholesterol (♂+♀), decreased serum glucose and triglycerides (♂), decreased serum AST (♀, see Table A6_3-1).
4.5.3	Urinalysis	At 1000 mg/kg: slightly decreased pH (♂+♀, see Table A6_3-1).
4.6	**Sacrifice and**	

Section 6.3.1 Repeated dose toxicity

Annex Point IIA VI.6.3 6.3.1 Five-week dietary toxicity study in rats

	pathology		
4.6.1	Organ weights	At ≥ 300 mg/kg: increased relative liver weight (♂+♀), increased relative kidney weight (♂)	
		At 1000 mg/kg: increased absolute liver weight (♀, see Table A6_3-2)	
4.6.2	Gross and histopathology	Gross lesions: none detected	
		Histology (see Table A6_3-2) :	
		At ≥ 300 mg/kg: Liver: diffuse hepatocellular hypertrophy (♀, minimal to moderate); Kidney: slight multifocal bilateral hyaline degenerative nephropathy (♂)	
		At 1000 mg/kg: Liver: diffuse hepatocellular hypertrophy (♂, minimal) Adrenal: slight bilateral vacuolisation in the adrenal cortex (♂)	X
4.7	**Other**	–	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The oral short-term repeated-dose toxicity of Icaridin to Sprague-Dawley rats was investigated. The study was performed according to OPPTS Guideline 870.3050 which is equivalent to OECD Guideline 407. Deviating from this guideline, the exposure period was 5 weeks instead of 4 weeks based on practical considerations.	
5.2	**Results and discussion**	Body weight gain was impaired in both sexes of the high-dose group.	
		Treatment-related clinical chemistry changes were found in the high-dose group only and included decreased glucose and triglyceride levels in males, decreased aspartate aminotransferase in females, and increased serum cholesterol in both sexes. High-dose males had increased platelet counts, but this parameter was not increased in the 13-week feeding study in rats (Annex Point IIA6.4.1).	
		Other in-life parameters were not affected by exposure to Icaridin.	
		Organ weight changes were suggested in the 300- and 1000-mg/kg groups. Liver weights were increased in both sexes, whereas kidney weight changes were noted in males only.	
		Histopathologically, hepatocellular hypertrophy was noted in 1000-mg/kg males and 300- and 1000-mg/kg females. This finding, along with the increased liver weight, is indicative of an adaptive response to increased metabolic demands caused by administration of the test substance. The liver findings are considered non-adverse since they are not associated with a functional perturbation of clinical chemistry parameters. Other histopathological findings included increased vacuolisation of the adrenal cortex in high-dose males, and hyaline degenerative nephropathy in 300- and 1000-mg/kg males. The kidney findings at 300 mg/kg were considered non-adverse since this effect was not apparent at the same dose level in the subchronic oral study. The hyaline deposition was consistent with an α2μ-globulin-induced nephropathy.	X
		Gross pathological changes attributable to exposure to Icaridin were not noted.	

Section 6.3.1 Repeated dose toxicity

Annex Point IIA VI.6.3 6.3.1 Five-week dietary toxicity study in rats

5.3 Conclusion		
5.3.1	LO(A)EL	LOAEL = 1034 mg/kg bw/day, based on nephropathy in males.
5.3.2	NO(A)EL	NOAEL = 308 mg/kg bw/day
5.3.3	Other	–
5.3.4	Reliability	2
5.3.5	Deficiencies	Yes The clinical observations were performed once weekly and not daily. However, these observations did not reveal any indication for substance-related abnormalities and the lower-than-recommended frequency of these observations is not critical for the interpretation of the results. Likewise, functional observations of behaviour were not conducted although suggested by OECD 407. In the absence of abnormal clinical observations, however, it is unlikely that substance-related changes in functional behavioural parameters would have been detected. However, special neurotoxicity studies have been conducted with dermally applied Icaridin and are summarised under Annex Point IIIA VI 1

Section 6.3.1 Repeated dose toxicity

Annex Point IIA VI.6.3 6.3.1 Five-week dietary toxicity study in rats

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	02 October 2006/Nov 2018
Materials and Methods	Applicant's version is adopted.
Results and discussion	Bodyweight gains were decreased in the high dose group by 10.8% in males (statistically significant) and 8.4% in female rats (not statistically significant) at study termination. Relevant histopathological findings for LOAEL/NOAEL derivation were increased occurrence of hyaline degenerative nephropathy in males from 308 mg/kg bw/day, reaching statistical significance at 1034 mg/kg bw/day, and statistically increased incidences of hepatocellular hypertrophy at 360 and 1141 mg/kg bw/d in females and at 1034 mg/kg bw/day in males. The mechanism of action for the nephropathy is not proven to be α2-microglobulin. Therefore, the nephropathy in the high dose group cannot be disregarded.
Conclusion	LOAEL: 308 mg/kg bw/day based based on nephropathy in males. NOAEL: 152 mg/kg bw/day based on nephropathy in males
Reliability	2
Acceptability	Acceptable
Remarks	

Section 6.3.1 Repeated dose toxicity

Annex Point IIA VI.6.3 6.3.1 Five-week dietary toxicity study in rats

Table A6_3-1. Results of clinical chemistry, haematology and urinalysis

Parameter changed	Unit	Controls	100 mg/kg	150 mg/kg	300 mg/kg	1000 mg/kg
Weeks after start of treatment		4 (blood) 3 (urine)	4 (blood) 3 (urine)	4 (blood) 3 (urine)	4 (blood) 3 (urine)	4 (blood) 3 (urine)
Males						
Platelet count	$10^3/mm^3$	–	–	–	–	↑*
Serum glucose	mg/dL	–	–	–	–	↓*
Serum triglyceride	mg/dL	–	–	–	–	↓*
Serum cholesterol	mg/dL	–	–	–	–	↑*
Urinary pH		–	–	–	–	↓*
Females						
Serum AST	U/L	–	–	–	–	↓*
Urinary pH		–	–	–	–	↓
Serum cholesterol	mg/dL	–	–	–	–	↑

* $p < 0.05$

Section 6.3.1 Repeated dose toxicity

Annex Point IIA VI.6.3 6.3.1 Five-week dietary toxicity study in rats

Table A6_3-2. Results of repeated dose toxicity study

Parameter	Control		100 mg/kg		150 mg/kg		300 mg/kg		1000 mg/kg		Dose-response +/–	
	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m	f
Number of animals examined	10	10	10	10	10	10	10	10	10	10		
Mortality	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10	–	–
Clinical signs	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	–	–
Body weight, terminal [% contr.]	100.0	100.0	96.7	100.4	99.6	102.0	101.8	99.0	89.2*	91.6	+	+
Clinical chemistry												
Glucose	–	–	–	–	–	–	–	–	↓*	–	+	–
Triglyceride	–	–	–	–	–	–	–	–	↓*	–	+	–
Cholesterol	–	–	–	–	–	–	–	–	↑*	↑	+	+
AST	–	–	–	–	–	–	–	–	–	↓*	–	+
Haematology												
Platelet count	–	–	–	–	–	–	–	–	↑*	–	+	–
Urinalysis												
pH	–	–	–	–	–	–	–	–	↓*	↓	+	+
Liver												
Organ weight												
Relative (%)	4.37	4,14	4.40	4.25	4.48	4.47	4.91*	4.860*	5.51*	5.63*	+	+
Absolute (g)	19.52	10.18	19.21	10.32	19.85	11.22	22.06	11.59	21.66	12.27*	–	+
Histopathology												
hypertrophy	0/10	3/10	n.e.	4/10	0/10	5/10	1/10	9/10*	10/10*	8/10*	+	+
Kidney												
Organ weight												
Relative (%)	0.80	0.88	0.84	0.88	0.82	0.88	0.87*	0.91	0.90*	0.99	+	–
Absolute(g)	3.57	2.16	3.63	2.11	3.64	2.20	3.93	2.15	3.55	2.14	–	–
Histopathology												
nephropathy, protein-droplet	2/10	0/10	n.e.	n.e.	3/10	n.e.	6/10	n.e.	10/10*	0/10	+	–
Adrenal gland												
Histopathology												
vacuolisation	0/10	0/10	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	3/10	0/10	–	–

* $p < 0.05$

[a] number of animals affected/total number of animals
n.e.: not examined

Section 6.3.2	Subacute toxicity	
Annex Point IIA VI.6.3	6.3.2 28-day dermal toxicity study in rabbits	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	A 90-day dermal study in rats has been performed and is submitted. On these grounds, a separate subacute dermal study is not required.	
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	02 October 2006	
Evaluation of applicant's justification	Applicant's justification is acceptable.	
Conclusion	Applicant's justification is acceptable	
Remarks		

Section 6.3.3	Subacute toxicity	
Annex Point IIA VI.6.3	**6.3.3 Subacute inhalation toxicity study in rats**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [X]	**Other justification [ ]**	
Detailed justification:	A repeated-exposure inhalation study has not been conducted. Exposure towards Icaridin by inhalation is negligible compared to the main routes of exposure which are direct dermal contact and oral uptake via hand-to-mouth contact. Both relevant routes have been addressed with repeated-dose studies. Although the vapour pressure of Icaridin at 20°C is slightly higher than 1×10^{-2} Pa (3.4×10^{-2} Pa), exposure to Icaridin vapours is considered to be low because of the use pattern of Icaridin-containing products. Mosquito repellents will be used either outdoors or in well-ventilated rooms during the summer time. Exposure estimations (for details *cf.* Doc. II-B, Section 3.2) demonstrate that the systemic exposure via inhalation during pump spray application of Icaridin is only 0.0118 mg/kg bw/day for a 60-kg user. For comparison, the expected systemic exposures via the dermal and oral route are 0.59 and 0.65 mg/kg bw/day, respectively. This corresponds to less than 2% of each of the non-inhalational routes. Therefore, by submitting repeated-dose studies for the dermal and oral route, the relevant routes of exposure have been covered.	X
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	02 October 2006	
Evaluation of applicant's justification	Applicant’s justification is agreed upon. Exposure estimations might be changed by CA but is still considered inhalation to be negligible.	
Conclusion	Applicant's justification is acceptable	
Remarks		

Section 6.4.1	Subchronic toxicity	
Annex Point IIA VI.6.4	6.4.1 90-day oral toxicity study in dogs	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	A subchronic oral toxicity study in dogs has not been performed. Conducting such a study is unjustified because the existing data base is sufficient for the identification and assessment of potential hazards for human health posed by Icaridin. Results from an existing dermal long-term study in dogs can be read across to a subchronic oral exposure situation. Additional animal testing is thus unnecessary and should be avoided in conformity with Directive 86/609/EEC.	X
	Icaridin is used in products intended solely for dermal application and dermal penetration will certainly be the dominant route of uptake. Consequently, dermal exposure has been chosen as the method of administration for a one-year toxicity study in Beagle dogs ([redacted] 1995). No adverse effects were caused by exposure to 200 mg/kg of the test substance, which was the highest dose used.	X
	In rats, the results of the dermal and oral subchronic studies were almost identical ([redacted] 1995; [redacted] 2001b). In both studies, hypertrophy of liver and kidney was found in the highest dose groups. The NOELs established in both studies were virtually identical with the dermal and oral NOEL being 200 and 194 mg/kg, respectively. Hence, there is no indication for a marked difference in sensitivity associated with either route of administration.	
	Since the subchronic dermal NOELs in the rat and dog are identical, it can be reasonably assumed that both species are similarly sensitive towards Icaridin. It is thus highly unlikely that oral administration of Icaridin to dogs over a 90-day period will produce adverse effects that, if any are observed, differ in nature or degree from those observed in rats.	X
	This expectation is also based on the metabolism study in rats in which the metabolites formed following i.v. or dermal application were essentially identical ([redacted] 1997). Additionally, the i.v. metabolites in rat did not differ qualitatively from those found in a dermal metabolism study in human volunteers indicating negligible inter-species variations ([redacted] 1997). This supports the assumption that Icaridin does not elicit a route-specific response and that extrapolation from the dermal one-year study in dogs is indeed justified.	
	Lastly, Icaridin-containing products have been widely used for the last 12 years with sales in Europe and beyond exceeding [redacted] 100 mL bottles. Based on the very low number of complaints that have been received over the years, there is little concern that Icaridin bears a yet-unknown potential for adverse health effects that would warrant an additional study in dogs.	
	References:	
	[redacted] (1997) [Hydroxmethyl-1-^{14}C]KBR 3023: Human volunteer metabolism study after dermal application. Report No. PF 4187	
	[redacted]. (1997) [Hydroxyethyl-1-^{14}C]KBR 3023: Rat metabolism study after intravenous injection and after dermal	

Section 6.4.1	Subchronic toxicity
Annex Point IIA VI.6.4	6.4.1 90-day oral toxicity study in dogs
	application. Report No. PF-4178 [redacted] (1995) A chronic percutaneous toxicity study in the Beagle dog. Report No. 107155 [redacted] (1995) A repeated dose 90-day dermal toxicity study with technical grade KBR 3023 in rats. Report No. 90-122-HC [redacted] (2001b) Technical grade KBR 3023: A subchronic toxicity study in the rat (14-week interval). Report No. 110223
Undertaking of intended data submission [ ]	
	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	22 November 2006
Evaluation of applicant's justification	Applicant's justification is accepted with below mentioned remarks. In the rats the identical NOAELs values in the dermal and oral subchronic studies could also be a result of poor oral absorption. Since no oral ADME study is available this is actually unknown . The argument regarding essentially identical metabolites in rats following i.v or dermal application do not give information about what happens after oral absorption and a route specific response. I.v and dermal are more alike as no first pass metabolism effect in the liver will occur and the substance will enter the blood system right after passing the skin (where metabolism also could occur). It is therefore more relevant to compare with i.v metabolism than oral metabolism due to the intended use of the substance on the skin.
Conclusion	Justification accepted.
Remarks	No information is available on dermal uptake of Icaridin in dogs. It is thus impossible to draw conclusions on systemic effects of Icaridin in dogs from a dermal study in which no effect was noted. However it can be accepted that the dermal study will give information about the dermal toxicity of icaridin in sufficiently high doses (compare to doses used on humans) because the foreseeable route of systemic exposure for this dermally applied insect repellent is though dermal absorption.

Section 6.4.1 Repeated dose toxicity

Annex Point IIA VI.6.4 6.4.1 Fourteen-week dietary toxicity study in rats

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted]. (2001b), Technical Grade KBR 3023: A Subchronic Toxicity Testing Study in the Rat (14-Week Interval). [redacted] Report No. 110223, 2001-04-05 (unpublished)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Saltigo GmbH	
1.2.2	Company with letter of access	–	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes	
		OPPTS Guideline No. 870.3100 ≅ OECD Guideline 408 (1998)	X
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes, deviations from OECD 408 included: – Exposure duration was 14 weeks instead of 90 days. – Clinical observations were performed on a weekly basis and not daily. – Ophthalmological examination was only performed on study day 76, not prior to exposure and at study termination.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in Section 2 of dossier.	
3.1.1	Lot/Batch number	Lot# Pt 030693	
3.1.2	Specification	As given in Section 2 of dossier.	
3.1.2.1	Description	Clear liquid	
3.1.2.2	Purity	97.1%	
3.1.2.3	Stability	> 6 months when stored at room temperature Stability and homogeneity in feed: confirmed	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Sprague-Dawley (Crl:CD®(SD)IGS BR)	
3.2.3	Source	Charles River Laboratories, Inc., Raleigh, NC, USA	
3.2.4	Sex	♂ + ♀	
3.2.5	Age/weight at study initiation	8 weeks; ♂: ~264 g, ♀: ~174 g	
3.2.6	Number of animals	10 per sex and group	

Section 6.4.1 Repeated dose toxicity

Annex Point IIA VI.6.4 6.4.1 Fourteen-week dietary toxicity study in rats

	per group		
3.2.7	Control animals	Yes	
3.3	**Administration/ Exposure**	Oral	
3.3.1	Duration of treatment	14 weeks	
3.3.2	Frequency of exposure	Daily	
3.3.3	Post-exposure period	–	
3.3.4	Oral		
3.3.4.1	Type	In food	
3.3.4.2	Concentration	Nominal doses: 100, 150, 300, 1000 mg/kg bw Actual doses: 1433, 2175, 4423, 14,315 ppm ♂: 100, 149, 301, 1033 mg/kg bw; ♀: 126, 194, 382, 1192 mg/kg bw ad libitum	
3.3.4.3	Controls	Plain diet	
3.4	**Examinations**		
3.4.1	Observations		
3.4.1.1	Clinical signs	Yes, once weekly.	
3.4.1.2	Mortality	Yes, once daily.	
3.4.2	Body weight	Yes, once weekly.	
3.4.3	Food consumption	Yes, once weekly.	
3.4.4	Water consumption	No	
3.4.5	Ophthalmoscopic examination	Yes, on day 76 of exposure	
3.4.6	Haematology	Yes, all surviving animals after 13 weeks on study Parameters: haematocrit, haemoglobin concentration, erythrocyte count, total and differential leukocyte count, platelet count, MCV, MCH, MCHC, reticulocyte and Heinz body counts, erythrocyte morphology	X
3.4.7	Clinical chemistry	Yes, all surviving animals after 13 weeks on study Parameters: sodium, potassium, chloride, calcium, phosphorus, fasting glucose, total cholesterol, total bilirubin, triglyceride, urea, uric acid, blood urea nitrogen, total bilirubin, creatinine, total protein, albumin, globulin, creatine kinase, alanine aminotransferase, aspartate aminotransferase (AST), alkaline phosphatase, gamma-glutamyl transpeptidase, lactate dehydrogenase	X
3.4.8	Urinalysis	Yes, all surviving animals after 12 weeks on study Parameters: appearance, volume, clarity, colour, osmolality, specific gravity, pH, protein, glucose, ketones, blood, urobilinogen, leukocytes, nitrite, microscopic observation of solids	X
3.5	**Sacrifice and**		

Section 6.4.1 Repeated dose toxicity

Annex Point IIA VI.6.4 6.4.1 Fourteen-week dietary toxicity study in rats

	pathology		
3.5.1	Organ weights	Yes Organs: liver, kidneys, adrenals, testes, epididymides, uterus, ovaries, thymus, spleen, brain, heart, lungs, thyroid	
3.5.2	Gross and histopathology	Yes. Gross pathology: all dose groups; Organs: adrenals, aorta, bone, bone marrow, brain, cervix, clitoral gland, epididymides, eyes, exorbital lachrymal gland, gonads, gross lesions, Harderian glands, heart, kidneys, liver, lungs, lymph nodes, mammary gland, muscle, oesophagus, pancreas, parathyroid, peripheral nerve, pituitary, preputial gland, prostate, rectum, salivary glands, seminal vesicles, skin, small and large intestines, spinal cord, spleen, stomach, thymus, thyroid, trachea, urinary bladder, uterus, vagina, Zymbal's gland Histopathology: control and high dose group, tissues with effects were examined at lower dose levels to establish NOELs Organs: as above, except: exorbital lachrymal gland, preputial and clitoral gland, vagina	
3.5.3	Other examinations	–	
3.5.4	Statistics	Continuous data: Evaluation of homogeneity of variance was done with Bartlett's test. Group means were analysed by one-way ANOVA followed by Dunnett's test. In the event of unequal variances, data were analysed by Kruskal-Wallis ANOVA followed by the Mann-Whitney-U test. Frequency data: chi-square and Fisher exact test.	
3.6	**Further remarks**	–	
		4 RESULTS AND DISCUSSION	
4.1	**Observations**		
4.1.1	Clinical signs	No effects	
4.1.2	Mortality	No treatment-related effect on mortality	
4.2	**Body weight gain**	Reduced body weight gain in both sexes of the high-dose group (see Table A6_4-2).	
4.3	**Food consumption and compound intake**	No effects	
4.4	**Ophtalmoscopic examination**	No effects	
4.5	**Blood analysis**		
4.5.1	Haematology	At 1000 mg/kg: slight decline in haematocrit, MCV, MCH, lymphocyte count (♀), slightly increased neutrophil count (♀)	X
4.5.2	Clinical chemistry	At ≥ 300 mg/kg: decreased triglycerides (♂) At 1000 mg/kg: increased serum cholesterol (♂), decreased serum glucose (♂), decreased serum AST (♀, see Table A6_4-1).	X
4.5.3	Urinalysis	No effects	

Section 6.4.1 Repeated dose toxicity

Annex Point IIA VI.6.4 6.4.1 Fourteen-week dietary toxicity study in rats

4.6	**Sacrifice and pathology**	
4.6.1	Organ weights	At ≥ 150 mg/kg: increased relative kidney weight (♂) At ≥ 300 mg/kg: increased relative liver weight,+ 7% (♀) At 1000 mg/kg: increased absolute liver weight (♀), increased relative liver weight, +47% (♂), increased relative kidney weight (♀, see Table A6_4-2)
4.6.2	Gross and histopathology	Gross lesions: none detected Histology (see Table A6_4-2) : At ≥ 300 mg/kg: Liver: diffuse hepatocellular hypertrophy (♀); At 1000 mg/kg: Liver: diffuse hepatocellular hypertrophy (♂); Kidney: slight multifocal bilateral hyaline degenerative nephropathy (♂, not significant)
4.7	**Other**	–

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The oral subchronic toxicity of Icaridin to Sprague-Dawley rats was investigated. The study was performed according to OPPTS Guideline 870.3100 which is equivalent to OECD Guideline 408. Deviating from this guideline, the exposure period was 14 weeks instead of 13 weeks.
5.2	**Results and discussion**	Body weight gain was impaired in both sexes of the high-dose group. Haematological changes suggestive of a treatment-related effect were noted in high-dose females only and included slight declines in haematocrit, MCV, MCH, lymphocyte count, and increased neutrophil count. Clinical chemistry parameters that were affected by Icaridin included decreased glucose in 1000-mg/kg males, decreased triglycerides in 300- and 1000-mg/kg males, decreased AST in 1000 mg/kg females, and increased cholesterol in 1000-mg/kg males. Other in-life parameters were not affected by exposure to Icaridin. Absolute organ weight changes were only noted in livers of high-dose females. Relative organ weight changes included kidneys of males of the 150-mg/kg group and all higher dose groups and high-dose females. Increased kidney weights were not associated with any significant microscopical alterations and are therefore considered to be of no toxicological relevance. Relative liver weights were increased in 300- and 1000-mg/kg females and 1000-mg/kg males. Increases in the relative weight of other organs in the high-dose groups were considered secondary to Icaridin-induced reductions in terminal body weight. Histopathologically, hepatocellular hypertrophy was noted in 1000-mg/kg males and 300- and 1000-mg/kg females. This finding, along with the increased liver weight, is indicative of an adaptive response to increased metabolic demands caused by administration of the test substance. Hyaline degenerative nephropathy, the incidence of which

Section 6.4.1 Repeated dose toxicity

Annex Point IIA VI.6.4 6.4.1 Fourteen-week dietary toxicity study in rats

		did not reach statistical significance, was noted in some 1000-mg/kg males. The hyaline deposition was consistent with an α2μ-globulin-induced nephropathy. Gross pathological changes attributable to exposure to Icaridin were not noted.
5.3	**Conclusion**	
5.3.1	LO(A)EL	LOEL = 382 mg/kg bw/day, based on hepatocellular hypertrophy in females (less than 15% rel.wt. increase) LOAEL = 1000 mg/kg bw/day, based on kidney effects in males
5.3.2	NO(A)EL	NOEL = 194 mg/kg bw/day NOAEL = 301 mg/kg bw/day
5.3.3	Other	–
5.3.4	Reliability	2
5.3.5	Deficiencies	Yes The clinical observations were performed once weekly and not daily. However, these observations did not reveal any indication for substance-related abnormalities and the lower-than-recommended frequency of these observations is not critical for the interpretation of the results. Likewise, the ophthalmological examinations were performed only once close to study termination. Since no remarkable findings were made at this point, results of pre-exposure examinations are not critical for the interpretation of the results of this study.

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Annex Point IIA VI.6.4 6.4.1 Fourteen-week dietary toxicity study in rats

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	12 December 2010/amended 2019 in relation to CLH-report, discrepancies with conclusion in doc IIA regarding NOAEL/LOAEL after WG meetings.
Materials and Methods	Applicant's version is adopted
Results and discussion	The mean body weights in the high dose group were at study termination decreased by 11.4% in males and 16.2% in females. Both results were statistically significant.
Conclusion	LOAEL: 382 mg/kg bw/day, based on relative liver weight ↑(♀ (1.4 %)), reduced MCV& MCH (♀) and hepatocellular hypertrophy (♀). NOAEL: 149 mg/kg bw/day.
Reliability	2
Acceptability	Acceptable
Remarks	-

Section 6.4.1 Repeated dose toxicity

Annex Point IIA VI.6.4 6.4.1 Fourteen-week dietary toxicity study in rats

Table A6_4-1. Results of clinical chemistry and haematology.

Parameter changed	Unit	Controls	100 mg/kg	150 mg/kg	300 mg/kg	1000 mg/kg
Weeks after start of treatment		13	13	13	13	13
Males						
Serum glucose	mg/dL	–	–	–	–	↓*
Serum triglyceride	mg/dL	–	–	–	↓*	↓*
Serum cholesterol	mg/dL	–	–	–	–	↑*
Females						
Serum AST	U/L	–	–	–	–	↓*
Serum cholesterol	mg/dL	–	–	–	–	↑
Serum triglyceride	mg/dL	–	–	–	–	↓
Haematocrit	%	–	–	–	–	↓*
MCV	µm³	–	–	–	↓*	↓*
MCH	pg	–	–	–	↓*	↓*
Segmented neutrophils	%	–	–	–	–	↑*
Lymphocytes	%	–	–	–	–	↓*

* $p < 0.05$

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Annex Point IIA VI.6.4 6.4.1 Fourteen-week dietary toxicity study in rats

Table A6_4-2. Results of repeated dose toxicity study

Parameter	Control		100 mg/kg		150 mg/kg		300 mg/kg		1000 mg/kg		Dose-response +/–	
	♂[a]	♀[a]	♂[a]	♀[a]	♂[a]	♀[a]	♂[a]	♀[a]	♂[a]	♀[a]	♂	♀
Number of animals examined	10	10	10	10	10	10	10	10	10	10		
Mortality	1/10	0/10	0/10	0/10	0/10	0/10	1/10	0/10	0/10	0/10	–	–
Clinical signs	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	–	–
Body weight (91 d) [% of control]	100.0	100.0	97.1	100.5	98.1	99.7	94.7	96.1	88.6*	83.8*	+	+
Clinical chemistry												
Glucose	–	–	–	–	–	–	–	–	↓*	–	+	–
Triglyceride	–	–	–	–	–	–	↓*	–	↓*	↓	+	+
Cholesterol	–	–	–	–	–	–	–	–	↑*	–	+	–
AST	–	–	–	–	–	–	–	–	–	↓*	–	+
Haematology												
Haematocrit	–	–	–	–	–	–	–	–	–	↓*	–	–
MCV	–	–	–	–	–	–	–	↓*	–	↓*	–	+
MCH	–	–	–	–	–	–	–	↓*	–	↓*	–	–
Neutrophils	–	–	–	–	–	–	–	–	–	↑*	–	+
Lymphocytes	–	–	–	–	–	–	–	–	–	↓*	–	+
Liver												
Organ weight												
Relative (%)	3.84	3.84	3.88	3.77	3.94	4.06	4.23	4.08	4.95*	5.62*	+	+
Absolute (g)	23.26	11.24	22.40	11.03	22.76	11.80	23.85	11.40	25.45	13.881	–	+
Histopathology												
hypertrophy	0/10	0/10	n.e.	n.e.	0/10	1/10	3/10	5/10*	5/10*	10/10*	+	+
Kidney												
Organ weight												
Relative (%)	0.67	0.78	0.72	0.79	0.80*	0.79	0.76*	0.80	0.90*	0.86*	+	+
Absolute (g)	4.02	2.27	4.14	2.295	4.543	2.29	4.28	2.23	4.56	2.14	–	–
Histopathology												
nephropathy, protein-droplet	0/10	0/10	n.e.	n.e.	n.e.	n.e.	0/10	n.e.	3/10	0/10	+	–

* $p < 0.05$
[a] number of animals affected/total number of animals
n.e.: not examined

Section 6.4.2 Repeated dose toxicity

Annex Point IIA VI.6.4.2 6.4.2 Thirteen-week dermal toxicity study in rats

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	1 REFERENCE	
1.1	**Reference**	[redacted] (1995), A Repeated Dose 90-Day Dermal Toxicity Study with Technical Grade KBR 3023 in Rats. Bayer Corporation, Agriculture Division, Toxicology, Stilwell, KS, USA, Study No. 90-122-HC, 1995-11-01 (unpublished)
1.2	**Data protection**	Yes
1.2.1	Data owner	Saltigo GmbH
1.2.2	Company with letter of access	–
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I
	2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes US-EPA FIFRA §82-3, November 1984 OECD Guideline 411, May 1981 Japanese MAFF, 59 NohSan No. 4200, January 1985
2.2	**GLP**	Yes
2.3	**Deviations**	Yes – The test substance was applied non-occlusively without dressing.
	3 MATERIALS AND METHODS	
3.1	**Test material**	As given in Section 2 of dossier.
3.1.1	Lot/Batch number	19001/90
3.1.2	Specification	As given in Section 2 of dossier.
3.1.2.1	Description	Clear, viscous liquid
3.1.2.2	Purity	99.2%
3.1.2.3	Stability	Stability was confirmed by analyses at study initiation and termination.
3.2	**Test Animals**	
3.2.1	Species	Rat
3.2.2	Strain	Sprague-Dawley (Sas:CD(SD) BR)
3.2.3	Source	Sasco, Inc., St. Louis, MO, USA
3.2.4	Sex	♂ + ♀
3.2.5	Age/weight at study initiation	♂: 8 weeks; 248 - 250 g, ♀: 10 weeks; 211 - 214 g
3.2.6	Number of animals per group	10 per sex and group
3.2.7	Control animals	Yes

Section 6.4.2 Repeated dose toxicity

Annex Point IIA VI.6.4.2 6.4.2 Thirteen-week dermal toxicity study in rats

3.3	**Administration/ Exposure**	Dermal	
3.3.1	Duration of treatment	13 weeks + 2-4 additional applications during the 14th week	
3.3.2	Frequency of exposure	Five days per week	
3.3.3	Post-exposure period	Four weeks for control and high-dose satellite groups (10 animals per sex and group).	
3.3.4	Dermal		
3.3.4.1	Area covered	16 cm², >16 cm² at the two highest dose levels	
3.3.4.2	Occlusion	Non-occlusive, without dressing. Elizabethan collars were used to prevent oral uptake of the test material.	
3.3.4.3	Vehicle	Undiluted	
3.3.4.4	Concentration in vehicle	–	
3.3.4.5	Dose levels	Nominal daily doses: 80, 200, 500, 1000 mg Icaridin/kg bw/day	X
		Actual average doses: 107, 268, 669, 1339 mg Icaridin/kg bw/day (see Figs. A6_4-1 though -4).	
3.3.4.6	Duration of exposure	24 h	
3.3.4.7	Removal of test substance	No	
3.3.4.8	Controls	Yes, untreated	
3.4	**Examinations**		
3.4.1	Observations		
3.4.1.1	Clinical signs	Yes, once daily	
3.4.1.2	Mortality	Yes, once daily	
3.4.2	Body weight	Yes, once weekly	
3.4.3	Food consumption	Yes, once weekly	
3.4.4	Water consumption	No	
3.4.5	Ophthalmoscopic examination	Yes, pre-exposure and pre-terminal	
3.4.6	Haematology	Yes, all surviving animals prior to sacrifice	
		Parameters: haematocrit, haemoglobin concentration, erythrocyte count, total and differential leukocyte count, platelet count, MCV, MCH, MCHC, Heinz body counts, erythrocyte morphology	
3.4.7	Clinical chemistry	Yes, all surviving animals prior to sacrifice.	
		Three animals per sex and dose level were inadvertently not fasted prior to blood sampling. All other rats were fasted overnight prior to blood sampling.	
		Parameters: sodium, potassium, chloride, calcium, phosphorus, fasting glucose, total cholesterol, total bilirubin, triglyceride, urea, uric acid,	

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		blood urea nitrogen, total bilirubin, creatinine, total protein, albumin, globulin, creatine kinase, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma-glutamyl transpeptidase, lactate dehydrogenase, T4, T3, T3 uptake, IgG, IgA, IgM
3.4.8	Urinalysis	Yes, all surviving animals one week prior to sacrifice Parameters: clarity, colour, osmolality, specific gravity, pH, protein, glucose, ketones, blood, bilirubin, microscopic observation of solids
3.5	**Sacrifice and pathology**	
3.5.1	Organ weights	Yes Organs: liver, kidneys, adrenals, testes, uterus, ovaries, thymus, spleen, brain, heart, lungs, lymph node (lumbar), thyroid
3.5.2	Gross and histopathology	Yes. Gross pathology: all dose groups; Organs: adrenals, aorta, bone, bone marrow, brain, cervix, clitoral gland, epididymides, eyes, exorbital lachrymal gland, gonads, gross lesions, Harderian glands, heart, kidneys, larynx, liver, lungs, lymph nodes, mammary gland, muscle, oesophagus, pancreas, parathyroid, peripheral nerve, pituitary, preputial gland, prostate, salivary glands, seminal vesicles, skin, skull, small and large intestines, spinal cord, spleen, stomach, thymus, thyroid, trachea, urinary bladder, uterus, vagina, Zymbal's gland Histopathology: control and high dose group; skin, liver, kidneys and gross lesions were evaluated for all dose groups. Organs: as above, except: exorbital lachrymal gland, preputial, Zymbal's and clitoral gland, vagina
3.5.3	Other examinations	–
3.5.4	Statistics	Continuous data: Evaluation of homogeneity of variance was done with Bartlett's test. If homogenous, group means were analysed by ANOVA followed by Dunnett's test. In the event of unequal variances, data were analysed by Kruskal-Wallis ANOVA followed by the Mann-Whitney-U test. Frequency data: chi-square and one-tailed Fisher exact test. All significant differences are reported at the 95% confidence level.
3.6	**Further remarks**	–
		4 RESULTS AND DISCUSSION
4.1	**Observations**	
4.1.1	Clinical signs	Treatment-related clinical signs were limited to topical lesions at the dose site. These included scabs and red foci that developed over time in all dose groups and resolved in the satellite groups by 16 days after cessation of treatment. Other clinical observations can be attributed to the wearing of Elizabethan collars for a prolonged period. These observations probably resulted from either the physical contact with the collar material or from the animals' impaired ability to groom them. These effects included stains on the head and body; lacrimation and eye irritation; scabs, sores

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		and alopecia on the head, neck and body; evidence of inflammation involving penis and urethra; and a swollen digit and forefoot. Two days after removal of the collars, all but two of these signs had resolved in the satellite animals.	
4.1.2	Mortality	No mortalities occurred.	
4.2	**Body weight gain**	No effects	
4.3	**Food consumption**	No effects	
4.4	**Ophtalmoscopic examination**	No effects	
4.5	**Blood analysis**		
4.5.1	Haematology	No effects	
4.5.2	Clinical chemistry	No effects	
4.5.3	Urinalysis	At ≥ 669 mg/kg: decreased pH (♂+♀)	X
		At 1339 mg/kg: decreased urobilinogen (♂)	
		Both parameters had returned to control values in satellite groups.	
4.6	**Sacrifice and pathology**		
4.6.1	Organ weights	At ≥ 500 mg/kg: increased relative liver (♂+♀) and kidney weights (♂), increased absolute liver weights (♀)	
		At 1000 mg/kg: increased absolute liver and kidney weights (♂, see Table A6_4-2)	
		None of these changes were apparent in satellite animals 4 weeks post exposure.	
4.6.2	Gross and histopathology	Gross lesions: none detected	
		Histology (see Table A6_4-2) :	
		At ≥ 80 mg/kg: Treated skin: acanthosis, hyperkeratosis, hypertrophy of the sebaceous glands around the hair follicle (♂+♀); also present in some animals of the control groups and considered of no toxicological significance.	
		At ≥ 500 mg/kg: Liver: diffuse hepatocellular hypertrophy (♂); Kidney: slight hyaline degenerative nephropathy (♂)	
		At 1000 mg/kg: Liver: diffuse hepatocellular hypertrophy (♀); Kidney: tubular degeneration and chronic inflammation (♂)	
		All changes were absent in satellite animals 4 weeks post exposure.	
4.7	**Other**	In the two highest dose groups, the test material spread out beyond the intended dose site. At the 1000 mg/kg level, the hair lateral to the dose site was moistened with test material. This afforded the possibility for higher dermal absorption than if the test material had been confined to the intended 10% of the body surface. Additionally, the test material reached parts of the coat where the rats could potentially orally ingest test material through grooming.	

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		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The dermal subchronic toxicity of Icaridin to Sprague-Dawley rats was investigated in a 90-day study using a 5-days-per-week application scheme. The study was performed according to OECD Guideline 411. Deviating from this guideline, rats were exposed to the test substance openly, without wrapping. Instead, Elizabethan collars were used to prevent ingestion of the test compound. Other than proposed in OECD 411, dermal exposures were not terminated after 6 h by washing or wiping, but the doses were left on the application site and the next daily dose is applied on top of the previous dose. A small fraction of the applied icaridin is lost due to evaporation. This fraction was measured in the rat dermal absorption study (Warren & Sturdivant, 1997). Based on these findings, it is assumed that this loss by evaporation will be no higher than 6% within 24 h. About half of the applied dose (47.2%) is absorbed within 24 h. Thus, 6% + 47.2% = 53.2% of the dose from the preceding day have dissipated from skin by evaporation and absorption. The remainder, 46.8% of the preceding dose, is still present on skin when the next dose is applied. As a result, the actual dose on skin increases during the five weekdays of dosing while it decreases during the weekend dosing hiatus. If the actual daily doses on skin are averaged throughout the dosing period (averaging of doses throughout a study period is common practice for both feeding and gavage studies), mean actual doses of 268 and 669 mg/kg bw/day are achieved, in the 268- and 669-mg/kg bw dose group, respectively. This is depicted in the Figures A6_4-1 though -4. Therefore, the actually applied doses in this study were 107, 268, 669, and 1339 mg/kg bw/day.	X
5.2	**Results and discussion**	Topical skin reactions (acanthosis and hyperkeratosis) were the only clinical signs that were noted in treated rats. The skin alterations were reversible in the 4-week post-exposure period. These lesions are common findings with repeated exposure to a variety of treatments (including water or medical petrolatum) and are considered to be of no toxicological relevance. Treatment-related findings included reduced urinary pH and increased liver and kidney weights that were found at doses of 500 mg/kg and above. These organ weight changes were accompanied by histopatho-logical alterations in the respective tissues. Liver enlargement, caused by diffuse hepatocellular hypertrophy (not: hyperplasia!), was not accompanied by perturbations of hepatic function. Clinical chemistry and haematology parameters were not different from untreated controls. The liver enlargement was fully reversible within a 4-week non-exposure period. Hepatocellular hypertrophy is very likely a transient and adaptive response, and thus non-adverse by definition. Increased kidney weights were noted in males only. Histopathologically, the kidney presented with slight hyaline degenerative nephropathy at 500 mg/kg bw/day and as tubular degeneration and chronic inflammation at 1000 mg/kg bw/day, again in males only.	X

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The only parameter related to kidney function that was changed at 500 mg/kg bw/day was the urinary pH. This change presented as a dose-dependent lowering of pH, from 8.4±0.4 in controls to 7.8±0.3 and 7.5±0.5 at 669 and 1339 mg/kg bw/day, respectively.

The rat's urinary pH has a physiological range of 7.3-8.5 (Gad and Chengelis, Animal Models in Toxicology. Marcel Dekker: New York, 1992). Hence, the urinary pH of icaridin-treated rats is within the rat's normal range and the observed change is not considered adverse. Thus, the macroscopic and microscopic kidney alterations observed at 500 mg/kg bw/day are considered non-adverse. The microscopic kidney alterations were described by the study pathologist as "slight hyaline degenerative nephropathy" which progressed into "tubular degeneration and chronic inflammation" at the top dose. Since these findings were restricted to male rats (no kidney response in dogs, mice or rabbits), it is most probable that kidney pathology is in fact an α2μ-globulin nephropathy. However, since mechanistic data to support this are not available, the kidney effects at the top dose are considered adverse and relevant.

In the two highest dose groups, the skin area exposed to the test substance was greater than intended. Thus, a much larger area of skin was exposed, affording the opportunity for more extensive absorption. Additionally, it is likely that rats in these dose groups ingested some of the spread test material. Thus, the systemic exposure might have been higher than if the exposed skin area had been limited to 10% of the body surface.

5.3 Conclusion

5.3.1	LO(A)EL	topical LOAEL ≤ 80 mg/kg bw/day, based on acanthosis systemic LOAEL = 1000 mg/kg bw/day, based on the kidney effects in male rats.
5.3.2	NO(A)EL	topical NOAEL < 80 mg/kg bw/day systemic NOAEL = 500 mg/kg bw/day
5.3.3	Other	–
5.3.4	Reliability	1
5.3.5	Deficiencies	No

Section 6.4.2 Repeated dose toxicity

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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 December 2010
Materials and Methods	Re 3.3.4.5 The cumulative dose calculations proposed by the applicant is not accepted by the RMS. The methodology is unusual. No measurements of actual dose on the skin or absorbed doses were performed. The extent of DA is not supported by data at the higher doses. The RMS will base calculations on the nominal doses.
Results and discussion	Point 5.2 Topical skin reactions (acanthosis and hyperkeratosis) were the only clinical signs that were noted in treated rats. These lesions are common findings with repeated exposure to a variety of treatments (including water or medical petrolatum) and are in general then not considered to be of no toxicological relevance. However since the intended use involves repeated dermal exposure over a prolonged period of time the effect could therefore be relevant. Although the kidney effects may be related to α2μ-globulin, the mechanism of nephrotoxicity is not demonstrated . The findings in the kidney are thus considered relevant for NOAEL setting in this study and must be considered in the human health risk assessment.
Conclusion	LOAEL for local effect = 80 mg/kg bw/day, based on acanthosis NOAEL for local effect < 80 mg/kg bw/day LOAEL for systemic effect = 500 mg/kg bw/day, based on nephropathy and hepatocellular hypertrophy in males. NOAEL for systemic effect = 200 mg/kg bw/day.
Reliability	1
Acceptability	Acceptable
Remarks	

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Table A6_4-1. Results of clinical chemistry haematology and urinalysis

				Dose: nominal (calculated)							
Parameter changed	**Unit**	**Controls**		**80 (107) mg/kg**		**200 (268) mg/kg**		**500 (669) mg/kg**		**1000 (1339) mg/kg**	
Weeks after start of treatment		13	17 [a]	13	17 [a]	13	17 [a]	13	17 [a]	13	17 [a]
Males											
Urine	pH	–	–	–	n.d.	–	n.d.	↓*	–	↓*	–
Urine urobilinogen	Eu/dL	–	–	–	n.d.	–	n.d.	–	–	↓*	–
Females											
Urine	pH	–	–	–	n.d.	–	n.d.	↓*	–	↓*	–

* $p < 0.05$
[a] Satellite groups, 4 weeks after cessation of treatment
n.d. = not determined

Section 6.4.2 Repeated dose toxicity

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Table A6_4-2. Results of repeated dose toxicity study

Parameter	Control		80 (107) mg/kg		200 (268) mg/kg		500 (669) mg/kg		1000 (1339) mg/kg		Dose-response +/–	
	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m	f
Number of animals examined	20	20	10	10	10	10	10	10	20	20		
Body weight (91 d) [% of control]	100.0	100.0	101.2	101.4	100.9	102.5	98.1	98.9	99.8	96.8	–	–
Mortality	0/20	0/20	0/10	0/10	0/20	0/20	0/20	0/20	0/20	0/20	–	–
Clinical signs												
Dose site, scabs	0/10	1/10	2/10	1/10	5/10	5/10	6/10	5/10	8/10	8/10	+	+
Dose site, red foci	0/10	1/10	1/10	1/10	3/10	0/10	4/10	1/10	5/10	2/10	+	–
Treated skin												
Histopathology												
acanthosis, 13 wk	1/10	0/10	10/10*	10/10*	10/10*	9/10*	10/10*	10/10*	10/10*	9/10*	+	+
acanthosis, 17 wk	1/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	–	–
hyperkeratosis, 13 wk	0/10	0/10*	3/10	8/10*	2/10	10/10*	3/10	8/10*	6/10	10/10*	+	+
hyperkeratosis, 17 wk	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	–	–
hypertrophy, glandular	1/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	–	–
Liver												
Organ weight												
relative., 13 wk	3.81	3.34	3.84	3.35	3.78	3.44	4.21	3.81*	4.83*	4.17	+	+
relative, 17 wk	3.54	3.11	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	3.56	3.33	–	–
Absolute, 13 wk	15.12	8.33	15.49	8.51	15.075	8.76	16.26	9.47*	19.36*	10.28*	+	+
absolute, 17 wk	14.89	7.87	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	14.00*	8.00	–	–
Histopathology												
hypertrophy, 13 wk	0/10	0/10	0/10	0/10	2/10	1/10	9/10*	2/10	10/10*	4/10	+	–
hypertrophy, 17 wk	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	–	–
Kidney												
Organ weight												
relative, 13 wk	0.85	0.82	0.84	0.80	0.83	0.77	0.97*	0.84	1.05*	0.85	+	–
relative17 wk	0.82	0.76	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	0.85	0.82*	–	–
absolute, 13 wk	3.34	2.04	3.36	2.03	3.32	1.95	3.75	2.09	4.20*	2.09	+	–
absolute, 17 wk	3.45	1.92	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	3.34	1.95	–	–
Histopathology												
degeneration, hyaline 13 wk	1/10	0/10	0/10	0/10	0/10	0/10	7/10*	0/10	8/10*	0/10	+	–
degeneration, hyaline 17 wk	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	–	–

* $p < 0.05$
[a] number of animals affected per ten animals
n.e. not examined

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Figure A6_4-1: Development of actual dermal dose in rats receiving 80 mg icaridin/kg bw/day

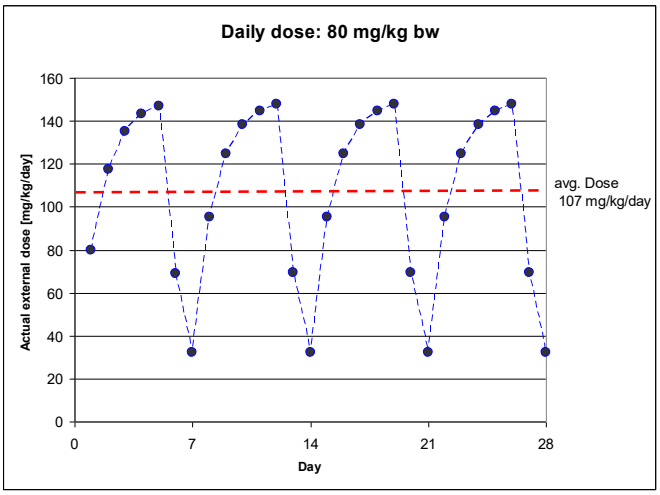
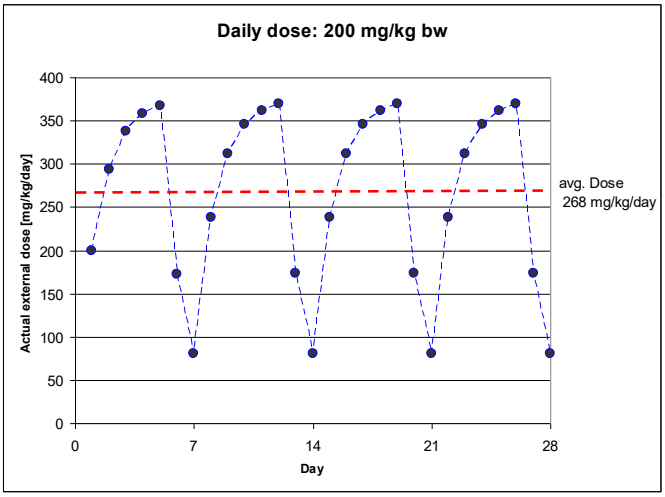


Figure A6_4-2: Development of actual dermal dose in rats receiving 200 mg icaridin/kg bw/day



Section 6.4.2 Repeated dose toxicity

Annex Point IIA VI.6.4.2 6.4.2 Thirteen-week dermal toxicity study in rats

Figure A6_4-3: Development of actual dermal dose in rats receiving 500 mg icaridin/kg bw/day

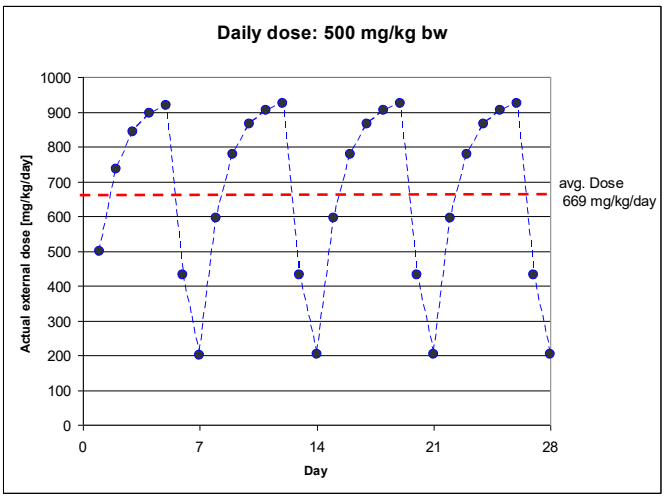
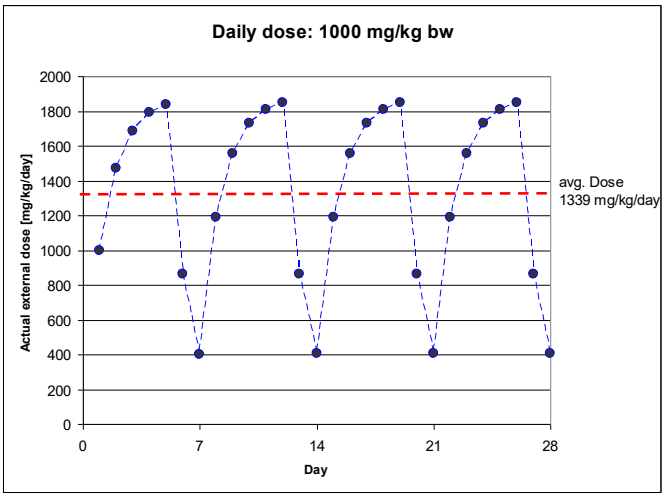


Figure A6_4-4: Development of actual dermal dose in rats receiving 1000 mg icaridin/kg bw/day



Section 6.4.3	Subchronic toxicity	
Annex Point IIA VI.6.4	**Subchronic inhalation toxicity study in rats**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [X]	**Other justification [ ]**	
Detailed justification:	A repeated-exposure inhalation study has not been conducted. Exposure towards Icaridin by inhalation is negligible compared to the main routes of exposure which are direct dermal contact and oral uptake via hand-to-mouth contact. Both relevant routes have been addressed with subchronic studies. Although the vapour pressure of Icaridin at 20°C is slightly higher than 1×10^{-2} Pa (3.4×10^{-2} Pa), exposure to Icaridin vapours is considered to be low because of the use pattern of Icaridin-containing products. Mosquito repellents will be used either outdoors or in well-ventilated rooms during the summer time. Exposure estimations (for details *cf.* Doc. II-B, Section 3.2) demonstrate that the systemic exposure via inhalation during pump spray application of Icaridin is only 0.0118 mg/kg bw/day for a 60-kg user. For comparison, the expected systemic exposures via the dermal and oral route are 0.59 and 0.65 mg/kg bw/day, respectively. This corresponds to less than 2% of each of the non-inhalational routes. Therefore, by submitting subchronic studies for the dermal and oral route, the relevant routes of exposure have been covered.	X
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	11 October 2006	
Evaluation of applicant's justification	Applicant’s justification is agreed upon. However, exposure estimations might be changed by CA.	
Conclusion	Applicant’s justification is acceptable	
Remarks		

Section 6.5 Chronic toxicity

Annex Point IIA VI.6.5 6.5 One-year dermal toxicity study in dogs

Official use only

1 REFERENCE

1.1	**Reference**	[redacted] (1995), Technical Grade KBR 3023: A Chronic Percutaneous Toxicity Study in the Beagle Dog. [redacted] Report No. 107155, 1995-12-01 (unpublished)
1.2	**Data protection**	Yes
1.2.1	Data owner	Saltigo GmbH
1.2.2	Company with letter of access	–
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes, compliance with the following guidelines was claimed: US-EPA FIFRA §§83-1 and 82-2, November 1984 US-EPA-TSCA Section 798.3320, July 1989 OECD Guideline 453, May 1981 Japanese MAFF, 59 NohSan No. 4200, January 1985 Deviating from the guideline statement, the study was found to comply with OECD Guideline 452 (May 1981). The claim of compliance with OECD 453 can be considered erroneous.
2.2	**GLP**	Yes
2.3	**Deviations**	No deviations from OECD 452 were noted.

3 MATERIALS AND METHODS

3.1	**Test material**	As given in Section 2 of dossier.
3.1.1	Lot/Batch number	PT030693
3.1.2	Specification	As given in Section 2 of dossier.
3.1.2.1	Description	Clear, viscous liquid
3.1.2.2	Purity	98.1%
3.1.2.3	Stability	Stability was confirmed by analyses at study initiation and termination.
3.2	**Test Animals**	
3.2.1	Species	Dog
3.2.2	Strain	Beagle
3.2.3	Source	White Eagle Laboratories, Doylestown, PA, USA
3.2.4	Sex	Males and females
3.2.5	Age/weight at study initiation	23-25 weeks ♂: 7.7-10.0 kg; ♀: 5.9-8.2 kg
3.2.6	Number of animals	4 per sex and group

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	per group	
3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	Dermal
3.3.1	Duration of treatment	1 year
3.3.2	Frequency of exposure	Five days per week
3.3.3	Post-exposure period	None
3.3.4	Dermal	
3.3.4.1	Area covered	10% of body surface
3.3.4.2	Occlusion	Open, without dressing. The test substance was applied to a nuchal crest-interscapular site that was out of reach for licking.
3.3.4.3	Vehicle	Undiluted
3.3.4.4	Concentration in vehicle	–
3.3.4.5	Dose levels	50, 100, 200 mg Icaridin/kg bw/day
3.3.4.6	Duration of exposure	24 h
3.3.4.7	Removal of test substance	No
3.3.4.8	Controls	Yes, untreated
3.4	**Examinations**	
3.4.1	Observations	
3.4.1.1	Clinical signs	Yes, once daily
3.4.1.2	Mortality	Yes, once daily
3.4.2	Body weight	Yes, once weekly
3.4.3	Food consumption	Yes, once daily
3.4.4	Water consumption	No
3.4.5	Ophthalmoscopic examination	Yes, pre-exposure, at 3, 6, and 9 months, and just prior to study termination.
3.4.6	Haematology	Yes, all animals. Twice prior to dosing, at 30, 60, 90, 180, 270 and 365 days of the study. Parameters: haematocrit, haemoglobin concentration, erythrocyte count, total and differential leukocyte count, platelet count, MCV, MCH, MCHC, erythrocyte morphology, reticulocyte count, Heinz bodies
3.4.7	Clinical chemistry	Yes, all animals. Twice prior to dosing, at 30, 60, 90, 180, 270 and 365 days of the study. Parameters: sodium, potassium, chloride, calcium, phosphorus, fasting

Section 6.5 Chronic toxicity

Annex Point IIA VI.6.5 6.5 One-year dermal toxicity study in dogs

		glucose, total cholesterol, total bilirubin, triglyceride, urea, uric acid, blood urea nitrogen, total bilirubin, creatinine, total protein, albumin, globulin, creatine kinase, alanine aminotransferase, aspartate amino-transferase, alkaline phosphatase, gamma-glutamyl transpeptidase, lactate dehydrogenase, T4, T3
3.4.8	Urinalysis	Yes, all animals. Twice prior to dosing, and at 30, 60, 90, 180, 270 and 365 days of exposure. Parameters: clarity, colour, osmolality, specific gravity, pH, protein, glucose, ketones, blood, bilirubin, urobilinogen, nitrite, leukocytes, microscopic observation of solids
3.5	**Sacrifice and pathology**	
3.5.1	Organ weights	Yes, all animals. Organs: liver, kidneys, adrenals, testes, uterus, ovaries, pituitary, spleen, brain, heart, lungs, thyroid
3.5.2	Gross and histopathology	Yes. Gross pathology: all animals; Organs: adrenals, aorta, bone, bone marrow, brain, cervix, clitoral gland, ears (internal structures), epididymides, eyes, 3rd eyelid/lachrymal gland, gall bladder, gonads, gross lesions, heart, joint (knee), kidneys, larynx, liver, lungs, lymph nodes, mammary gland, muscle, nasal structures, nasopharynx, oesophagus, pancreas, parathyroid, peripheral nerve, pituitary, prostate, salivary glands, seminal vesicles, skin, small and large intestines, spinal cord, spleen, stomach, thymus, thyroid, trachea, urinary bladder, uterus Histopathology: all animals Organs: as above, except: ears and nasal structures
3.5.3	Other examinations	Prior to commencement of dosing, neuroexamination of mental status/behaviour, gait characteristics, postural status and reactions, and spinal/cranial reflex tests were conducted on all animals. In addition, thoracic auscultation of heart and lungs was conducted, and rectal body temperature was measured. These examinations were repeated at 3 months and just prior to sacrifice. ECGs and blood pressure measurements were performed on all animals once prior to dosing, at 3, 6 and 9 months, and just prior to study termination. Determination of hepatic cytochrome P450 enzyme: cytochrome P450 protein, O-demethylase activity, N-demethylase activity
3.5.4	Statistics	Continuous data were analysed by ANOVA followed by Student's *t*-test if statistical differences were suggested. Frequency data: chi-square followed by Fisher's Exact test. All significant differences are reported at the 95% confidence level. A probability value of $p \leq 0.05$ was considered significant.
3.6	**Further remarks**	The initial duration of the study was 90 days, however, due to animal welfare considerations and the absence of toxicity during 90 days of exposure, the duration was extended to one year.

Section 6.5 Chronic toxicity

Annex Point IIA VI.6.5 6.5 One-year dermal toxicity study in dogs

		4 RESULTS AND DISCUSSION	
4.1	**Observations**		
4.1.1	Clinical signs	Treatment-related clinical signs were not observed.	
4.1.2	Mortality	No mortalities occurred.	
4.2	**Body weight gain**	No effects	
4.3	**Food consumption**	No effects	
4.4	**Ophtalmoscopic examination**	No effects	
4.5	**Blood analysis**		
4.5.1	Haematology	No compound-related effects	
4.5.2	Clinical chemistry	No compound-related effects	
4.5.3	Urinalysis	No compound-related effects	
4.6	**Sacrifice and pathology**		
4.6.1	Organ weights	No compound-related effects. There was an increase in the relative and absolute spleen weight however without statistically significance (see Table A6_4-2).	X
4.6.2	Gross and histopathology	Gross lesions: No significant difference between the incidence of gross lesions in control and treated animals was found. The most frequent lesions were discolouration of the lungs and raised zones on the spleen.	
		Histology: No compound-related lesions were noted.	
4.7	**Other**	Neurological examination: no clinical abnormalities	
		ECG/blood pressure measurements: no effects	
		Hepatic cytochrome P450: no compound-related effects	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The dermal subchronic toxicity of Icaridin to Beagle dogs was investigated in a one-year study using a 5-days-per-week application scheme. Compliance with OECD Guideline 453 “Combined Chronic Toxicity/Carcinogenicity Studies” was claimed, but the study methods are better described by OECD Guideline 452 “Chronic Toxicity Studies”.	
		Male and female Beagle dogs were treated for one year with undiluted Icaridin (0, 50, 100, 200 mg/kg bw/day) in a fashion that prevented oral uptake of the test material by the dogs. This was achieved by application of the test material to the nuchal crest/ interscapular area.	
		Body weights, feed consumption, clinical observations, ophthalomo-logical examinations, a battery of clinical pathology tests, terminal body and organ weights, gross pathology and histopathology evaluations were determined/performed. In addition, neurological as well as ECG/blood pressure testing was performed.	
5.2	**Results and**	There were no treatment-related observations or findings from the in-	

Section 6.5 Chronic toxicity

Annex Point IIA VI.6.5 6.5 One-year dermal toxicity study in dogs

	discussion	life phase, nor were there any gross pathology or histopathology observations at the doses of Icaridin tested.	
		The absence of signs of toxicity may lead to the conclusion that Icaridin is not systemically absorbed via skin. However, systemic uptake has been demonstrated in rats and humans. It can be surmised that absorption of Icaridin through dog skin is going to be at least as high as through human skin. This assumption is founded in the principle that hair follicles constitute a significant entry pore for lipophilic compounds. The follicle is coated with sebum along which the compound can migrate through the skin. In a furry animal like the dog, the follicle density is multiple times higher than in human skin (96 vs. 11 follicles per cm²).	X
		In humans, systemic exposure after dermal application has been demonstrated in a study on volunteers. A dermal absorption of 1.7% within 8 h was observed with undiluted Icaridin. In the present dog study, exposure times are practically infinite, since the application site is never cleared from the test substance before the next dose is applied. Assuming conservatively that the skin of dogs is equally permeable for undiluted Icaridin as is human skin, one can estimate that the penetration of Icaridin through dog skin within 24 h is about 6%.	
		The total absence of systemic effects at 200 mg/kg/day was also a result of the oral subchronic study in the rat. It thus appears that the NOAEL of 200 mg/kg/day in the chronic dog study is more indicative of the low toxicity of Icaridin than of insufficient exposure.	X
5.3	**Conclusion**	The NOAEL of 200 mg/kg/day is more than 10 times in excess of the expected external exposure of humans (~18 mg/kg/day).	X
		Considering that the dogs were exposed for one year without ever removing the material from the dose site, it can be concluded that the study allows an adequate hazard assessment for dermally applied Icaridin even if excessive use by humans is anticipated.	
5.3.1	LO(A)EL	LOAEL > 200 mg/kg bw/day, based on the lack of substance-related effects in the highest dose group	
5.3.2	NO(A)EL	NOAEL = 200 mg/kg bw/day	
5.3.3	Other	–	
5.3.4	Reliability	1	X
5.3.5	Deficiencies	No.	
		The study design deviates significantly from the cited OECD Guideline 453 and does not meet the requirements of a carcinogenicity study. However, the study was conducted in accordance with the relevant OECD Guideline 452 which applies to one-year toxicity studies. The study can therefore be considered valid for risk assessment in context of Annex Point IIA 6.4.2.	

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Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	22 November 2006
Materials and Methods	Applicant's version is adopted
Results and discussion	Point 4.6.1: There was an increase in the relative and absolute spleen weight however without statistically significance. The dermal absorption of the dog skin is unknown as no documentation/studies has been submitted on this issue which jeopardise the study as such as it is not known for certain that the substance is actually absorbed and no systemic effect were seen in the highest dose. If it is anticipated that there is no reasons to believe that the dog should be different from other species (with more or less hair) with respect to be ability to absorb substance over the skin the question is then would dermal absorption then be comparable to the mouse, rat or human. According to an article (Suzuki *et al.* 2001. Iontophoretic pulsatile transdermal delivery of human parathyroid hormone (1-34. Journal of Pharmacy and Pharmacology, 2001, 53:1227-1234) investigating transdermal absorption of human parathyroid in SD rats, hairless rats and beagle dogs, dogs had a lower absorption than the rat but higher than humans (based on a correlation between absorption rates and the ratios of skin porosity to the dermal thickness; transport believed to occur mainly via the hair-follicle route). Hence the dermal absorption in dogs would then be between 4-17% as this was the average dermal absorption for human and rats, respectively, exposed to 200 mg/kg bw/day. The systemic absorbed dose tested would then be 20 mg/kg bw/day (anticipating 10% dermal absorption for the dog). In general it must be emphasized that the highest tested dose of 200 mg/kg bw/day is too low to give information on the systemic inherent toxicological properties of the substance and this end point has therefore not been covered in this dossier. However it can be accepted that the dermal study will give information about the dermal toxicity of icaridin in sufficiently high doses (compare to doses used on humans) because the foreseeable route of systemic exposure for this dermally applied insect repellent is through dermal absorption. Correction: In the 14 weeks oral rat study the NOAEL were based on effects on liver and kidney. Since no oral ADME studies in rats have been performed a default of 100% oral absorption has been set. However it is not known if very limited oral absorption is responsible for the few systemic effects seen in the oral rat study.
Conclusion	-
Reliability	2
Acceptability	Acceptable

Section 6.5 Chronic toxicity

Annex Point IIA VI.6.5	6.5 One-year dermal toxicity study in dogs
Remarks	In the range finding study (28-Day Preliminary Dermal Study: KBR 3023 in one beagle dog) where dermal application of up to 750 mg/kg bw was tested in one female beagle dog during a 28 day period no systemic toxicity was noted. Neither was any analyses performed on systemic uptake from the skin of the dog.

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Annex Point IIA VI.6.5 6.5 One-year dermal toxicity study in dogs

Table A6_4-1. Results of clinical chemistry, haematology and urinalysis

No treatment-related changes were observed in any of the investigated parameters.

Table A6_4-2. Results of repeated dose toxicity study

Parameter	Control		50 mg/kg		100 mg/kg		200 mg/kg		Dose-response +/–	
	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m	f
Number of animals examined	4	4	4	4	4	4	4	4		
Body weight (1 yr) [% of control]	100.0	100.0	110.4	91.6	108.1	96.2	103.6	91.3	–	–
Bw gain (1 yr) [% of control]	100.0	100.0	143.6	74.6	135.3	105.5	127.3	89.1	–	–
Spleen										
abs. weight [% of control]	100.0	100.0	128.8	78.3	99.2	94.2	148.0	107.2	–	–
rel. weight [% of control]	100.0	100.0	123.1	86.6	95.4	104.7	147.6	122.5	–	–

* $p < 0.05$
[a] number of animals affected/total number of animals

Section A6.5 / A6.7 Chronic Toxicity / Carcinogenicity

Annex Points IIA VI.6.5/6.7	6.5/6.7 Combined chronic dermal toxicity/carcinogenicity study in the rat

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted]. (1996a), Technical Grade KBR 3023: A Combined Chronic Toxicity/Oncogenicity Testing Study in the Rat. [redacted] Report No. 107432, 1996-12-17, amended by Report No. 107432-1, 1997-05-12 (unpublished)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Saltigo GmbH	
1.2.2	Company with letter of access	–	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, compliance with the following guidelines was claimed: US-EPA FIFRA §83-5 US-EPA-TSCA Section 798.3320 OECD Guideline 453, May 1981 Japanese MAFF, 59 NohSan No. 4200	
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes, the following deviations from OECD 453 were noted: – Animals were > 6 weeks of age when dosing commenced. – Two-year survival was less than 50% in all groups. – The highest dose level caused no signs of toxicity.	X
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in Section 2 of dossier.	
3.1.1	Lot/Batch number	030693	
3.1.2	Specification	As given in Section 2 of dossier.	
3.1.2.1	Description	Clear liquid	
3.1.2.2	Purity	96.7–98.5%	X
3.1.2.3	Stability	Stability was confirmed by six separate analyses conducted prior to, during and after the exposure period.	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Sprague-Dawley	
3.2.3	Source	Charles River Breeders, Portage, MI, USA	

Section A6.5 / A6.7 Chronic Toxicity / Carcinogenicity

Annex Points IIA VI.6.5/6.7	6.5/6.7 Combined chronic dermal toxicity/carcinogenicity study in the rat

3.2.4	Sex	♂ + ♀
3.2.5	Age/weight at study initiation	8 weeks; ♂: ~182 g, ♀: ~150 g
3.2.6	Number of animals per group	
3.2.6.1	at interim sacrifice	One-year sacrifice group: 20 per sex in the control and high dose group 10 per sex in the low and intermediate dose groups.
3.2.6.2	at terminal sacrifice	Two-year sacrifice group: 50 per sex in all dose groups
3.2.7	Control animals	Yes
3.2.8	Replacement group	For approx. the first month of treatment, an additional 5 rats/dose/sex were placed on study as part of the 1- and 2-year sacrifice groups. These animals served as potential replacements for any animals that died or developed non-treatment-related problems very early in the study.
3.3	**Administration/ Exposure**	Dermal
3.3.1	Duration of treatment	104 weeks
3.3.2	Interim sacrifice(s)	After 52 weeks
3.3.3	Final sacrifice	After 104 weeks
3.3.4	Frequency of exposure	5 days/week
3.3.5	Postexposure period	None
		Dermal
3.3.6	Area covered	10% of body surface
3.3.7	Occlusion	Non-occlusive, without dressing. Elizabethan collars were used to prevent oral uptake of the test material.
3.3.8	Vehicle	Undiluted
3.3.9	Concentration in vehicle	–
3.3.10	Dose levels	50, 100, 200 mg Icaridin/kg bw/day
3.3.11	Duration of exposure	24 h
3.3.12	Removal of test substance	No
3.3.13	Controls	Yes, shaved but untreated
3.4	**Examinations**	
3.4.1	Body weight	Yes, once weekly
3.4.2	Food consumption	Yes, once weekly

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3.4.3	Water consumption	No
3.4.4	Clinical signs	Yes, twice daily (once on weekends and holidays)
3.4.5	Macroscopic investigations	Palpable masses, external surface areas, orifices, posture, general behaviour, respiration, excretory products
3.4.6	Ophthalmoscopic examination	Yes, pre-exposure and prior to sacrifice
3.4.7	Haematology	Yes
	No. of animals:	20 animals/sex/dose of the 2-year sacrifice group
	Time points:	After 3, 6, 12, 18, 24 months of treatment
	Parameters:	Haematocrit, haemoglobin concentration, erythrocyte count, total and differential leukocyte count, platelet count, MCV, MCH, MCHC, Heinz bodies, reticulocyte counts, erythrocyte morphology
3.4.8	Clinical Chemistry	Yes
	No. of animals:	20 animals/sex/dose of the 2-year sacrifice group
	Time points:	After 3, 6, 12, 18, 24 months of treatment
	Parameters:	Sodium, potassium, chloride, calcium, phosphorus, fasting glucose, total cholesterol, bilirubin, triglyceride, urea, uric acid, blood urea nitrogen, total bilirubin, creatinine, total protein, albumin, globulin, creatine kinase, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma-glutamyl transpeptidase, lactate dehydrogenase
3.4.9	Urinalysis	Yes
	No. of animals:	20 animals/sex/dose of the 2-year sacrifice group
	Time points:	After 3, 6, 12, 18, 24 months of treatment
	Parameters:	Clarity, colour, specific gravity, pH, protein, glucose, ketones, blood, bilirubin, urobilinogen, nitrite, microscopic observation of solids
3.4.10	Pathology	Yes
3.4.10.1	Organ Weights	Yes
	from:	All surviving animals at interim + terminal sacrifice
	Organs:	Liver, kidneys, adrenals, testes, ovaries, spleen, brain, heart, lungs
3.4.11	Histopathology	Yes
	from:	All surviving animals at interim + terminal sacrifice
	Organs:	Adrenals, aorta, bone, bone marrow, brain, cervix, epididymides, eyes, gonads, gross lesions, Harderian gland, heart, joint (knee), kidneys, larynx, liver, lungs, lymph nodes, mammary gland, muscle, oesophagus, pancreas, parathyroid, peripheral nerve, pituitary, prostate, salivary glands, seminal vesicles, skin, skull, small and large intestines, spinal cord, spleen, stomach, thymus, thyroid, trachea, urinary bladder, uterus, Zymbal's gland
3.4.12	Other examinations	–

Section A6.5 / A6.7 Chronic Toxicity / Carcinogenicity

Annex Points IIA VI.6.5/6.7	6.5/6.7 Combined chronic dermal toxicity/carcinogenicity study in the rat
3.5 Statistics	Continuous data: Evaluation of homogeneity of variance was done with Bartlett's test. If homogenous, group means were analysed by one-way ANOVA followed by Dunnett's test. In the event of unequal variances, data were analysed by Kruskal-Wallis ANOVA followed by the Mann-Whitney-U test. Frequency data: chi-square and Fisher exact test. For the Bartlett test, $p \leq 0.001$ was considered significant; for all other tests, $p \leq 0.05$ was considered statistically significant.
3.6 Further remarks	–

Section A6.5 / A6.7 Chronic Toxicity / Carcinogenicity

Annex Points IIA VI.6.5/6.7 6.5/6.7 Combined chronic dermal toxicity/carcinogenicity study in the rat

		4 RESULTS AND DISCUSSION	
4.1	**Body weight**	No effects	
4.2	**Food consumption**	No effects	
4.3	**Water consumption**	Not assessed	
4.4	**Clinical signs**	There were no treatment-related clinical signs.	
4.5	**Macroscopic investigations**	No compound-related abnormalities were noted. Mortality was unaffected by administration of Icaridin (see Table A6_7-2). Non-healing open wounds developed around the collar area in ~20% of the male animals in all dose groups. This problem was managed by removal of the collar for up to 3 weeks and coating of the affected areas with corn oil. Animals of the 2-year sacrifice group that continued to be affected were sacrificed at 1 year in lieu of the corresponding animals of the 1-year sacrifice group which in turn became part of the 2-year sacrifice groups. Problems also developed with lesions affecting the hind feet of ~40% of male rats. All dose groups and controls had similar incidences of this lesion that was therefore considered not compound-related. Resolution of this problem was attempted by moving the affected animals from wire-bottom cages to flat-bottom cages with bedding, but the bedding material contributed to the irritation of the wounds and impaired the healing process. Finally, the situation was greatly improved by a 2-week, daily treatment regimen with an anti-inflammatory ointment.	
4.6	**Ophthalmoscopic examination**	No effects	
4.7	**Haematology**	No effects	
4.8	**Clinical Chemistry**	No effects	
4.9	**Urinalysis**	No effects	
4.10	**Pathology**	No effects	
4.10.1	Organ Weights	No effects	
4.10.2	Histopathology	One year At ≥ 50 mg/kg: Treated skin: acanthosis, hyperkeratosis (♂) Two years: At ≥ 50 mg/kg: Treated skin: acanthosis, hyperkeratosis (♀) At 200 mg/kg: Treated skin: acanthosis, hyperkeratosis (♂) Liver: increased incidence of cystic degeneration (♂); this lesion was considered of no toxicological significance since it occurs spontaneously in ageing rats at a low incidence. The seeming dose-relation is probably a coincidence. Liver weight or functional parameters were not altered.	X

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4.10.3 Time to tumours	No evidence of chemically induced neoplastic lesions in any tissue at any dosage.	
4.11 Other	–	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	The dermal chronic toxicity and oncogenicity of Icaridin to Sprague-Dawley rats was investigated in a 2-year study with a 1-year interim sacrifice using a 5-days-per-week application scheme. The study was performed according to OECD Guideline 453. Male and female Sprague-Dawley rats were treated for up to two years with undiluted Icaridin (0, 50, 100, 200 mg/kg bw/day). Elizabethan collars were used to prevent ingestion of the test compound. Animals were sacrificed after one or two years of exposure. Body weights, feed consumption, clinical observations, ophthalmological examinations, a battery of clinical pathology tests, terminal body and organ weights, gross pathology and histopathology evaluations were determined/performed.	
5.2 Results and discussion	Lesions of the treated skin (acanthosis and hyperkeratosis) were the only clearly treatment-related alterations that were observed. These lesions are common findings with repeated exposure to a variety of treatments (including water or medical petrolatum) and are considered to be of no toxicological relevance. Microscopic liver lesions (increased incidence of cystic degeneration) at the highest dose level (200 mg/kg bw/day) occurred in male rats with an apparent dose-related incidence (see Table A6_7-2). However, the severity of the lesions did not correlate with the dose. It is doubtful that the lesion is substance-related since it occurs spontaneously in ageing rats at a low incidence. The seeming dose-relation is probably a coincidence. The fact that the lesion was not noted at the interim sacrifice clearly points towards a geriatric effect. Although the liver is a target organ for Icaridin in the rat, the liver effects noted in the subchronic dermal study in rats (hypertrophy, weight increase at ≥ 500 mg/kg/day) do not resemble the findings in the 2-yr sacrifice males. The cystic degeneration in 2-yr males was not accompanied by hepatocellular hypertrophy which seems to be the most sensitive indicator for a hepatic response towards Icaridin. Cystic degeneration in ageing male rats is therefore unlikely to be related to hepatic findings detected in the short-term studies in rats. In addition, one can state that the cystic degeneration is not an adverse effect. First, liver weight or functional parameters were not altered. One definition of an adverse effect includes the criteria that the ability of the organism to maintain homeostasis will be impaired or that the susceptibility of the organism to the deleterious effects of other environmental influences is enhanced. This is apparently not the case as is underscored by the fact that the mortality rate was not significantly higher in high-dose males. Secondly, an effect only occurring at the highest dose in one sex of one species is not relevant for human risk assessment, especially since the	X

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		rat has a known sensitivity for liver alterations. The increased incidence of hepatic cystic degeneration in high-dose males should thus be disregarded for LOAEL/NOAEL setting. There were no treatment-related neoplasias in any organ or tissue of any dose group.	
5.3	**Conclusion**		
5.3.1	LO(A)EL	LOAEL > 200 mg/kg bw/day, based on the absence of treatment-related adverse effects	X
5.3.2	NO(A)EL	NOAEL = 200 mg/kg bw/day	X
5.3.3	Other	–	
5.3.4	Reliability	2	
5.3.5	Deficiencies	Yes. Dosing was commenced when animals were 8 weeks of age. This is 2 weeks older than recommended but necessary to obtain animals of sufficient size to facilitate the conduct of the test. The two-year survival was less than 50% in all dose groups. Nevertheless, none of the animals that died during the second year of treatment were lost for pathological analysis. The highest dose (200 mg/kg bw/day) did not cause signs of toxicity. The subchronic dermal toxicity study in rats demonstrated that doses greater than 200 mg/kg would spread out beyond the intended area of exposure and would therefore be difficult to interpret. The selection of the highest dose level was made after consultations with US-EPA toxicologists. These deficiencies do not affect the overall validity of the study.	X

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Annex Points IIA VI.6.5/6.7	6.5/6.7 Combined chronic dermal toxicity/carcinogenicity study in the rat

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	12 December 2010
Materials and Methods	2.3 Since NOAEL and LOAEL values are changed this deviation is no longer applicable.
Results and discussion	Revised version: 4.12. One year At ≥ 50 mg/kg: Treated skin: acanthosis, hyperkeratosis (♂) Two years: At ≥ 50 mg/kg: Treated skin: acanthosis, hyperkeratosis (♀) At 200 mg/kg: Treated skin: acanthosis, hyperkeratosis (♂and ♀) Liver: increased incidence of cystic degeneration (♂) with clear dose response. Liver weight or functional parameters were not altered. 5.2 Lesions of the treated skin (acanthosis and hyperkeratosis) were clearly treatment-related; Especially the acanthosis was dose dependent and occurred at high level in the highest dose group. These lesions are common findings with repeated exposure to a variety of treatments (including water or medical petrolatum) and are in general then not considered to be of no toxicological relevance. However since the intended use involves repeated dermal exposure, the effect on the skin could therefore be relevant. Liver lesions (increased incidence of cystic degeneration) at the highest dose level (200 mg/kg bw) occurred in male rats with a dose response relationship. There were no treatment-related neoplasias in any organ or tissue of any dose group.

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Annex Points IIA VI.6.5/6.7	6.5/6.7 Combined chronic dermal toxicity/carcinogenicity study in the rat
Conclusion	5.3.1 LOAEL 200 mg/kg bw/day, based on liver lesions (cystic degeneration) in male rats. LOAEL >200 mg/kg bw/day based on no observed effects in female rats. 5.3.2 NOAEL 100 mg/kg bw/day for male rats. NOAEL 200 mg/kg bw/day for females rats. NOAEL for local effects on the application site <50 mg/kg bw/day Other conclusions: Liver lesions (increased incidence of cystic degeneration) at the highest dose level (200 mg/kg bw/day) occurred in male rats with a dose response relationship. The toxicological significance of this finding is unknown. The liver weight or related functional parameters were not affected in this study. However, it cannot be excluded that the dose-related increased incidence of cystic degeneration is treatment related. Should the effect be solely related to the aging of the rats it seems peculiar that after 2 years only 8/47 of controls were effects compared to 20/47 of the high dose males. A comparison of human relevance for rat specific effects in general has no relevance when setting NOAEL values for the investigated species in the respective studies. The relevance of the species specific effects for humans are then taken into account in the human risk assessments. There were no treatment-related neoplasias in any organ or tissue of any dose group. 5.3.5. As NOAEL and LOAEL settings are changed, this deficiency is no longer applicable.
Reliability	2
Acceptability	Acceptable
Remarks	Point 3.1.2.2. The lowest purity in the study is below the declared purity specifications of substance. Point 4.12. The increased incidence of cystic degeneration with dose relationship cannot be disregarded and may be treatment related. Point 5.3.5. The slightly lower survival (<50%) of the animals by the end of the study is not considered to trigger a demand for new studies.

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Annex Points IIA VI.6.5/6.7	6.5/6.7 Combined chronic dermal toxicity/carcinogenicity study in the rat

Table A6_7-1. Table for clinical chemistry, haematology and urinalysis

No treatment-related changes were observed in any of the investigated parameters.

Table A6_7-2. Results of carcinogenicity study

Parameter	Control		50 mg/kg		100 mg/kg		200 mg/kg		Dose-response + / –	
	♂[a]	♀[a]	♂	♀	♂	♀	♂	♀	♂	♀
Number of animals examined	50	50	50	50	50	50	50	50		
Mortality	27/50 (54%)	30/50 (60%)	32/50 (64%)	25/50 (50%)	27/50 (54%)	32/50 (64%)	32/50 (64%)	33/50 (66%)	–	–
First year	3/50	1/50	4/50	2/50	1/50	2/50	3/50	3/50	–	–
Second year	24/47	29/49	28/46	23/48	26/49	30/48	29/47	30/47	–	–
Body weight [% of control]										
54 week sacrifice	100.0	100.0	102.6	98.0	100.5	98.7	98.0	97.9	–	–
104 week sacrifice	100.0	100.0	101.6	103.4	105.6	103.5	102.2	107.5	–	–
Treated skin (2-yr) Histopathology										
acanthosis	0/47	1/49	5/46*	4/48	4/49	6/48*	16/47*	12/47*	+	+
hyperkeratosis	22/47	3/49	24/46	10/48*	32/49	10/48*	37/47*	22/47*	+	+
Liver										
degeneration, cystic										
First year (avg. severity score)	0/20 (–)	0/19 (–)	0/10 (–)	0/10 (–)	0/10 (–)	0/9 (–)	0/18 (–)	0/20 (–)	–	–
Second year (avg. severity score)	8/47 (2.3)	1/49 (2.0)	11/46 (2.0)	1/48 (3.0)	14/49 (2.1)	1/48 (2.0)	20/47* (2.4)	2/47 (2.0)	+ –	– –
There were no treatment-related effects on tumour incidence in any group.										

[a] Number of animals affected/number of animals investigated
* $p < 0.05$

Section A6.6.1 Genotoxicity in vitro

Annex Point IIA VI.6.6.1 6.6.1 Mutagenicity testing in bacteria – Salmonella/microsome test

			Official use only
		1 REFERENCE	
1.1	**Reference**	Herbold, A. (1990), KBR 3023 – Salmonella/ Microsome Test. Bayer AG, Toxicology, Wuppertal, Germany, Report No. 18917, 1990-03-16 (unpublished)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Saltigo GmbH	
1.2.2	Company with letter of access	–	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No guideline statement Methods used were in accordance with OECD Guideline 471.	X
2.2	**GLP**	Yes	
2.3	**Deviations**	None	X
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in Section 2 of dossier.	
3.1.1	Lot/Batch number	19009/89	
3.1.2	Specification	As given in Section 2 of dossier.	
3.1.2.1	Description	Clear viscous liquid	
3.1.2.2	Purity	99.1%	
3.1.2.3	Stability	Until January 24, 1990 Icaridin in the vehicle was stable at room temperature for at least 4 h.	
3.2	**Study Type**	Bacterial reverse mutation test	
3.2.1	Organism/cell type	S. typhimurium: TA 1535, TA 1537, TA 98, TA 100	
3.2.2	Deficiencies / Proficiencies	Histidine auxotroph	
3.2.3	Metabolic activation system	S9 mix from livers of Aroclor-1254-treated Sprague-Dawley rats (single i.p. injection, 500 mg/kg bw, 5 days prior to sacrifice)	
3.2.4	Positive control	TA 1535: sodium azide, 10 µg/plate (–S9) TA 100: nitrofurantoin, 0.2 µg/plate (–S9), TA 1537, TA 98: 4-nitro-1,2-phenylene diamine, 0.5 µg/plate (–S9) All strains: 2-aminoanthracene, 3 µg/plate (+S9)	
3.3	**Application of test substance**		
3.3.1	Concentrations	0, 8, 40, 200, 1000, 5000 µg/plate	
3.3.2	Way of application	Icaridin was dissolved in ethanol	

Section A6.6.1 Genotoxicity in vitro

Annex Point IIA VI.6.6.1 6.6.1 Mutagenicity testing in bacteria – Salmonella/microsome test

3.3.3	Pre-incubation time	Direct plate incorporation	
3.3.4	Other modifications	–	
3.4	**Examinations**	See tables in appendix for examinations and results.	
		4 RESULTS AND DISCUSSION	
4.1	**Genotoxicity**		
4.1.1	without metabolic activation	No	
4.1.2	with metabolic activation	No	
4.2	**Cytotoxicity**	Yes, at 5000 µg/plate	X
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Icaridin was investigated using the Salmonella/microsome test for point mutagenic effects at doses up to 5000 µg/plate using direct plate incorporation.	
		The test was performed according to OECD Guideline 471, although this compliance was not stated in the study report.	X
		Two independent tests were performed in all four tester strains. The incubation time was 48 h at 37°C. Strain-specific positive controls were assessed concurrently.	
		A reproducible and dose-related increase in mutant counts of at least one strain is considered a positive result. A doubling (tripling for TA 1537) of control revertant counts should be reached.	
5.2	**Results and discussion**	None of the tester strains showed a dose-related and biologically relevant increase in revertant counts over those of the negative controls both in the presence or absence of S9 mix	
		There was no indication for a cytotoxic effect of Icaridin except for a weak, strain-specific bacteriotoxicity at 5000 µg/plate.	X
5.3	**Conclusion**	Icaridin is non-mutagenic in the Salmonella/microsome test with and without metabolic activation.	
5.3.1	Reliability	1	X
5.3.2	Deficiencies	No	

Section A6.6.1 Genotoxicity in vitro

Annex Point IIA VI.6.6.1 6.6.1 Mutagenicity testing in bacteria – Salmonella/microsome test

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	12 November 2006
Materials and Methods	
Results and discussion	Revisions, inclusions or suggested changes in text in italics. 4.2 Yes, bacteriotoxicit*y* at 5000 µg/plate 5.1 §2 The test was performed according to OECD Guideline 471 *(1983),* although this compliance was not stated in the study report. 5.2 §2 The only cytotoxic effect of Icaridin was weak bacteriotoxicity at the highest dose 5000 µg/plate (+S9 mix) that did not impair reading of the plates.
Conclusion	Applicant's version is adopted
Reliability	2
Acceptability	Acceptable
Remarks	2.1 Methods used were in accordance with OECD Guideline 471 (1983). 2.3 Deviations from current guideline OECD 471(1997): Only four bacterial strains were used. Fifth strain (e.g. *E.coli* WP2 or *E.coli* WP2 (pKM101) or *S. typhimurium* TA102 to detect cross-linking mutagens is missing.

Section A6.6.1 Genotoxicity in vitro

Annex Point IIA VI.6.6.1 6.6.1 Mutagenicity testing in bacteria – Salmonella/microsome test

Table A6_6_1-1: Table for gene mutation assay, first test

Concentration [µg/plate]	Number of mutant cells (average number of revertants per plate ± SD, n=4)							
	TA 1535		TA 100		TA 1537		TA 98	
	–S9	+S9	–S9	+S9	–S9	+S9	–S9	+S9
0	15 ± 4	29 ± 8	93 ± 11	91 ± 12	6 ± 1	7 ± 1	13 ± 3	26 ± 1
8	15 ± 2	20 ± 4	81 ± 10	82 ± 12	6 ± 2	5 ± 3	16 ± 5	28 ± 4
40	21 ± 8	22 ± 3	89 ± 10	79 ± 2	5 ± 2	7 ± 2	15 ± 5	24 ± 4
200	14 ± 4	18 ± 2	91 ± 8	83 ± 15	6 ± 2	5 ± 3	12 ± 2	23 ± 4
1000	15 ± 2	25 ± 5	75 ± 12	89 ± 18	5 ± 1	6 ± 3	18 ± 3	18 ± 9
5000	12 ± 3	17 ± 3 [a]	64 ± 7	34 ± 17 [a]	3 ± 1	3 ± 1 [a]	8 ± 2	14 ± 3 [a]
Positive control	1235 ±39*	86 ±25*	295 ± 17*	370 ± 19-	37 ± 9*	33 ± 3*	73 ± 14*	367 ± 20*

[a] bacteriotoxic effect observed by titre determination
* mutagenic effect

Table A6_6_1-2: Table for gene mutation assay, second test

Concentration [µg/plate]	Number of mutant cells (average number of revertants per plate ± SD, n=4)							
	TA 1535		TA 100		TA 1537		TA 98	
	–S9	+S9	–S9	+S9	–S9	+S9	–S9	+S9
0	9 ± 4	12 ± 4	56 ± 10	61 ± 13	6 ± 4	6 ± 1	14 ± 3	20 ± 3
8	6 ± 2	11 ± 3	62 ± 10	52 ± 10	4 ± 1	7 ± 3	14 ± 2	18 ± 5
40	8 ± 1	13 ± 2	51 ± 14	49 ± 10	5 ± 3	5 ± 3	13 ± 3	21 ± 5
200	10 ± 2	10 ± 4	55 ± 10	47 ± 13	6 ± 4	7 ± 2	13 ± 4	23 ± 5
1000	9 ± 3	14 ± 3	49 ± 4	50 ± 9	4 ± 1	4 ± 2	16 ± 3	19 ± 4
5000	5 ± 1	7 ± 2	51 ± 7	58 ± 4 [a]	3 ± 1	3 ± 1 [a]	6 ± 3	18 ± 7 [a]
Positive control	1191 ±40*	47 ± 6*	200 ± 20*	439 ± 72*	45 ± 7*	64 ± 7*	61 ± 12*	479 ± 86*

[a] bacteriotoxic effect observed by titre determination
* mutagenic effect

Section A6.6.2 Genotoxicity in vitro

Annex Point IIA VI.6.6.2 6.6.2 *In-vitro* mammalian chromosome aberration test in CHO cells (1)

Official use only

		1 REFERENCE
1.1	**Reference**	Gahlmann, R. (1996), KBR 3023 – In Vitro Mammalian Chromosome Aberration Test with Chinese Hamster Ovary (CHO) Cells. Bayer AG, Toxicology, Wuppertal, Germany, Report No. 25019, 1996-04-30 (unpublished)
1.2	**Data protection**	Yes
1.2.1	Data owner	Saltigo GmbH
1.2.2	Company with letter of access	–
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes OECD Guideline 473 (May 1983) US-EPA FIFRA §84-2 (July 1986)
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	190012/89
3.1.2	Specification	As given in Section 2 of dossier.
3.1.2.1	Description	Clear viscous liquid
3.1.2.2	Purity	99.5%
3.1.2.3	Stability	Until May 3, 1993 Stability of Icaridin in the vehicle was confirmed.
3.2	**Study Type**	*In-vitro* mammalian chromosome aberration test
3.2.1	Organism/cell type	Chinese hamster ovary (CHO)
3.2.2	Metabolic activation system	S9 mix from livers of Aroclor-1254-treated Wistar rats.
3.2.3	Positive control	–S9: mitomycin C (0.08 – 1.0 µg/mL) +S9: cyclophosphamide (5.0 – 25.0 µg/mL)

Section A6.6.2 Genotoxicity in vitro

Annex Point IIA VI.6.6.2 6.6.2 *In-vitro* mammalian chromosome aberration test in CHO cells (1)

3.3	**Application of test substance**	
3.3.1	Concentrations	First test: –S9: 0, 4, 20, 100 µg/mL +S9: 0, 100, 700, 1400 µg/mL Second test: –S9: 0, 400, 800, 1200 µg/mL +S9: 0, 800, 1200, 1600 µg/mL
3.3.2	Way of application	Dissolved in ethanol
3.3.3	Exposure times	2, 7, 18, 27 h
3.3.4	Harvest times	7, 18, 27 h after initiation of exposure
3.4	**Examinations**	See tables in appendix for examinations and results
3.4.1	Number of cells evaluated	200 per culture

4 RESULTS AND DISCUSSION

4.1	**Genotoxicity**	
4.1.1	without metabolic activation	Yes at ≥ 4 µg/mL
4.1.2	with metabolic activation	Yes at ≥ 1200 µg/mL
4.2	**Cytotoxicity**	Yes –S9: at ≥ 4 µg/mL, ≥ 7 h exposure time +S9: at ≥ 700 µg/mL, ≥ 18 h exposure time

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The clastogenic potential of Icaridin was evaluated in a chromosome aberration test in Chinese hamster ovary (CHO) cells according to OECD Guideline 473. In a first test, CHO cells were continuously exposed for 7, 18, and 27 h to concentrations of 4, 20, and 100 µg/mL in the absence of S9 mix. In the presence of S9 mix, cells were treated for 2 h with 100, 700 and 1400 µg/mL. In both cases, with and without S9 mix, cells were harvested 7, 18 or 27 h after beginning of the treatment. In a repeat test, cells were treated with Icaridin for 2 h at concentrations of 400, 800 and 1200 µg/mL (–S9) and 800, 1200, 1600 µg/mL (+S9). Cells were then harvested 18 or 27 h after beginning of exposure.

Section A6.6.2 Genotoxicity in vitro

Annex Point IIA VI.6.6.2 6.6.2 *In-vitro* mammalian chromosome aberration test in CHO cells (1)

5.2	**Results and discussion**	Significant dose-dependent cytotoxicity was induced by Icaridin treatment, both with and without S9 mix. In the repeat test, a significant number of metaphases with chromosome disintegration were detected among cells that had been treated with 1600 µg/mL in the presence of S9 mix. There were clear dose-related increases of numbers of metaphases with aberrations (including and excluding gaps) among cells treated with 1400 µg/mL in the presence of S9 mix. Cells harvested after 18 and 27 h revealed this effect. Increased numbers of aberrations were also observed in the absence of S9 mix but effects were not dose-dependent. In the repeat test, increased numbers of chromosome aberrations were induced by the highest doses of Icaridin with and without S9 mix, 1600 and 1200 µg/mL, respectively. Thus, Icaridin induced chromosome aberrations at cytotoxic concentrations, with and without metabolic activation. The positive controls, mitomycin C and cyclophosphamide, were clearly clastogenic demonstrating the sensitivity of the test system.
5.3	**Conclusion**	Icaridin induced chromosome aberrations in CHO cells at cytotoxic concentrations, with and without metabolic activation
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Section A6.6.2 Genotoxicity in vitro

Annex Point IIA VI.6.6.2 6.6.2 *In-vitro* mammalian chromosome aberration test in CHO cells (1)

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	1 November 2006/revised according to WGI 2017 decision
Materials and Methods	Applicant's version can be adopted when positive control data have been included in the reporting (in tables A6_6_2-1 to A6_6_2-7)
Results and discussion	Applicant's version is adopted
Conclusion	*Icaridin induced chromosome aberrations in CHO cells with and without metabolic activation*
Reliability	1
Acceptability	Acceptable
Remarks	Positive control data has been included in tables A6_6_2-1 to A6_6_2-7. -S9: positive at ≥4 µg/mL +S9: positive at ≥1200 µg/mL Cytotoxicity with mitotic index < 50% of control: –S9: at ≥ 50 µg/mL, at 27 h exposure time +S9: at ≥1400 µg/mL, ≥7 h exposure time

Commented [ABP1]: Has been corrected according WG discussion and decision

Section A6.6.2 Genotoxicity in vitro

Annex Point IIA VI.6.6.2 6.6.2 *In-vitro* mammalian chromosome aberration test in CHO cells (1)

Table A6_6_2-1 First pre-test for cytotoxicity

Concentration	S9 Mix	Length of Treatment*	Harvest Time*	Mitotic Index[1]
0.2	-	7	7	87
2.0	-	7	7	78
20.0	-	7	7	74
200.0	-	7	7	37
2000.0	-	7	7	< 1
0.2	+	2	7	104
2.0	+	2	7	96
20.0	+	2	7	65
200.0	+	2	7	110
2000.0	+	2	7	< 1
0.2	-	18	18	47
2.0	-	18	18	47
20.0	-	18	18	50
200.0	-	18	18	40
2000.0	-	18	18	< 1
0.2	+	2	18	125
2.0	+	2	18	117
20.0	+	2	18	92
200.0	+	2	18	118
2000.0	+	2	18	< 1
0.2	-	27	27	60
2.0	-	27	27	65
20.0	-	27	27	79
200.0	-	27	27	16
2000.0	-	27	27	< 1
0.2	+	2	27	75
2.0	+	2	27	53
20.0	+	2	27	47
200.0	+	2	27	47
2000.0	+	2	27	< 1

*in hours
[1] relative to solvent controls (%)

Section A6.6.2 Genotoxicity in vitro

Annex Point IIA VI.6.6.2 6.6.2 *In-vitro* mammalian chromosome aberration test in CHO cells (1)

Table A6_6_2-2 Second pre-test for cytotoxicity

Concentration	S9 Mix	Length of Treatment*	Harvest Time*	Mitotic Index[1]
2	-	7	7	62
10	-	7	7	52
50	-	7	7	40
100	+	2	7	102
200	+	2	7	71
400	+	2	7	98
800	+	2	7	< 1
2	-	18	18	17
10	-	18	18	21
50	-	18	18	12
100	+	2	18	82
200	+	2	18	80
400	+	2	18	82
800	+	2	18	22
2	-	27	27	79
10	-	27	27	67
50	-	27	27	12
100	+	2	27	131
200	+	2	27	106
400	+	2	27	131
800	+	2	27	74

*in hours
[1] relative to solvent controls (%)

Table A6_6_2-3 Third pre-test for cytotoxicity

Concentration	S9 Mix	Length of Treatment*	Harvest Time*	Mitotic Index[1]
400	-	2	18	115
800	-	2	18	77
1200	-	2	18	67
1600	-	2	18	< 1
2000	-	2	18	< 1

*in hours
[1] relative to solvent controls (%)

Section A6.6.2 Genotoxicity in vitro

Annex Point IIA VI.6.6.2 6.6.2 *In-vitro* mammalian chromosome aberration test in CHO cells (1)

Table A6_6_2-4 Table for cytogenetic *in-vitro* test: chromosomal analysis (continuous Icaridin exposure until harvest, without activation)

		Mitomycin C 1 µg/mL			Solvent control			Icaridin [µg/mL]								
								4			20			100		
Time of harvest [h]		**7**	**18**	**27**	**7**	**18**	**27**	**7**	**18**	**27**	**7**	**18**	**27**	**7**	**18**	**27**
Cytotoxicity (mitotic indices)		Yes	Yes	Yes	No	No	No	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes
Chromatid aberrations	gaps	23 [a]	4	17	4	7	5	8	4	12	8	8	6	10	14	12
	breaks	5	8	13	0	0	0	2	3	4	1	0	1	3	3	2
	deletions	4	0	0	0	0	1	0	0	2	0	0	0	0	0	0
Iso-chromatid aberrations	gaps	7	5	8	0	1	0	2	2	1	2	0	0	1	1	2
	breaks	6	8	25	1	1	0	3	0	0	2	0	1	1	1	1
	deletions	6	3	8	1	0	2	8	5	9	7	3	2	5	4	6
Mitotic index [mitotic nuclei per 1000 cells]		41.0	33.5	27.5	93.5	85.5	44.0	82.5	77.5	34.0	57.0	88.0	17.5	71.0	57.0	21.0
Mitotic index [% control]		43.9	39.2	62.5	100.0	100.0	100.0	88.2	90.6	77.3	61.0	102.9	39.8	75.9	66.7	47.7
Metaphases with aberrations, excluding gaps [%]		11.0**	9.5**	30.0**	1.0	0.5	1.5	7.0**	5.0**	7.5**	6.5**	1.5	2.0	5.0*	4.5**	8.5**

[a] total incidence in 200 cells scored
*p<0.05; **p<0.01

Table A6_6_2-5 Table for cytogenetic *in-vitro* test: chromosomal analysis (2 h exposure to Icaridin, with activation)

		Endoxan 25 µg/mL			Solvent control			Icaridin [µg/mL]								
								100			700			1400		
Time of harvest [h]		**7**	**18**	**27**	**7**	**18**	**27**	**7**	**18**	**27**	**7**	**18**	**27**	**7**	**18**	**27**
Cytotoxicity (mitotic indices)		Yes	Yes	No	No	No	No	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes
Chromatid aberrations	gaps	18	5	12	5	4	2	2	1	5	2	4	6	18	7	12
	breaks	6	4	6	0	0	0	0	0	2	1	2	1	15	0	2
	deletions	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Iso-chromatid aberrations	gaps	4	3	5	0	0	0	1	0	1	1	0	4	7	0	2
	breaks	9	14	15	0	0	5	3	1	2	1	1	3	32	12	1
	deletions	7	3	18	3	3	5	1	5	3	6	3	10	3	11	6
Mitotic index [mitotic nuclei per 1000 cells]		42.5	32.5	59.0	106.5	85.0	48.5	78.0	62.5	40.5	108.0	52.5	37.0	37.5	29.5	39.0
Mitotic index [% control]		39.9	38.2	121.6	100.0	100.0	100.0	73.2	73.5	83.5	101.4	61.8	76.3	35.2	34.7	80.4
Metaphases with aberrations, excluding gaps [%]		15.0 **	17.0 **	24.0 **	3.5	2.0	2.0	6.5	2.5	3.5	3.5	4.0	3.5	7.0	28.5**	22.0**

[a] total incidence in 200 cells scored
*p<0.05; **p<0.01

Table A6_6_2-6 Table for cytogenetic in-vitro test: chromosomal analysis (2 h exposure to Icaridin, without activation)

		Solvent control		Mitomycin 2 µg/mL	Icaridin [µg/mL]			
					400	800	1200	
Time of harvest [h]		**18**	**27**	**18**	**18**	**18**	**18**	**27**
Cytotoxicity (mitotic indices)		No	No	Yes	No	No	No	Yes
Chromatid aberrations	gaps	1 [a]	0	10	1	2	0	3
	breaks	0	1	32	0	1	2	6
	deletions	0	0	3	0	1	0	1
Isochromatid aberrations	gaps	1	5	9	4	0	3	5
	breaks	1	0	47	2	3	2	23
	deletions	6	4	4	4	3	5	20
Mitotic index [mitotic nuclei per 1000 cells]		62.5	38.0	11.0	65.5	55.0	64.5	25.5
Mitotic index [% control]		100.0	100.0	17.6	104.8	88.0	103.2	67.1

Metaphases with aberrations, excluding gaps [%]	4.0	3.5	87.5**	3.5	4.5	7.5	38.0**

[a] total incidence in 200 cells scored
*p≤ 0.05; **p≤ 0.01

Table A6_6_2-7 Table for cytogenetic in-vitro test: chromosomal analysis (2 h exposure to Icaridin, <u>with</u> activation)

		Solvent control		Endoxan 10 µg/mL	Icaridin [µg/mL]			
					800	1200	1600	
Time of harvest [h]		**18**	**27**	**18**	**18**	**18**	**18**	**27**
Cytotoxicity (mitotic indices)		No	No	Yes	No	Yes	Yes	Yes
Chromatid aberrations	gaps	0 [a]	4	10	1	6	10	5
	breaks	1	0	15	0	1	6	23
	deletions	0	0	1	1	0	1	0
Isochromatid aberrations	gaps	0	0	15	2	7	7	10
	breaks	1	0	37	2	2	35	32
	deletions	4	10	5	3	6	9	5
Mitotic index [mitotic nuclei per 1000 cells]		58.0	35.0	40.0	69.5	45.0	8.5	16.5
Mitotic index [% control]		100.0	100.0	69.0	119.8	77.6	14.7	47.1
Metaphases with aberrations, excluding gaps [%]		3.0	5.0	49.0**	3.0	9.0**	49.0**	40.5**

[a] total incidence in 200 cells scored
*p≤ 0.05; **p≤ 0.01

Section A6.6.2 Genotoxicity in vitro

Annex Point IIA VI.6.6.2 6.6.2 *In-vitro* mammalian chromosome aberration test in CHO cells (2)

Official use only

		1 REFERENCE
1.1	**Reference**	Gudi, R. and Schadly, E.H. (1997), Chromosome Aberrations in Chinese Hamster Ovary (CHO) Cells. Microbiological Associates, Inc., Rockville, MD, USA Report No. 107777, 1997-08-04 (unpublished)
1.2	**Data protection**	Yes
1.2.1	Data owner	Saltigo GmbH
1.2.2	Company with letter of access	–
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes US-EPA FIFRA §84-2 (≅ OECD Guideline 473)
2.2	**GLP**	Yes
2.3	**Deviations**	Yes – Dose spacing is too large in the test system with S9-activation. Mitotic indices are reduced by 97% at 2000 µg/mL and by only 12% at the next lower concentration, 1000 µg/mL. A reduction by approx. 50% is desirable at the highest evaluated concentration.
		3 MATERIALS AND METHODS
3.1	**Test material**	As given in Section 2 of dossier.
3.1.1	Lot/Batch number	030693
3.1.2	Specification	As given in Section 2 of dossier.
3.1.2.1	Description	Clear, colourless liquid
3.1.2.2	Purity	97.1%
3.1.2.3	Stability	Stability of Icaridin was confirmed by pre- and post-testing analyses.
3.2	**Study Type**	*In-vitro* mammalian chromosome aberration test
3.2.1	Organism/cell type	Chinese hamster ovary (CHO)
3.2.2	Metabolic activation system	S9 mix from livers of Aroclor-1254-treated male Sprague-Dawley rats (single i.p. injection, 500 mg/kg bw, five days prior to sacrifice).
3.2.3	Positive control	–S9: mitomycin C (0.08 µg/mL) +S9: cyclophosphamide (10 µg/mL)
3.3	**Application of test substance**	
3.3.1	Concentrations	–S9: 0, 125, 250, 1000 µg/mL +S9: 0, 250, 500, 1000, 2000 µg/mL
3.3.2	Way of application	Dissolved in dimethylsulphoxide (DMSO)

3.3.3	Exposure times	–S9: 20 h +S9: 4 h
3.3.4	Harvest times	–S9: 20 h after initiation of treatment +S9: 12 h after initiation of treatment
3.4	**Examinations**	See tables in appendix for examinations and results
3.4.1	Number of cells evaluated	2 flasks, 100 cells per flask

4 RESULTS AND DISCUSSION

4.1	**Genotoxicity**	
4.1.1	without metabolic activation	Yes, at ≥ 500 µg/mL
4.1.2	with metabolic activation	No
4.2	**Cytotoxicity**	Yes –S9: at ≥ 250 µg/mL +S9: at ≥ 2000 µg/mL

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The clastogenic potential of Icaridin was evaluated in a chromosome aberration test in Chinese hamster ovary (CHO) cells according to US-EPA FIFRA §84-2 which is comparable to OECD Guideline 473. Based on preliminary cytotoxicity assays, CHO cells were continuously exposed for 20 h to concentrations of 125, 250, 500, and 1000 µg/mL in the absence of S9 mix. In the presence of S9 mix, cells were treated for 4 h with 250, 500, 1000 and 2000 µg/mL. Cells were harvested 20 and 12 h after initiation of exposure in the absence and presence of S9 mix, respectively. Cell harvest times were determined after evaluation of the average generation time measured in the preliminary assays.
5.2	**Results and discussion**	The test article was soluble in treatment medium at all concentrations tested. Toxicity (cell growth inhibition) was approximately 46 and 49% at the highest dose levels evaluated for chromosome aberrations, 1000 and 2000 µg/mL, in the non-activated and S9-activated assays, respectively. Statistically significant increases in chromosome aberrations were observed in the non-activated test system relative to the solvent control group at concentrations of 500 and 1000 µg/mL ($p \leq 0.01$, Fisher's exact test). The Cochran-Armitage test was also positive for a dose response ($p \leq 0.05$). These positive results were observed at toxic dose levels. No statistically significant increases in chromosome aberrations were observed in the S9-activated test system, regardless of test concentration. The positive controls, mitomycin C and cyclophosphamide, were clearly clastogenic demonstrating the sensitivity of the test system.
5.3	**Conclusion**	Icaridin was positive for chromosome aberrations in CHO cells at cytotoxic concentrations without metabolic activation and negative after metabolic activation.
5.3.1	Reliability	1

5.3.2	Deficiencies	No The overall conclusion “positive for cytogenicity at cytotoxic concentrations” is not affected by the low number of metaphases that were evaluated for the highest concentration in the S9-activated test system.

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	1 November 2006/ amended 2017 after WG discussion and correction made .
Materials and Methods	Applicant's version can be adopted.
Results and discussion	Applicant's version is adopted
Conclusion	*Overall assessment: Positive.* This is based upon positive results on chromosome aberrations without metabolic activation (20 h harvest) obtained in the absence of excessive cytotoxicity (well below 50% according to OECD 473). Tables A6_6_2-1 and A6_6_2-2, shows that cytotoxicity based upon mitotic index was 26% (mean of flasks A and B) without metabolic activation for both the concentration 500 µg/ml and 1000 µg/ml, respectively. Based on the values for cell growth inhibition cytotoxicity was 32% and 46% for the concentration 500 and 1000 µg/ml, respectively.
Reliability	1
Acceptability	Applicant's version can be adopted.
Remarks	Applicant's version is adopted

Table A6_6_2-1 Table for cytogenetic *in-vitro* test: chromosomal analysis without activation (continuous Icaridin exposure until harvest, 20 h)

		MMC 0.08 µg/mL		Solvent control		Icaridin [µg/mL]							
						125		250		500		1000	
Flask		**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**
Cytotoxicity (cell growth inhibition %)		20		0		0		12		32		46	
Cytotoxicity (cell growth)		No	No	No	No	No	No	No	No	No	No	No	No
Cell count (% solvent control)		72	80	101	99	95	105	88	86	49	82	61	44
Mitotic index [% mitotic nuclei]		3.0	3.4	5.4	6.0	4.2	6.6	5.0	4.4	4.6	3.8	5.2	3.2
Mitotic index [% solvent control]		53	60	95	105	74	116	88	77	81	67	91	56
Chromatid-type aberrations	gaps	4	7	2 [a]	2	3	3	1	0	1	1	8	6
	breaks	40	52	2	1	4	0	1	2	8	6	5	8
	exchanges	29	28	0	0	1	0	0	3	4	2	0	4
Chromosome-type aberrations	breaks	7	2	1	0	0	5	1	1	5	4	0	0
	dicentric	2	2	0	0	1	0	0	1	1	1	3	1
	ring	0	0	0	0	0	0	0	0	0	0	0	0
Metaphases with aberrations, excluding gaps [%]		49	47	3	1	4	2	2	6	13**	10**	8**	13**

[a] total incidence in 100 cells scored
*p<0.05; **p<0.01, Fisher's exact test

Table A6_6_2-2 Table for cytogenetic *in-vitro* test: chromosomal analysis with activation (4 h exposure to Icaridin, harvest 12 h after start of exposure)

		CP 10 µg/mL		Solvent control		Icaridin [µg/mL]							
						250		500		1000		2000	
Flask		**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**	**A** [b]	**B** [c]
Cytotoxicity (cell growth)		No	No	No	No	No	No	No	No	No	No	Yes	Yes
Cell count (% solvent control)		110	161	97	103	107	125	162	135	96	118	51	50
Mitotic index [% mitotic nuclei]		1.2	0.8	6.6	5.4	4.8	5.8	5.4	5.2	5.6	6.4	0.2	0.2
Mitotic index [% solvent control]		20	13	110	90	80	97	90	87	93	107	3	3
Chromatid-type aberrations	gaps	0	2	2 [a]	3	1	0	4	1	2	1	0	0
	breaks	35	33	1	1	0	0	2	2	2	2	4	0
	exchanges	19	21	1	1	0	0	0	0	0	0	3	0
Chromosome-type aberrations	breaks	0	1	2	1	0	0	0	0	1	0	0	1
	dicentric	0	2	0	2	1	2	0	1	1	1	0	1
	ring	0	0	0	0	0	2	0	0	1	0	0	0
Metaphases with aberrations, excluding gaps [%]		37	36	3	4	1	2	2	2	5	3	13	9

[a] total incidence in 100 cells scored
[b] total incidence in 8 cells scored
[c] total incidence in 23 cells scored
*p<0.05; **p<0.01

Section A6.6.3 Genotoxicity in vitro

Annex Point IIA VI.6.6.3 6.6.3 HGPRT-forward-mutation assay in V79 cells

			Official use only
		1 REFERENCE	
1.1	**Reference**	Herbold, B. (1999), KBR 3023 – V79/HPRT-Test in Vitro for the Detection of Induced Forward Mutations. Bayer AG, Toxicology, Wuppertal, Germany Report No. 29220, 1999-10-19 (unpublished)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Saltigo GmbH	
1.2.2	Company with letter of access	–	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes EC Method B.17 (1988) OECD Guideline 476 (1984) US-EPA OPPTS 870.5300 (1998)	X
2.2	**GLP**	Yes	
2.3	**Deviations**	None	
		3 MATERIALS AND METHODS	
2.4	**Test material**	As given in Section 2 of dossier.	
2.4.1	Lot/Batch number	898711001	
2.4.2	Specification	As given in Section 2 of dossier.	
2.4.2.1	Description	Colourless liquid	
2.4.2.2	Purity	98.7%	
2.4.2.3	Stability	Until February 13, 2000. Stability in vehicle (ethanol) was confirmed.	
2.5	**Study Type**	*In-vitro* mammalian cell gene mutation test	
2.5.1	Organism/cell type	Chinese hamster lung fibroblasts (V79)	
2.5.2	Metabolic activation system	S9 mix from livers of Aroclor-1254-treated male Sprague-Dawley rats.	
2.5.3	Positive control	–S9: ethylmethanesulphonate (EMS), 900 µg/mL +S9: dimethylbenzanthracene (DMBA), 20 µg/mL	
2.6	**Application of test substance**		
2.6.1	Concentrations	–S9: 400, 600, 800, 1000, 1200, 1400, 1500, 1600 µg/mL +S9: 400, 600, 800, 1000, 1200, 1300, 1400, 1500, 1600 µg/mL	
2.6.2	Way of application	Dissolved in ethanol	
2.6.3	Exposure time	5 h	

Section A6.6.3 Genotoxicity in vitro

Annex Point IIA VI.6.6.3 6.6.3 HGPRT-forward-mutation assay in V79 cells

2.6.4	Other modifications	–
2.7	**Examinations**	See tables in appendix for examinations and results
2.7.1	Number of cells evaluated	200 cells per dish for determination of cloning efficiency 3×10^5 cells per dish for mutant selection
		3 RESULTS AND DISCUSSION
3.1	**Genotoxicity**	
3.1.1	without metabolic activation	No
3.1.2	with metabolic activation	No
3.2	**Cytotoxicity**	Yes –S9: at 1200 µg/mL: relative survival ~58% of vehicle control +S9: at 1300 µg/mL: relative survival ~34% of vehicle control
		4 APPLICANT'S SUMMARY AND CONCLUSION
4.1	**Materials and methods**	The ability of Icaridin to induce forward mutations of the HGPRT locus in V79 Chinese hamster lung fibroblasts was investigated. The study was conducted according to OECD Guideline 476. Concentrations up to 1600 µg/mL were tested, with and without metabolic activation with S9 mix.
4.2	**Results and discussion**	With and without activation, Icaridin induced decreases in survival to treatment and decreases in relative population growth. These results revealed a significant concentration-related cytotoxicity of Icaridin. Precipitation of the test substance in the culture medium was not observed. There was no biologically significant increase in mutant frequency above that of vehicle controls, neither with nor without metabolic activation.
4.3	**Conclusion**	Icaridin is non-mutagenic in the V79/HGPRT forward mutation assay, both with and without metabolic activation.
4.3.1	Reliability	1
4.3.2	Deficiencies	None

Section A6.6.3 Genotoxicity in vitro

Annex Point IIA VI.6.6.3 6.6.3 HGPRT-forward-mutation assay in V79 cells

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	16 November 2006
Materials and Methods	Applicant's version is adopted
Results and discussion	Applicant's version is adopted
Conclusion	Applicant's version is adopted
Reliability	1
Acceptability	Acceptable
Remarks	2.1 Guideline: the valid OECD guideline at the time of testing and reporting is dated July 1997.

Section A6.6.3 Genotoxicity in vitro

Annex Point IIA VI.6.6.3 6.6.3 HGPRT-forward-mutation assay in V79 cells

Table A6_6_3-1 Table for gene mutation assay: CHO-HPRT-test, non-activation

Concentration [µg/mL]	1st trial				2nd trial			
	Flask #1		Flask #2		Flask #1		Flask #2	
	MF [a]	RS [b]	MF	RS	MF	RS	MF	RS
Vehicle control	0.7	100.0	1.2	100.0	0.6	100.0	1.5	100.0
400	0.6	109.9	0.8	109.5	Not tested			
600	1.4	75.8	1.1	109.5	0.8	108.9	3.5	177.2
800	0.7	118.7	0.0	126.3	0.5	137.9	1.3	74.6
1000	0.0	67.9	0.0	74.5	0.5	76.1	0.5	71.4
1200	0.7	48.5	0.6	88.1	0.5	75.0	0.4	23.0
1400	0.0	36.9	0.6	47.0	0.6	195.2	C	C
1500	Not tested				0.4	37.5	N	60.6
1600	N	1.1	N	1.3	N	0.3	N	0.0
Positive control	475.4*	41.6	491.4*	43.0	578.0*	103.5	706.0*	66.2

[a] Mutation frequency [$\times 10^{-6}$]
[b] Survival to treatment [% vehicle control]
C = one dish lost due to contamination
N = not cloned due to cytotoxicity
* Significant increase, ($\alpha = 0.05$, one-sided Dunnett test)

Table A6_6_3-2 Table for gene mutation assay: CHO-HPRT-test, activation

Section A6.6.3 Genotoxicity in vitro

Annex Point IIA VI.6.6.3 6.6.3 HGPRT-forward-mutation assay in V79 cells

Concentration [µg/mL]	1st trial				2nd trial			
	Flask #1		Flask #2		Flask #1		Flask #2	
	MF [a]	RS [b]	MF	RS	MF	RS	MF	RS
Vehicle control	3.0	100.0	3.3	100.0	1.0	100.0	0.0	100.0
400	1.1	110.7	0.5	76.0	2.0	144.9	0.6	114.6
600	1.2	81.5	0.7	63.0	2.3	124.7	0.9	100.0
800	0.5	109.2	0.7	69.6	0.7	169.6	1.0	100.2
1000	1.8	63.1	3.2	69.2	0.7	158.5	2.0	69.5
1200	0.0	110.7	0.9	31.3	1.9	103.4	2.6	75.3
1300	3.4	53.2	4.4	18.4	3.3	34.2	0.0	31.1
1400	1.4	61.8	2.1	13.5	1.2	16.1	4.8	19.5
1500	0.0	8.6	N	0.8	N	0.5	N	0.4
1600	N	2.6	N	N	N	0.2	N	0.0
Positive control	65.2*	51.3	71.6*	46.7	44.4*	122.7	63.9*	89.1

[a] Mutation frequency [$\times 10^{-6}$]
[b] Survival to treatment [% vehicle control]
N = not cloned due to cytotoxicity
* Significant increase, ($\alpha = 0.05$, one-sided Dunnett test)

Section A6.6.4 Genotoxicity in vivo

Annex Point IIA VI.6.6.4 6.6.4 Micronucleus test on the mouse

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1994), KBR 3023 – Micronucleus Test on the Mouse. [redacted] Report No. 23291, 1994-08-29 (unpublished)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Saltigo GmbH	
1.2.2	Company with letter of access	–	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes EC Method B.12 (1992) OECD Guideline 474 (1983)	
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes	
		No vehicle controls were tested at 16 and 48 h.	X
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in Section 2 of dossier.	
3.1.1	Lot/Batch number	010393	
3.1.2	Specification	As given in Section 2 of dossier.	
3.1.2.1	Description	Clear, colourless liquid	
3.1.2.2	Purity	99.0%	
3.1.2.3	Stability	Until October 26, 1994. Test material was stable in vehicle for at least 24 h at room temperature.	
3.1.2.4	Maximum tolerable dose	350 mg/kg bw	
3.2	**Test Animals**		
3.2.1	Species	Mouse	
3.2.2	Strain	Hsd/win: NMRI	
3.2.3	Source	Harlan Winkelmann GmbH, Borchen, Germany	
3.2.4	Sex	♂ and ♀	
3.2.5	Age/weight at study initiation	6-12 weeks of age, 27-49 g bw	
3.2.6	Number of animals per group	5 ♂ + 5 ♀ per dose and sampling time	
3.2.7	Control animals	Yes	

Section A6.6.4 Genotoxicity in vivo

Annex Point IIA VI.6.6.4 6.6.4 Micronucleus test on the mouse

3.3	**Administration/ Exposure**	Intraperitoneal
3.3.1	Number of applications	1
3.3.2	Interval between applications	Not applicable
3.3.3	Postexposure period	16, 24, 48 h after treatment
		Intraperitoneal
3.3.4	Vehicle	0.5% aqueous Cremophor
3.3.5	Concentration in vehicle	35 mg/mL
3.3.6	Total volume applied	10 mL/kg bw
3.3.7	Dose applied	350 mg/kg bw
3.3.8	Substance used as positive control	Cyclophosphamide (CP), 20 mg/kg bw
3.3.9	Controls	Vehicle
3.4	**Examinations**	
3.4.1	Clinical signs	Yes
3.4.2	Tissue	Bone marrow
	Number of animals:	All animals
	Number of cells:	1000
	Time points:	16, 24, 48 h after treatment
	Type of cells	Erythrocytes in bone marrow
	Parameters:	Polychromatic/normochromatic erythrocytes ratio
3.5	**Further remarks**	–
		4 RESULTS AND DISCUSSION
4.1	**Clinical signs**	Treated animals showed the following symptoms until sacrifice: apathy, bristled coat, lateral recumbency, spasms, extension spasms, leaping spasms, twitching, laboured breathing, closed eyelids Three out of 40 treated animals died during the test period. No symptoms or mortalities were recorded for the control groups.
4.2	**Haematology**	There was a dose-dependent increase in the ratio of normochromatic vs. polychromatic erythrocytes (see Table 6_6_4-1 in appendix). This is an indirect proof of the test substance reaching the bone marrow.
4.3	**Genotoxicity**	No
4.4	**Other**	–

Section A6.6.4 Genotoxicity in vivo

Annex Point IIA VI.6.6.4 6.6.4 Micronucleus test on the mouse

		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The micronucleus test was applied to investigate the clastogenic potential on the chromosomes of bone-marrow erythroblasts in male and female mice. The study was conducted according to OECD Guideline 474. The animals were treated with a single i.p. injection of the maximum tolerated dose of 350 mg/kg bw. Femoral bone marrow smears were prepared 16, 24 and 48 later. Negative and positive control animals were sacrificed 24 h after dosage.	
5.2	**Results and discussion**	The Icaridin-treated mice showed clinical signs of toxicity after administration. Three out of 40 animals in the test substance group died prior to sacrifice. There was an altered ratio between polychromatic and normochromatic erythrocytes. This is indicative of bone marrow cell depression. No indications of a clastogenic effect of Icaridin were found at any sampling time. The positive control, cyclophosphamide, significantly increased the number of micronucleated polychromatic erythrocytes. The number of micronucleated normochromatic erythrocytes did not increase in any of the groups.	X
5.3	**Conclusion**	Icaridin was negative for the induction of micronuclei in bone-marrow erythroblasts in mice.	
5.3.1	Reliability	1	
5.3.2	Deficiencies	No	

Section A6.6.4 Genotoxicity in vivo

Annex Point IIA VI.6.6.4 6.6.4 Micronucleus test on the mouse

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	16 November 2006
Materials and Methods	Applicant's version is adopted
Results and discussion	Applicant's version is adopted with one supplementary information. The results of the microscopic investigations showed that Icaridin had reached the femoral bone marrow as it should which is a condition for detecting an eventual clastogenic effect of the substance.
Conclusion	Applicant's version is adopted
Reliability	1
Acceptability	Acceptable
Remarks	2.3 The control data with respect to sacrifice at other time points than 24 hours after dosing are scarce/lacking.. But this is not assumed to affect the validity of the study.

Section A6.6.4 Genotoxicity in vivo

Annex Point IIA VI.6.6.4 6.6.4 Micronucleus test on the mouse

Table A6_6_4-1. Table for Micronucleus Test In Vivo

	Vehicle control	350 mg Icaridin/kg			CP (positive control) 20 mg/kg
Number of cells evaluated	**10,000**	**10,000**			**10,000**
Sampling time (h)	**24**	**16**	**24**	**48**	**24**
Number of normochromatic erythrocytes per 1000 poly–chromatic erythrocytes	719 ± 188	1047 ± 241	1434 ± 521	1514±739*	743 ± 163
Micronucleated cells per 1000 normo-chromatic erythrocytes	1.7 ± 1.6	1.6 ± 1.7	1.5 ± 0.6	1.6 ± 1.4	1.7 ± 2.3
Micronucleated cells per 1000 poly-chromatic erythrocytes	1.3 ± 1.1	1.2 ± 0.8	1.5 ± 1.2	1.4 ± 1.1	12.3 ± 5.8*

*$p < 0.01$ in non-parametric Wilcoxon rank test

Section A6.7 Carcinogenicity

Annex Points IIA VI.6.7 6.7 Dermal carcinogenicity study in the mouse

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1996b), Technical Grade KBR 3023: An Oncogenicity Dermal Toxicity Study in the Mouse. [redacted], Report No. 107433, 1996-12-18, amended by Report No. 107433-1, 1997-06-03 (unpublished)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Saltigo GmbH	
1.2.2	Company with letter of access	–	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, compliance with the following guidelines was claimed: US-EPA FIFRA §83-2 US-EPA-TSCA Section 798.3300 OECD Guideline 451, May 1981 Japanese MAFF, 59 NohSan No. 4200	
2.2	**GLP**	Yes	
2.3	**Deviations**		X
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in Section 2 of dossier.	
3.1.1	Lot/Batch number	030693	
3.1.2	Specification	As given in Section 2 of dossier.	
3.1.2.1	Description	Clear liquid	
3.1.2.2	Purity	96.7–98.5%	X
3.1.2.3	Stability	Stability was confirmed by four separate analyses conducted prior to, during and after the exposure period.	
3.2	**Test Animals**		
3.2.1	Species	Mouse	
3.2.2	Strain	CD-1[ICR]/BR	
3.2.3	Source	Charles River Breeders, Portage, MI, USA	
3.2.4	Sex	♂ + ♀	
3.2.5	Age/weight at study initiation	8 weeks; ♂: ~28 g, ♀: ~25 g	
3.2.6	Number of animals per group		

Section A6.7 Carcinogenicity

Annex Points IIA VI.6.7 6.7 Dermal carcinogenicity study in the mouse

3.2.6.1	at interim sacrifice	–
3.2.6.2	at terminal sacrifice	50 per sex and dose group
3.2.7	Control animals	Yes
3.2.8	Replacement group	For approx. the first month of treatment, an additional 5 ± 2 mice/ dose/ sex were placed on study as part of the 18-month sacrifice group. These animals served as potential replacements for any animals that died or developed non-treatment-related problems very early in the study.
3.3	**Administration/ Exposure**	Dermal
3.3.1	Duration of treatment	18 months
3.3.2	Interim sacrifice(s)	Not performed
3.3.3	Final sacrifice	18 months
3.3.4	Frequency of exposure	5 days/week
3.3.5	Postexposure period	None
		Dermal
3.3.6	Area covered	4–5 cm²
3.3.7	Occlusion	Non-occlusive, without dressing. Elizabethan collars were used to prevent oral uptake of the test material.
3.3.8	Vehicle	Undiluted
3.3.9	Concentration in vehicle	–
3.3.10	Dose levels	50, 100, 200 mg Icaridin/kg bw/day
3.3.11	Duration of exposure	24 h
3.3.12	Removal of test substance	No
3.3.13	Controls	Yes, shaved but untreated
3.4	**Examinations**	
3.4.1	Body weight	Yes, once weekly
3.4.2	Food consumption	Yes, once weekly
3.4.3	Water consumption	No
3.4.4	Clinical signs	Yes, twice daily (once on weekends and holidays)
3.4.5	Macroscopic investigations	Palpable masses, external surface areas, orifices, posture, general behaviour, respiration, excretory products

Section A6.7 Carcinogenicity

Annex Points IIA VI.6.7 6.7 Dermal carcinogenicity study in the mouse

3.4.6	Ophthalmoscopic examination	Yes
	No. of animals:	All animals
	Time points:	After 9 months of treatment
3.4.7	Haematology	Yes
	No. of animals:	10 animals/sex/dose
	Time points:	After 12 and 18 months of treatment
	Parameters:	Haematocrit, haemoglobin concentration, erythrocyte count, total and differential leukocyte count, platelet count, MCV, MCH, MCHC, Heinz bodies, reticulocyte counts, erythrocyte morphology
3.4.8	Clinical Chemistry	No
3.4.9	Urinalysis	No
3.4.10	Pathology	Yes
3.4.10.1	Organ Weights	Yes
	from:	All surviving animals at sacrifice
	Organs:	Liver, kidneys, adrenals, testes, ovaries, spleen, brain, heart, lungs
3.4.10.2	Histopathology	Yes
	from:	All surviving animals at sacrifice
	Organs:	Adrenals, aorta, bone, bone marrow, brain, cervix, epididymides, eyes, gall bladder, gonads, gross lesions, Harderian gland, heart, joint (knee), kidneys, larynx, liver, lungs, lymph nodes, mammary gland, muscle, oesophagus, pancreas, parathyroid, peripheral nerve, pituitary, prostate, salivary glands, seminal vesicles, skin, skull, small and large intestines, spinal cord, spleen, stomach, thymus, thyroid, trachea, urinary bladder, uterus, Zymbal's gland
3.4.11	Other examinations	–
3.5	**Statistics**	Continuous data: Evaluation of homogeneity of variance was done with Bartlett's test. If homogenous, group means were analysed by one-way ANOVA followed by Dunnett's test. In the event of unequal variances, data were analysed by Kruskal-Wallis ANOVA followed by the Mann-Whitney-U test. Frequency data: chi-square and Fisher exact test. For the Bartlett test, $p \leq 0.001$ was considered significant; for all other tests, $p \leq 0.05$ was considered statistically significant.
3.6	**Further remarks**	–
		4 RESULTS AND DISCUSSION
4.1	**Body weight**	No effects
4.2	**Food consumption**	No effects
4.3	**Water consumption**	Not assessed
4.4	**Clinical signs**	There were no treatment-related clinical signs.

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4.5	**Macroscopic investigations**	No compound-related abnormalities were noted. Mortality was unaffected by administration of Icaridin (see Table A6_7-2).	
4.6	**Ophthalmoscopic examination**	At approximately 9 months, an increased incidence of an ulcerative-like lesion of the eye was noted in both sexes and in all dose groups, including controls. Corneal ulceration (8-24%), corneal oedema (2-16%), corneal vascularisation (8-24%), and corneal opacity (52-72%) were observed. There were no statistically significant differences in the incidences of these lesions between control and treated groups. It was postulated that the condition was associated with the use of Elizabethan collars that restricted normal grooming activities and decreased the ability to remove foreign matter from their eyes and was not of toxicological concern.	
4.7	**Haematology**	No effects	
4.8	**Pathology**	No effects	
4.9	**Organ Weights**	No effects	
4.10	**Histopathology**	No evidence of a chemically-induced neoplastic or non-neoplastic effect was observed in this study. Lesions were generally limited to those associated with the ageing CD-1 mouse.	
4.11	**Other examinations**	–	
4.12	**Time to tumours**	No evidence of chemically induced neoplastic lesions in any tissue at any dosage.	
4.13	**Other**	–	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The dermal oncogenicity of Icaridin to CD-1 mice was investigated in a 18-month study using a 5-days-per-week application scheme. The study was performed according to OECD Guideline 451. Male and female CD-1 mice were treated for up to 18 months with undiluted Icaridin (0, 50, 100, 200 mg/kg bw/day). Elizabethan collars were used to prevent ingestion of the test compound.	X
		Animals were sacrificed after 18 months of exposure. Body weights, feed consumption, clinical observations, haematology, terminal body and organ weights, gross pathology and histopathology evaluations were determined/performed.	
5.2	**Results and discussion**	There were no treatment-related alterations of any in-life or post-mortem parameter investigated.	X
		There were no treatment-related neoplasias in any organ or tissue of any dose group. The only neoplastic changes observed were a tendency towards higher incidence of alveolar/bronchial adenomas in male rats in the high dose. However the increase was not statistically significant and considered to be a normal variation in ageing CD-1 mice (see Table A6_7-2).	
		It is especially noteworthy that no neoplastic alterations were found at the application site in spite of the frequent shaving of this skin area	

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		which can be regarded as a potential tumour promoting event. The absence of systemic effects does not allow the direct conclusion that Icaridin was systemically absorbed. However, the *in vivo* dermal absorption of undiluted Icaridin has been determined in the rat. In the dermal ADME study by Ecker & Weber (1997), rats were exposed to undiluted Icaridin at single or repeated doses of 20 mg/kg bw (0.5 mg/cm²) or a single dose of 200 mg/kg bw (5.0 mg/cm²). The applied dose was left in place for 7 days before sacrifice. A mean absorption value of 47.5% of applied dose was found after 7 days of exposure to 200 mg Icaridin/kg bw (5.0 mg/cm²).	X
		Absorption through murine skin is expected to be at least as high as through rat skin. The absolute thickness of the murine epidermis is lower than in the rat and the diffusion distance between stratum corneum and the sub-epidermal capillary bed is also shorter. Both factors are critical determinants of systemic uptake via skin. The study, although not achieving the MTD due to the low toxicity of Icaridin and the limitations inherent to the dermal route of application, demonstrates the absence of a carcinogenic potential of Icaridin at systemic dose levels that cannot be reached by the foreseeable application by humans.	
5.3	**Conclusion**	Icaridin is without carcinogenic potential, both topically and systemically, at systemic doses that greatly exceed the potential human exposure due to the use in repellent products.	X
5.3.1	LO(A)EL	LOAEL > 200 mg/kg bw/day, based on the absence of treatment-related effects	
5.3.2	NO(A)EL	NOAEL = 200 mg/kg bw/day	
5.3.3	Other	–	
5.3.4	Reliability	1	X
5.3.5	Deficiencies	Yes. Dosing was commenced when animals were 8 weeks of age. This is 2 weeks older than recommended but necessary to obtain animals of sufficient size to facilitate the conduct of the test. The highest dose (200 mg/kg bw/day) did not cause signs of toxicity. The subchronic dermal toxicity study in rats demonstrated that doses greater than 200 mg/kg would spread out beyond the intended area of exposure and would therefore be difficult to interpret. The selection of the highest dose level was made after consultations with US-EPA toxicologists. These deficiencies do not affect the overall validity of the study.	

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Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	01 November 2006
Materials and Methods	2.3 Yes, the following deviations from OECD 451 were noted: – Animals were > 6 weeks of age when dosing commenced. – The highest dose level caused no signs of toxicity. 5.1 Male and female CD-1 mice were treated for up to 18 months with undiluted Icaridin (0, 50, 100, 200 mg/kg bw/day).
Results and discussion	The dermal absorption of the mouse skin is unknown as no studies have been submitted. This jeopardise the study as such as it is not known for certain that the substance is actually absorbed and no systemic effect were seen in the highest dose to reassure that it has been absorbed. In general it must be emphasized that the highest tested dose of 200 mg/kg bw/day is to low to give information on the systemic inherent toxicological properties of the substance and this end point has therefore not been covered in this dossier. However it can be accepted that the dermal study will give information about the dermal toxicity of icaridin in sufficiently high doses (compare to doses used on humans) because the foreseeable route of systemic exposure for this dermally applied insect repellent is though dermal absorption. The study gives the very important information that the substance does not produce skin cancer. For mean dermal absorption values for the rats please refer to CA-evaluation of the respective study.
Conclusion	Icaridin is without carcinogenic potential to the skin.
Reliability	2
Acceptability	Acceptable
Remarks	3.1.2.2. Lowest purity in study below declared purity specifications of the substance No systemic toxicity apparent. No studies are available on the uptake of Icaridin through mouse skin. There is no data provided to prove the relevance of the study to the endpoint regarding systemic carcinogenic effect of Icaridin.

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Table A6_7-1. Table for haematology

No treatment-related changes were observed in any of the investigated haematology parameters.

Table A6_7-2. Results of carcinogenicity study

Parameter	Historical control[a]		Concurrent control		50 mg/kg		100 mg/kg		200 mg/kg		Dose-response + / –	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
No. of animals examined			50	50	50	50	50	50	50	50		
Mortality			11 (22%)	6 (12%)	8 (16%)	13 (26%)	3 (6%)	9 (18%)	7 (14%)	7 (14%)	–	–
found dead			4	3	2	4	1	2	2	1	–	–
unsched. kill			7	3	6	9	2	7	5	6	–	–
clinical signs												
corneal ulceration			4	5	12	7	6	4	12	6	–	–
corneal oedema			1	8	2	2	2	2	3	6	–	–
corneal vascularisation			4	5	11	7	8	4	10	6	–	–
corneal opacity			28	26	34	30	33	26	36	35	–	–
bw gain [g] (78 wks)			8.7	8.2	8.8	8.6	8.7	8.4	8.7	8.7	–	–
bw (78 wks) [% control]			100	100	101	101	99	99	98	99	–	–
food consumption [% control]			100	100	101	102	101	99	102	98	–	–
clin. chemistry			not examined									
haematology			No significant changes in any observed parameter								–	–
urinalysis			not examined									
Lung												
alveolar/ bronchiolar hyperplasia	0-4	0-5	1	0	6	5	3	4	6	3	–	–
alveolar/ bronchiolar adenoma	1-14	0-9	5	2	6	1	3	2	9	4	–	–
alveolar/ bronchiolar carcinoma	1-9	0-3	1	0	0	1	0	0	1	1	–	–
There were no treatment-related effects on tumour incidence in any group.												

[a] Studies in CD-mice conducted at Bayer Corp. (Stilwell, KS) between 1980-1991 (range of incidences per 50 animals)
* $p < 0.05$

Section A6.8.1 Teratogenicity Study

Annex Point IIA VI.6.8.1 6.8.1 Dermal teratogenicity study in the rabbit

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1996), Developmental Toxicity Study in Rabbits after Dermal Application. [redacted] Report No. 24928, 1996-03-22 (unpublished)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Saltigo GmbH	
1.2.2	Company with letter of access	–	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, compliance with the following guidelines was claimed: OECD Guideline 414, May 1981 EC Method B.31, February 1995 US-EPA FIFRA §83-3, November 1984 Japanese MAFF, 59 NohSan No. 4200	
2.2	**GLP**	Yes	
2.3	**Deviations**	The study was conducted according to the original version of OECD Guideline 414 (May 1981). It fulfils the requirements of the updated OECD Guideline 414 (January 2001), but with the following deviations: - Age of the animals was not reported - Room temperature deviated slightly from the desired range	X
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in Section 2 of dossier.	
3.1.1	Lot/Batch number	030693	
3.1.2	Specification	As given in Section 2 of dossier.	
3.1.2.1	Description	Clear, light yellow liquid	
3.1.2.2	Purity	97.7 to 97.8%	
3.1.2.3	Stability	6 months	
3.2	**Test Animals**		
3.2.1	Species	Rabbit	
3.2.2	Strain	Himalayan rabbit CHBB:HM	
3.2.3	Source	Dr. Karl Thomae GmbH, Biberach, Germany	
3.2.4	Sex	Females (untreated males were used as sires)	
3.2.5	Age/weight at study initiation	Age not given, 1892-2893 g bw	

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3.2.6	Number of animals per group	24 females
3.2.7	Control animals	Yes
3.2.8	Mating period	Day 0: between 7 a.m. and 10 a.m.
3.3	**Administration/ Exposure**	Dermal
3.3.1	Duration of exposure	Day 0-28 of gestation
		Dermal
3.3.2	Area covered	10% of body surface
3.3.3	Occlusion	Non-occlusive. Collars were used to prevent oral uptake of the test material.
3.3.4	Vehicle	Undiluted
3.3.5	Concentration in vehicle	–
3.3.6	Dose levels	0, 50, 100, 200 mg Icaridin/kg bw/day
3.3.7	Duration of exposure	24 h
3.3.8	Removal of test substance	Yes
3.3.9	Controls	Yes, shaved, treated with tap water
3.4	**Examinations**	
3.4.1	Body weight	Yes, daily on days 0 through 29 of gestation
3.4.2	Food consumption	Yes, every day from day 0 through 29 of gestation
3.4.3	Clinical signs	Yes, twice daily (general appearance, behaviour, excretory products, local reactions at the dose site)
3.4.4	Examination of uterine content	Gravid uterine weight Individual weights and appearance of placentas Number of corpora lutea Number of implantations
3.4.5	Maternal organ weights	-
3.4.6	Examination of foetuses	Yes individual weights of live foetuses; occurrence of external malformations and other findings deviating from normal; occurrence in findings in abdominal, pelvic and thoracic organs; findings in the brain and skeletal system

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3.4.6.1	General	Litter size, no. of live and dead foetuses, foetal weight, sex of live foetuses, malformations
3.4.6.2	Skeleton	Yes
3.4.6.3	Soft tissue	Yes
3.5	**Further remarks**	Animals were subjected to gross pathological evaluation during caesarean section on day 29 p.c. Animals which aborted were killed and necropsied after abortion was evident. Animals with uterine anomaly, without implantations and with abortion were not taken into account for statistical evaluation. Animals with total resorption were not taken into account for calculations of group mean values of body weights, body weight gains and feed intake.
		4 RESULTS AND DISCUSSION
4.1	**Maternal toxic effects**	No mortalities were observed. Local skin reactions appeared in a dose-dependent manner at all dose levels. In all treatment groups the females revealed squamous skin beginning on day 4 to 12 p.c. and lasting until necropsy. An increased incidence of slight erythema was evident at levels of 50 mg/kg bw/day and above. One animal of the 200 mg/kg group also revealed a moderate erythema. In most cases erythema already appeared on the first days of treatment and lasted until necropsy. Very slight or slight oedema transiently appeared in several animals of the 200 mg/kg group. Cracked skin appeared in single animals of the 50 mg/kg group (1 day only) and in the 100 mg/kg group (days 3 to 9) while most of the animals of the 200 mg/kg group revealed cracked skin (days 3 to 19).
4.2	**Teratogenic / embryo toxic effects**	No effects (see Tables A6_8_1-2 and A6_8_1-3)
4.3	**Other effects**	–
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	The developmental toxicity of Icaridin after dermal exposure was tested in pregnant Himalayan rabbits. The study was conducted according to OECD Guideline 414. The undiluted test substance was applied daily to a shaved dorsal area throughout the entire gestational period (day 0-28). Doses of 50, 100 or 200 mg/kg bw/day were applied. Rabbits wore collars to prevent ingestion of the test material. Does were sacrificed on gestational day 29 and foetuses were removed by Caesarean section and a gross maternal necropsy was performed. Investigations were performed on local and general tolerability of the test compound by the does as well as on its effect on intrauterine development.
5.2	**Results and discussion**	No mortalities were observed. Local reactions at the dose site of the females appeared in a dose dependent fashion at all dose levels. Slight erythema of the skin

Section A6.8.1	Teratogenicity Study	
Annex Point IIA VI.6.8.1	6.8.1 Dermal teratogenicity study in the rabbit	

(200 mg/kg: also moderate), squamous and cracked skin already appeared at the 50 mg/kg level while oedema (very slight or slight) of the skin occurred at the 200 mg/kg level.

Except from these local reactions at the dose site, appearance as well as behaviour and mortality of the females were unaffected by treatment at levels up to and including 200 mg/kg bw/day.

The feed intakes and body weight gains of the females were also unaffected by treatment at levels up to and including 200 mg/kg bw/day

An increased incidence of females revealed soft faeces at the 200 mg/kg level which was probably due to the stress caused by the local reactions at the dose site which were more pronounced (higher incidence, higher grade) in the 200 mg/kg group than in the other groups.

There were no treatment-related gross pathological findings at levels up to and including 200 mg/kg bw/day. See Table A 6_8_1-1.

With respect to intrauterine development, the fertility rate, the gestation rate, the number of corpora lutea, the pre-implantation loss and correspondingly the number of implantation sites, the post implantation loss and correspondingly the number of foetuses, foetal sex, foetal weight as well as appearance and weight of placentas were unaffected by treatment at levels up to and including 200 mg/kg bw/day.

External, visceral and skeletal evaluation of the foetuses revealed no effects of Icaridin on foetal morphology at levels up to and including 200 mg/kg bw/day. A teratogenic potential of Icaridin was not evident.

The lack of maternal toxicity at the highest dose raises concerns about a potentially insufficient degree of systemic exposure.

X

A dermal penetration study has been conducted *in vitro* using rabbit skin explants. This study showed a dermal penetration of 5% from a 11.5% solution of Icaridin in ethanol. A somewhat lower dermal absorption (3.7%) was found in human volunteers exposed to a 15% ethanolic solution of Icaridin. The surface concentration in the *in vitro* rabbit study was 6.3 mg Icaridin/cm² while the human *in vivo* study featured a surface concentration of 0.63 mg/cm². Thus, under similar exposure conditions, penetration through rabbit skin is going to be substantially higher than through human skin.

It is also relevant that Icaridin caused significant cracking of the skin in this teratology study; a condition that is very likely to further facilitate dermal uptake by high-dose females. The absorption-promoting effect of the skin lesions has been clearly shown in the dermal dose-finding study in rabbits.

It is also noteworthy that the exposure period covered the entire duration of pregnancy from conception to term. The early commencement of exposure ascertains that maternal body burdens of Icaridin had already reached the maximum attainable level at the onset of organogenesis around Day 6 of pregnancy.

In the rabbit teratology study, the highest external dose of 200 mg/kg/ day did not elicit adverse effects on maternal and developmental parameters. This dose therefore represents a clear NOAEL for these effects.

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		Nevertheless, exposure of does at this NOAEL will lead to substantially higher systemic doses in the pregnant doe than that expected for an adult woman: with an assumed 5% dermal penetration in the rabbit, the internal NOAEL for teratogenic effects in the rabbit is 10 mg/kg/day. For a 60-kg adult, the anticipated systemic dose is only 1.24 mg/kg/day, more than half of which originates from a very conservative oral uptake scenario (see Doc. II-B, Section 3.2.4).	
5.3	**Conclusion**	Icaridin does not possess a teratogenic or embryotoxic potential in the rabbit at the maximum dose attainable by dermal application. The resulting systemic dose level is about 10 times in excess of worst-case estimates for everyday users of Icaridin-containing repellents.	X
5.3.1	LO(A)EL maternal toxic effects	LOAEL > 200 mg/kg bw/day	
5.3.2	NO(A)EL maternal toxic effects	NOAEL = 200 mg/kg bw/day	
5.3.3	LO(A)EL embryo toxic / teratogenic effects	LOEL > 200 mg/kg bw/day	
5.3.4	NO(A)EL embryo toxic / teratogenic effects	NOEL = 200 mg/kg bw/day	
5.3.5	Reliability	1	X
5.3.6	Deficiencies	A seeming deficiency of this study is the lack of maternal toxicity at the highest dose and as a consequence concerns about a potentially insufficient degree of systemic exposure. The aim of toxicity at the highest dose is not an inflexible requirement of the relevant OECD Guideline 414: the necessity to induce "some developmental and/or maternal toxicity" is given, "unless limited by the physical/chemical nature […] of the test substance". The ability to apply doses greater than 200 mg Icaridin/kg/day is clearly limited by the physical/chemical nature of the substance. Thus, the absence of systemic effects at the top dose is not even a deviation from OECD 414. From the present study, teratogenic effects in the rabbit can be ruled out at doses excessive of normal human use. A NOAEL for both maternal and teratogenic effects of dermally applied Icaridin can be derived. Thus, the study serves both purposes of a teratogenicity study, identification of a potential developmental hazard and setting of a NOAEL for foetal and maternal effects.	X

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Annex Point IIA VI.6.8.1 6.8.1 Dermal teratogenicity study in the rabbit

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	01 November 2006
Materials and Methods	Applicant's version is adopted. Statistical significance in the 100 mg/kg bw/day was reached for the malformation" Arthrogryposis" when a Q^2 test (Fischer exact) was used.
Results and discussion	This dermal study is not performed in accordance with guidelines and does not give sufficient information about the teratogenic potential of the substance. The highest tested dose was too low as no maternal toxicity was seen and in reality only accounts for a systemic dose of 10 mg/kg bw/day (5% dermal absorption in the rabbit). As the rabbit is often regarded to be the most sensitive species with respect to teratogenicity the importance of a good rabbit test is obvoius. The absence of effects on foetus in one species cannot be extrapolated to another species. Therefore testing in two species is crucial. The massive use pattern of the substance raises concerns for potential developmental effects as it involves the general public hereunder adults, pregnant women and small children where risk reduction measures are not possible. The unexplained effects seen on the rabbit foetus raises concerns as it seems to be a rare malformation and occurs with higher foetus incidences in the dosed groups. The higher incidence of foetus with the rare external malformation arthrogryposis were seen in all dosed groups (0%, 2.7%, 3.6 and 1.4% in control, 50, 100 and 200 mg/kg bw/day, respectively,) without any dose-relationship. It is, however debatable how much weight on dose-relation and statistic on rare incidences. The incidence appears to be within the historical control incidence of 1.2%. The historical control data on foetus incidences presented in the table are, however of limited relevance since the time interval is outside the normally accepted deviation (±1 year from current study) from the performed study. Furthermore no reference has been made to the origin of the data. The dermal dose-range finding study indicates a steep dose-response curve (hence a narrow therapeutic index): Only 1/3 does delivered a litter at 1000 mg/kg bw/day while no maternel effects were seen at 700 mg/kg bw/day. The dosing regime in the main test is therefore not relevant. The inherent properties with respect to teratogenicity of the substance in rabbits should be clarified and an oral developmental study on rabbits should be performed according to current guidelines. The exposure considerations are of no relevance in this respect and include too many uncertainties. The dermal absorption can change considerably if the skin is not intact and more than 1 application of the product per day is not unrealistic (which would mean a daily exposure of 36-54 mg a.i/kg bw/day). The small margin of safety would be unacceptable.

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Conclusion	The study cannot be used in the risk assessment.
Reliability	3
Acceptability	Not acceptable to the endpoint systemic teratogenicity
Remarks	2.3 Deviation from guideline: No maternal toxicity or developmental toxicity at any dose level. *In vitro* absorption of Icaridin through rabbit skin is approx. 5%. No maternal toxicity is noted. No data on concentration of substance in fetuses is available.

Table A6_8_1-1. Table for teratogenic effects (separate data for all dosage groups)

Maternal effects

Parameter	Control data		50 mg/kg	100 mg/kg	200 mg/kg	Dose-response + / –
	historical (1988-1990)	study				
Number of does examined		24	24	24	24	
Clinical findings						
dosing site, **squamous skin**	–	0	24	24	24	+
erythema						
very slight **slight** **moderate**	–	10 1 0	13 11 0	4 20 0	0 23 1	+
oedema						
very slight **slight**	–	0 0	0 0	0 0	10 3	+
Cracked skin	–	0	2	4	18	+
Mortality of does [%]	0.0	0.0	0.0	0.0	0.0	–
Body weight gain [g] Day 0 – 29	215.6	250.3	216.6	280.8	214.8	–
Gravid uterus weight [g]	–	355.0	350.3	388.2	349.0	–
Food consumption, range [g/animal/day]	40-96	72-91	68-87	69-93	67-89	–
Pregnancies [%]	–	100	92	92	100	–

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Table A6_8_1-2. Table for teratogenic effects (separate data for all dosage groups)

Litter response (Caesarean section data)

Parameter	Control data		50 mg/kg	100 mg/kg	200 mg/kg	Dose-response + / –
	Historical (1988-1990)	Study				
Corpora lutea [total number]		189	177	192	176	–
Corpora lutea [total/no. of does with implantations]	8.0 – 9.8	8.6 ± 2.1	8.0 ±1.1	8.7 ± 1.6	7.7 ± 1.9	–
Implantations [total number]s		174	161	178	165	–
Implantations [total/number of does]	5.9 – 6.8	7.9 ± 2.2	7.3 ± 1.2	8.1 ± 1.6	7.2 ± 2.0	–
Total number of live foetuses		155	146	167	140	–
Total number of dead foetuses		0	0	0	0	–
Pre-implantation loss [total/no. of does with implantations]	1.5 – 3.1	0.7	0.7	0.6	0.5	–
Post-implantation loss [total/no. of does with implantations]		0.9	0.7	0.5	1.1	–
Total number of litters		22	22	22	23	
Foetus weight (mean) [g]	37.7-44	36.5	37.2	37.1	38.8	–
Placenta weight (mean) [g]	4.1-5.3	3.9	4.1	3.9	4.1	–
Foetal sex ratio (% male)	46.4-61.3	51	48	45	51	–

Significantly different from control: *p < 0.05; **p < 0.01

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Table A6_8_1-3. Table for teratogenic effects (separate data for all dosage groups)

Examination of the foetuses

Parameter	Control data		50 mg/kg	100 mg/kg	200 mg/kg	Dose-response + / –
	Historical (1992-1994)	study				
Number of foetuses per group		155	146	167	140	–
Total number of foetuses with malformations		0	6*	9*	2	–
% of foetuses with malformations	1.74 – 7.92	0	4.1	5.4	1.4	–
External malformations						
Arthrogryposis (total number)	5/404[#]	0	4	6*	2	–
Skeletal malformations	No treatment related-malformations					
Visceral malformations						
Cardiac septal defect (total number)		0	0	2	0	–
External and visceral deviations		0	1	1	0	–

Statistically significant difference to control: *p < 0.05

[#] Incidences in control groups from 1993-1994

Section A6.8.1 Teratogenicity Study

Annex Point IIA VI.6.8.1 6.8.1 Dermal teratogenicity study in the rat

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		1 REFERENCE
1.1	**Reference**	[redacted] (1996b), A Developmental Toxicity Study with KBR 3023 Technical in the Sprague-Dawley Rat. [redacted], Study No. 95-622-DI, 1996-09-11 (unpublished)
1.2	**Data protection**	Yes
1.2.1	Data owner	Saltigo GmbH
1.2.2	Company with letter of access	–
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes, compliance with the following guidelines was claimed: US-EPA FIFRA §83-3 US-EPA-TSCA Section 798.4900 OECD Guideline 414, May 1981 EC Method B.31, February 1995 Japanese MAFF, 59 NohSan No. 4200
2.2	**GLP**	Yes
2.3	**Deviations**	None
		3 MATERIALS AND METHODS
3.1	**Test material**	As given in Section 2 of dossier.
3.1.1	Lot/Batch number	030693
3.1.2	Specification	As given in Section 2 of dossier.
3.1.2.1	Description	Clear, colourless liquid
3.1.2.2	Purity	97.4–97.7%
3.1.2.3	Stability	At least six months
3.2	**Test Animals**	
3.2.1	Species	Rat
3.2.2	Strain	Sprague-Dawley
3.2.3	Source	Sasco, Inc., Omaha, NE, USA
3.2.4	Sex	Females (untreated males were used as sires)
3.2.5	Age/weight at study initiation	12-15 weeks of age, 207-286 g bw

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3.2.6	Number of animals per group	30 females
3.2.7	Control animals	Yes
3.2.8	Mating period	Until insemination was detected
3.3	**Administration/ Exposure**	Dermal
3.3.1	Duration of exposure	Day 0-20 of gestation
		Dermal
3.3.2	Area covered	10% of body surface
3.3.3	Occlusion	Non-occlusive, without dressing. Elizabethan collars were used to prevent oral uptake of the test material.
3.3.4	Vehicle	Undiluted
3.3.5	Concentration in vehicle	–
3.3.6	Dose levels	50, 200, 400 mg Icaridin/kg bw/day
3.3.7	Duration of exposure	24 h
3.3.8	Removal of test substance	No
3.3.9	Controls	Yes, shaved but untreated
3.4	**Examinations**	
3.4.1	Body weight	Yes, daily on days 0 through 20 of gestation
3.4.2	Food consumption	Yes, every other day from day 2 through 20 of gestation
3.4.3	Clinical signs	Yes, twice daily
3.4.4	Examination of uterine content	Gravid uterine weight Number of corpora lutea Number of implantations Number of resorptions
3.4.5	Maternal organ weights	Liver, thyroid
3.4.6	Examination of foetuses	

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3.4.6.1	General	Litter size, no. of dead foetuses, foetal weight, sex ratio	
3.4.6.2	Skeleton	Yes, about half of the foetuses	
3.4.6.3	Soft tissue	Yes, about half of the foetuses	
3.5	**Further remarks**	Dams suspected to be non-gravid on day 20 underwent gross necropsy. Patency of the cervical/uterine os was verified. If the openings were patent the uterus was excised and examined for implantation sites to confirm pregnancy. In addition, the ovaries were examined for corpora lutea. If present, the corpora lutea were counted and the dam was examined as usual. If the dam was not pregnant, no further examinations were performed.	
		4 RESULTS AND DISCUSSION	
4.1	**Maternal toxic effects**	No mortalities were observed.	X
		The majority of the clinical signs, primarily nasal stain, were observed independent of the dose and were ascribed to the wearing of Elizabethan collars. Scab formation at the dose site was observed in all groups and was not considered treatment-related. Scaly and/or sloughing skin was observed in all Icaridin-treated groups. Dermal irritation is a common response to repeated dermal exposure to a variety of substances, including water or petrolatum, and should not be regarded as a substance-related adverse effect (see Table A6_8_1-1).	
		Upon necropsy, increased relative and absolute liver weights were noted in dams of the high/dose group (see Table A6_8_1-1).	
4.2	**Teratogenic / embryotoxic effects**	A statistically significant increased incidence of skeletal variants was observed in the high/dose group. These variants included incomplete ossification and abnormal rib ossification centres. However, there was no clear dose/response relationship and the incidences were only marginally different from those observed in historical controls. Therefore, these findings are not considered as substance-related adverse effects.	X
		No further effects were noted (see Tables A6_8_1-2 and A6_8_1-3).	
4.3	**Other effects**	The test substance spread out beyond the intended dose site in the 200 and 400 mg/kg dose groups. A mean increase in dose site area of 12.4 and 34.6% was calculated for the 200 and 400 mg/kg dose groups, respectively.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	

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5.1	**Materials and methods**	The developmental toxicity of Icaridin after dermal exposure was tested in pregnant Sprague-Dawley rats. The study was conducted according to OECD Guideline 414.	
		The undiluted test substance was applied daily to a shaved dorsal area throughout the entire gestational period (day 0-20). Doses of 50, 200 or 400 mg/kg bw/day were applied. Rats wore Elizabethan collars to prevent ingestion of the test material.	
		Dams were sacrificed on gestational day 20 and foetuses were removed by Caesarean section and a gross maternal necropsy was performed. All foetuses were evaluated for external anomalies. Approximately half of each litter was examined for visceral effects; the other half underwent a skeletal examination.	
5.2	**Results and discussion**	No effects were observed on maternal body weight and food consumption. Dermal effects (scaling/sloughing) were observed at the dose site of all Icaridin-treated groups from around day 7 until the end of the study. These alterations were interpreted as an adaptive response to the cumulative exposure to the test material.	
		Compound-related necropsy findings were limited to the high-dose group and included increased liver weight. There were no statistically significant effects on any reproductive parameters or any embryologic endpoints. The incidence of malformations or developmental variants was not affected by exposure to Icaridin.	X
		A statistically significant decrease in the mean percentage of male foetuses per implantation site and the concomitant statistically significant increase in the mean number of females were observed in the high-dose group. These effects are thought to be incidental since the number of females per implantation site was not significantly elevated and the male-to-female ratio of the treated groups was close to that of historical controls.	
		An increased incidence of skeletal variants in foetuses of the high-dose group was not considered to be of toxicological relevance since there was no clear dose-response relationship and the incidences were within the range of those in historical controls.	X
5.3	**Conclusion**		
5.3.1	LO(A)EL maternal toxic effects	LOAEL = 400 mg/kg bw/day, based on increased liver weights	
5.3.2	NO(A)EL maternal toxic effects	NOAEL = 200 mg/kg bw/day	
5.3.3	LO(A)EL embryotoxic / teratogenic effects	LOAEL > 400 mg/kg bw/day	X

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5.3.4	NO(A)EL embryotoxic / teratogenic effects	NOAEL = 400 mg/kg bw/day	X
5.3.5	Reliability	1	
5.3.6	Deficiencies	No	

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Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	12 October 2006
Materials and Methods	Applicant's version is adopted
Results and discussion	Revised (Text in italics proposed changed). 4.1. No mortalities were observed. The majority of the clinical signs, primarily nasal stain, were observed independent of the dose and were ascribed to the wearing of Elizabethan collars. *Scab formation and scaly and/or sloughing skin was observed in all Icaridin-treated groups in a dose response manner (see Table A6_8_1-1).* The dermal irritation at the dose site was clearly treatment-related. These lesions are common findings with repeated exposure to a variety of treatments (including water or medical petrolatum) and are in general then not considered to be of no toxicological relevance. However since the use of the substance as a dermally applied repellent contact with the skin over a prolonged are expected and the effect could therefore be relevant. Upon necropsy, increased relative and absolute liver weights were noted. 5.2. § 2 and 4 revised (Text in italics proposed changed). § 2 Compound-related necropsy findings were limited to the high-dose group and included increased liver weight. There were no statistically significant effects on any reproductive parameters. The incidence of malformations was not affected by exposure to Icaridin. A dose-related (however not clear) increase in the incidence of delayed ossifications was noted receiving statistically significance in the highest dose group (see Table A6_8_1-3). § 4 The increased incidence of skeletal variants in foetuses of the high-dose group cannot be ruled out to be of toxicological relevance despite no clear dose response. The incidences were statistically significant and in two of three foci outside the range of those in the historical controls. The same type of delayed ossification was also noted in the highest dose group (500 mg/kg bw/day) in the oral dose range finding study in the rat (A6.8.1.(1). The increased incidence of delayed ossification is evaluated as an embryotoxic effect.

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Conclusion	Other conclusions: Revised version LO(A)EL embryotoxic / teratogenic effects: LOAEL = 400 mg/kg bw NO(A)EL embryotoxic / teratogenic effects: NOAEL = 200 mg/kg bw Maternal LOAEL = 400 mg/kg bw/day, based on increased liver weights Maternal NOAEL = 200 mg/kg bw/day
Reliability	1
Acceptability	Acceptable
Remarks	Comment: In the oral dose range finding study (single dose of 500 mg/kg bw/day) for embryotoxic effects in the rat (A6.8.1.(1)) the same delayed foetal ossifications were noted. The embryotoxic effect correlated with maternal toxicity (reduced weight gain during the entire study period,reduced feed intake and empty stomach observed after autopsy) and therefore considered as an unspecific secondary effect of maternal toxicity. In this study no such maternal toxicity was noted. It could very well be a coincidence as the historic range of this kind of finding is very wide but this is just an assumption. Until the background for the delayed ossifications is more elucidated it is considered an embryotoxic effect and should affect the settings of LOAEL and NOAEL. The endpoint differentiation of foetal skeletal alterations was not studied in the other two range finding studies performed before the key study.

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Table A6_8_1-1. Table for teratogenic effects

Maternal effects

Parameter	Control data		50 mg/kg	200 mg/kg	400 mg/kg	Dose-response + / –
	historical[+]	study				
Number of dams examined	187	24	29	27	28	
Clinical findings	–					
dosing site, scaling/sloughing (day 6-15)	–	0	3	20	24	+
nasal stain (day 6-15)	–	18	24	22	27	–
Mortality of dams [%]	0.0	0.0	0.0	0.0	0.0	–
Body weight gain [g] Day 0 - 20	–	131.3 ± 5.63	133.9 ± 3.67	140.2 ± 3.20	135.3 ± 4.80	–
Gravid uterus weight [g]	–	71.8	73.6	84.4*	78.1	–
Food consumption, range [g/kg bw/day]	–	88-94	87-98	87-98	87-98	–
Pregnancies [%]	85.6	100	100	100	100	–
Maternal liver weights						
absolute [g] [#]		15.3 ± 0.3	15.6 ± 0.3	16.1 ± 0.3	16.7 ± 0.3**	+
relative [%] [#]		4.14 ± 0.05	4.15 ± 0.05	4.19 ± 0.04	4.35 ± 0.05*	+

[+] Data from [redacted], studies were conducted between 1993 and 1995
[#] Mean ± S.E.
Significantly different from control: *p < 0.05; **p < 0.01

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Table A6_8_1-2. Table for teratogenic effects

Litter response (Caesarean section data)

Parameter	Control data		50 mg/kg	200 mg/kg	400 mg/kg	Dose-response + / –
	historical[#]	study				
Corpora lutea [total/number of dams]	16.4±0.22	14.4	14.8	15.7	15.5	–
Implantations [total/number of dams]	15.2±0.25	12.6	13.1	14.6	14.4	–
Resorptions [total/number of dams]	1.0±0.09	0.9	0.9	0.9	1.8	–
Total number of foetuses	2142	280	352	370	351	–
Pre-implantation loss [% per animal]	7.4±0.97	11.8	11.7	6.2	7.2	–
Post-implantation loss [% per animal]	7.1±0.82	7.7	6.6	6.3	12.8	–
Total number of litters	151	24	29	27	28	–
Foetuses / litter	–	11.67	12.14	13.70	12.54	–
Live foetuses / litter [ratio]	14.2±0.26	11.67	12.10	13.70	12.54	–
Dead foetuses / litter [ratio]	0.02	0.00	0.03	0.00	0.00	–
Foetus weight (mean) [g]	–	4.1	4.0	4.1	4.1	–
Placenta weight (mean) [g]	–	0.63	0.62	0.62	0.63	–
Foetal sex ratio						
ratio ♂/♀	1.037	1.373	1.041	0.968	0.867	+
% ♂ per implant	–	53.0	48.0	45.6*	40.6**	+
% ♀ per implant	–	39.4	45.4	48.0	46.6	–

Significantly different from control: *p < 0.05; **p < 0.01

[#] Data from [redacted], studies were conducted between 1993 and 1995

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Table A6_8_1-3. Table for teratogenic effects

Examination of the foetuses

Parameter	Control data		50 mg/kg	200 mg/kg	400 mg/kg	Dose-response + / –
	historical[#]	study				
External malformations [% of foetuses]	0.0	0.4	0.3	0.3	0.3	–
External variations [% of foetuses]	0.0	0.0	3.1**	0.3	0.0	–
Skeletal malformations [% of foetuses]	0.01	0.7	0.5	0.5	0.0	–
Skeletal variations [% of foetuses]	100.0	100.0	100.0	100.0	100.0	–
Incomplete ossification						
Supraoccipital	3.6-30.6	30.3	42.9	42.2	43.6*	–
Thoracic Centra	15.7-68.7	42.8	45.6	50.5	57.5*	–
Sternebrae #5	60.2-78.4	78.6	78.6	80.2	92.3*	–
Abnormal rib ossification	0.5-20.0	11.7	8.2	20.3	24.9**	–
Visceral malformations [% of foetuses]	0.01	3.0	0.6	1.7	1.2	–
Visceral variations [% of foetuses]	0.07	8.1	1.8*	5.1	11.8	–

Significantly different from control: *p < 0.05; **p < 0.01

[#] Data from [redacted], studies were conducted between 1993 and 1995

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			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted]. (2008): Icaridin – Developmental Toxicity Study in Rabbits after Oral Administration [redacted], Report No. AT05042, 2008-12-16 (unpublished)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Saltigo GmbH	
1.2.2	Company with letter of access	–	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, OECD Guideline 414 (January 2001)	
2.2	**GLP**	Yes	
2.3	**Deviations**	None	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in Section 2 of dossier.	
3.1.1	Lot/Batch number	CHCAEC0060	
3.1.2	Specification	As given in Section 2 of dossier.	
3.1.2.1	Description	Clear yellowish slightly viscous liquid	
3.1.2.2	Purity	98.9%	
3.1.2.3	Stability	approved for use until June 21, 2008	
3.2	**Test Animals**		
3.2.1	Species	Rabbit	
3.2.2	Strain	Himalayan rabbit CHBB:HM	
3.2.3	Source	Charles River Deutschland, Kißlegg, Germany	
3.2.4	Sex	Females (untreated males were used as sires)	
3.2.5	Age/weight at study initiation	Age: between 177 and 318 days old, bw: 2475 to 3455 g	X
3.2.6	Number of animals per group	22-25 females	
3.2.7	Control animals	Yes	
3.2.8	Mating period	Day 0: between 5 a.m. and 10 a.m.	

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3.3	**Administration/ Exposure**	**Oral**
3.3.1	Duration of exposure	Day 6-28 post mating
3.3.2	Postexposure period	n.a.
3.3.3	Type	Gavage
3.3.4	Doses	0, 100, 300, 1000 mg/kg bw
3.3.5	Vehicle	0.5% carboxymethylcellulose (CMC) in demineralised water
3.3.6	Concentration in vehicle	20, 60, and 200 mg/mL
3.3.7	Total volume applied	5 mL/kg bw
3.3.8	Controls	Vehicle
3.4	**Examinations**	
3.4.1	Body weight	On day 0 p.c. and daily from day 6 to day 29 p.c. Corrected body weight gain was determined by subtracting the uterus weight on day 29 from the body weight gain from days 0 to 29 p.c.
3.4.2	Food consumption	Days 0-3, 3-6, 6-9, 9-12, 12-15, 15-18, 18-20, 20-21, 21-24, 24-27, and 27-29 p.c.
3.4.3	Water consumption	Water intake was assessed daily by visual estimation of the quantities left over and reported together with clinical findings.
3.4.4	Clinical signs	Twice daily from days 0 to 29 p.c. twice daily (once daily only on weekends, on public holidays, and on day 29 p.c.)
3.4.5	Examination of uterine content	– number of corpora lutea and implantations – uterus weight – individual weights and appearance of the placentas
3.4.6	Maternal organ weights	liver and kidneys (pairwise)
3.4.7	Examination of foetuses	

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3.4.7.1	General	– number of live foetuses – number of early resorptions (only implantation site visible), late resorptions (foetal or placental remnant visible) and dead foetuses (foetuses without signs of life, but without maceration) – sex of live foetuses – individual weights of live foetuses – occurrence of external findings in the foetuses
3.4.7.2	Skeleton	Occurrence of findings in the skeletal system (clearing of the foetuses with diluted potassium hydroxide solution, staining of the skeletal system for skeletal evaluation including cartilaginous findings with Alcian Blue and Alizarin Red S, evaluation of the skeletal system (modified DAWSON/INOUYE technique)
3.4.7.3	Soft tissue	Occurrence of findings in abdominal, pelvic, and thoracic organs and in the brain (evisceration by the modified STAPLES technique including a transverse section through the brain)
3.5	**Further remarks**	–
		4 RESULTS AND DISCUSSION
4.1	**Maternal toxic effects**	
4.1.1	Mortality	Two females of the 1000 mg/kg group and one female of the 300 mg/kg group were found dead after they had shown clear signs of maternal toxicity.
4.1.2	Abortions	Eight females of the 1000 mg/kg group aborted after they had shown signs of maternal toxicity. A treatment-related effect on the abortion of one female of the 300 mg/kg group cannot be excluded since this doe displayed signs of maternal toxicity (markedly decreased feed intake, marked body weight loss after start of treatment -174 g, reduced amounts of faeces, decreased water consumption and thus decreased and discoloured urination).
4.1.3	Feed intake and excretory products	The females which were found dead or aborted at the 1000 mg/kg and 300 mg/kg levels showed slightly to severely decreased feed intake. Decreased mean feed intake occurred in females with viable foetuses at the 1000 mg/kg level, and individual feed intake was transiently markedly decreased in several females of the 300 mg/kg level (see Table A6_8_1-1). A slightly increased incidence of reduced amounts of faeces occurred in the 1000 mg/kg group, for which a treatment-related effect cannot be excluded.
4.1.4	Body weight	Body weight development was markedly impaired in females with viable foetuses at the 1000 mg/kg level (see Table A6_8_1-2). Furthermore, body weight loss occurred in three females with viable foetuses at the 300 mg/kg level during the treatment period, for which a treatment-related effect cannot be excluded. Body weight development was marginally to severely affected in females of the 300 mg/kg and 1000 mg/kg groups which were found dead or aborted.

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4.1.5	Necropsy	Two females of the 1000 mg/kg level which aborted or which were found dead, revealed findings of the abdominal cavity (e.g. enlarged stomach and caecum, hardened fatty tissue caecum with gaseous contents, light discoloured liver). One female of the 300 mg/kg group which was found dead showed a lung with haemorrhage, most likely of perimortal origin.
4.1.6	Organ weights	Table A6_8_1-3 shows that the mean absolute liver weights as well as the liver and kidneys weights to carcass weights ratios were statistically significantly increased at the 1000 mg/kg level.
4.1.7	Appearance and weight of placentas	A treatment-related effect on placentas with hardened and light or dark discoloured parts cannot be excluded at the 1000 mg/kg level, and placental weights were decreased at this dose level.
4.2	**Teratogenic / embryotoxic effects**	
4.2.1	Gestation rate	The gestation rate of the 1000 mg/kg group was markedly decreased by the abortion of eight females, and it was slightly decreased at the 300 mg/kg level by the abortion of one female, for which a treatment-related effect cannot be excluded (see Table A6_8_1-4), although one female of the control group also aborted. Other parameters of intrauterine development are summarised in Table A6_8_1-5.
4.2.2	Foetal weights	Foetal weights were distinctly decreased at the 1000 mg/kg level. The litter weight was also slightly decreased (without statistical significance) at the 1000 mg/kg level. Foetal weights at the 300 mg/kg level were slightly decreased when compared to concurrent controls (36.91 vs. 42.67 g) but lay well inside the range of historical control data (32.36 g – 41.32 g). In addition, the litter size and the mean litter weight at this dose level were higher than in the current control group. Incidentally, the foetal weights of the current control group were higher than the upper range of historical control data (see Table A6_8_1-5). Thus, the lower mean foetal weight in the 300 mg/kg group is thought to be without toxicological significance.
4.2.3	Foetal malformations	The overall incidences of foetuses or litters with malformations revealed the lowest percentages in the 1000 mg/kg and 300 mg/kg groups (see Table A6_8_1-6). The findings seen in the dose groups also occurred in the current control group (malposition of forelimb(s), fused ribs in the cartilaginous part with/without bifurcation or thickening, fusion of caudal vertebral bodies) or are known as spontaneous findings in the historical control data of the rabbit strain used (abdominal hernia with/without missing forelimbs), cleft palate, cardiac ventricular septal defect, cardiac ventricular septal defect with findings of the major vessels, multiple skeletal malformation, missing presacral vertebra). Although cleft palate is known as a spontaneous finding in the rabbit strain used (see above), and the father of the affected foetus also sired a foetus with a cleft palate in the low dose group of a recent study (conducted in 2008), an unspecific treatment-related effect for one foetus

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		with this finding at the 1000 mg/kg level cannot totally be excluded, as this foetus revealed a very low foetal weight (13.58 g).	
4.2.4	Foetal external and visceral deviations	A treatment-related effect on external and visceral deviations was not evident at dose levels up to and including 1000 mg/kg (see Table A6_8_1-7).	
4.2.5	Foetal skeletal deviations	Foetal examinations for skeletal retardations and variations revealed retarded ossification at the 1000 mg/kg level, for which a treatment-related effect is assumed due to the markedly decreased foetal weights at this dose level. A treatment-related effect cannot be excluded for retarded ossification of the 5th sternebrae and hyoid body at the 300 mg/kg level due to the slightly decreased foetal weight. Furthermore, a treatment-related effect is assumed for a slightly increased incidence of fused sternebrae at the 1000 mg/kg level.	X
4.3	**Other effects**	–	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Twenty-five (0 mg/kg group), 22 (100 mg/kg and 300 mg/kg groups) or 23 (1000 mg/kg group) female Himalayan rabbits each were treated daily by oral administration (gavage) with Icaridin (active ingredient: 98.9%) using 0.5% carboxymethylcellulose (CMC) in demineralized water as the vehicle from days 6 to 28 p.c. in the following doses: 0, 100, 300, and 1000 mg/kg body weight/day, respectively. On day 29 of gestation the foetuses were delivered by Caesarean section. The study was conducted in 2007-2008 following OECD guideline 414 (2001 version).	
5.2	**Results and discussion**	Two females of the 1000 mg/kg group and one female of the 300 mg/kg group were found dead after they had shown clear signs of maternal toxicity. Eight females of the 1000 mg/kg group aborted after they had shown signs of maternal toxicity. A treatment-related effect on the abortion of one female of the 300 mg/kg group cannot be excluded. The females which were found dead or aborted at the 1000 mg/kg and 300 mg/kg levels showed slightly to severely decreased feed intake. Decreased mean feed intake occurred in females with viable foetuses at the 1000 mg/kg level, and individual feed intake was transiently markedly decreased in several females of the 300 mg/kg level. Body weight development was markedly impaired in females with viable foetuses at the 1000 mg/kg level. Furthermore, body weight loss occurred in three females with viable foetuses at the 300 mg/kg level during the treatment period, for which a treatment-related effect cannot be excluded. Body weight development was marginally to severely affected in females of the 300 mg/kg and 1000 mg/kg groups which were found dead or	

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aborted.

Two females of the 1000 mg/kg level which aborted or which were found dead, revealed findings of the abdominal cavity (e.g. enlarged stomach and caecum, hardened fatty tissue caecum with gaseous contents, light discoloured liver). One female of the 300 mg/kg group which was found dead showed a lung with haemorrhage, most likely of perimortal origin (see below).

Mean absolute liver weights as well as the liver and kidneys weights to carcass weights ratios were increased at the 1000 mg/kg level.

The lung of the deceased female of the 300 mg/kg group, reported with haemorrhage at necropsy, showed slight acute congestion at histopathological examination, most likely of perimortal origin. All other females examined after the scheduled necropsy did not reveal any compound-related effects in their livers and kidneys at dose levels up to and including 1000 mg/kg.

The gestation rate of the 1000 mg/kg group was markedly decreased by the abortion of eight females, and it was slightly decreased at the 300 mg/kg level due to the abortion of one female, for which a treatment-related effect cannot be excluded.

A treatment-related effect on placentas with hardened and light or dark discoloured parts cannot be excluded at the 1000 mg/kg level, and placental weights were decreased at this dose level.

Postimplantation loss in females with viable foetuses at Caesarean section and correspondingly the mean number of foetuses as well as foetal sex distribution were unaffected at dose levels up to and including 1000 mg/kg.

The foetal weights were distinctly decreased at the 1000 mg/kg level. A treatment-related effect for the slightly decreased foetal weights at the 300 mg/kg level cannot totally be excluded. However, this finding is thought to be without toxicological significance since the foetal weights were within the range of historical control values.

A treatment-related effect on malformations was not evident at dose levels up to and including 300 mg/kg. A specific teratogenic potential is also excluded at the 1000 mg/kg level, but an unspecific treatment-related effect for the isolated finding of a cleft palate in a low weight foetus at this dose level cannot totally be excluded.

A treatment-related effect on external and visceral deviations was not evident at dose levels up to and including 1000 mg/kg.

Foetal examinations for skeletal retardations and variations revealed retarded ossification at the 1000 mg/kg level, for which a treatment-related effect is assumed due to the markedly decreased foetal weights at this dose level. A treatment-related effect cannot be excluded for retarded ossification of the 5th sternebrae and hyoid body at the 300 mg/kg level due to the slightly decreased foetal weight. Furthermore, a treatment-related effect is assumed for a slightly increased incidence of fused

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		sternebrae at the 1000 mg/kg level. Final evaluation of the stage of foetal development (weight, ossification) is not possible at the 300 mg/kg level, as the litter size was incidentally higher in this group.	
5.3	**Conclusion**	**Icaridin is not teratogenic or embryotoxic in the rabbit via the oral route.**	X
5.3.1	LO(A)EL maternal toxic effects	LOAEL = 300 mg/kg bw/day, based on reduced feed intake, bw loss, clinical signs and abortion	
5.3.2	NO(A)EL maternal toxic effects	NOAEL = 100 mg/kg bw/day	
5.3.3	LO(A)EL embryo toxic / teratogenic effects	LOEL = 300 mg/kg bw/day, based on reduced foetal weights compared to concurrent controls LOAEL =1000 mg/kg bw/day, based on reduced foetal weights compared to historical controls	
5.3.4	NO(A)EL embryo toxic / teratogenic effects	NOEL = 100 mg/kg bw/day NOAEL = 300 mg/kg bw/day	
5.3.5	Reliability	1	
5.3.6	Deficiencies	No	

Section A6.8.1 Teratogenicity Study

Annex Point IIA VI.6.8.1 6.8.1 Oral teratogenicity study in the rabbit

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	09.03.2009
Materials and Methods	3.2.5 …bw: 2475 to *3496* g.
Results and discussion	Applicant's version is adopted with the addition of table A6_8_1_8 on foetal skeletal deviation.
Conclusion	
	5.3 Conclusion
	5.3.7 $LOAEL_{maternal}$ = 300 mg/kg bw/day, based on reduced feed intake, bw loss, clinical signs and abortion.
	5.3.8 $NOAEL_{maternal}$ = 100 mg/kg bw/day
	5.3.9 $LOAEL_{foetal}$ = 300 mg/kg bw/day, based on reduced foetal weights and retarded ossification of 5^{th} sternebrae and hyoid body.
	5.3.10 $NOAEL_{foetal}$ = 100 mg/kg bw/day
Reliability	1
Acceptability	5.3.11 Acceptable
Remarks	5.3.12 Number of foetuses was below and foetal weight and placental weight was above historical controls in the control group in this study.

Section A6.8.1 Teratogenicity Study

Annex Point IIA VI.6.8.1 6.8.1 Oral teratogenicity study in the rabbit

Table A6_8_1-1 Mean Feed Intake

Mean feed intakes (g/animal/day)	Dose (mg/kg bw/day)				Dose response +/–
	0	100	300	1000	
days 0 - 3 p.c.	91.5	94.3	91.4	88.1	–
days 3 - 6 p.c.	84.9	84.3	80.9	81.1	–
days 6 - 9 p.c.	79.8	77.9	69.3	47.1**	+
days 9 - 12 p.c.	78.5	73.6	70.5	55.6**	+
days 12 - 15 p.c.	68.5	62.8	61.0	46.9**	+
days 15 - 18 p.c.	70.6	64.6	55.4	40.9**	+
days 18 - 20 p.c.	70.7	70.8	64.4	50.5	+
days 20 - 21 p.c.	66.4	65.4	60.0	48.8	+
days 21 - 24 p.c.	69.8	67.9	63.2	51.8	+
days 24 - 27 p.c.	76.4	71.1	69.0	60.3	+
days 27 - 29 p.c.	79.6	75.0	72.8	62.4*	+

Statistically significant difference to control * = $p < 0.05$
Statistically significant difference to control ** = $p < 0.01$

Table A6_8_1-2 Mean Body Weight Gain

	Historical control range 2000-2006	Dose (mg/kg bw/day)				Dose response +/–
		0	100	300	1000	
absolute bw gain (g) days 6 - 29 p.c	46.5-362.0	123.5	113.0	87.3	11.5*	+
absolute bw gain (g) days 0 - 29 p.c.	65.2-473.5	152.4	138.0	112.4	31.4	+
corrected bw gain (g) days 0 - 29 p.c.	+65.2 – -291.8	-192.0	-227.6	-264.3	-277.1	+

Statistically significant difference to control * = $p < 0.05$

Section A6.8.1 Teratogenicity Study

Annex Point IIA VI.6.8.1 6.8.1 Oral teratogenicity study in the rabbit

Table A6_8_1-3 Mean Absolute Liver and Combined Kidneys Weights (g) and Mean Liver and Kidneys Weights to Carcass Weights Ratios (%)

	Dose (mg/kg bw/day)				Dose response +/–
	0	100	300	1000	
Absolute weights					
Liver	57.283 g	58.608 g	60.184 g	66.733 g**	+
Kidneys	13.239 g	13.494 g	13.787 g	14.513 g	+
Relative weights					
Liver	2.1890%	2.1678%	2.2868%	2.5876%**	+
Kidneys	0.5062%	0.5028%	0.5230%	0.5617%**	+

Statistically significant difference to control ** = p < 0.01

Table A6_8_1-4 Gestation Rate

	Historical control range 2000-2006	Dose (mg/kg bw/day)				Dose response +/–
		0	100	300	1000	
Does with viable foetuses on day 29 p.c.	/					
n	/	19	22	19	12	+
in % of females with implantations	90-100	95	100	95	60	+
abortions, n	0-1	1	0	1	8	+
total resorptions, n	0-2	0	0	0	0	–

Section A6.8.1 Teratogenicity Study

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Table A6_8_1-5 Mean Values of the Parameters of Intrauterine Development

	Historical control range 2000-2006	Dose (mg/kg bw/day) 0	100	300	1000	Dose response +/–
number of females (without females displaying abortions)						
with implantations (a)	/	19	22	19	12	+
with viable foetuses (b)	/	19	22	19	12	+
mean values per female						
placental weight in g [b]	4.05-4.90	5.29	5.11	4.85	4.22**	+
number of foetuses [b]	5.9-8.2	5.2	6.0	6.7	6.8	+
postimplantation loss [a]	0.3-1.0	1.4	0.9	0.7	1.0	–
males in% [b]	41.2-57.1	46.0	53.7	46.8	40.4	–
foetal weight in g [b]	32.36-41.32	42.67	40.15	36.91**	29.71**	+
litter weight in g [b]	/	217.44	237.03	244.96	199.93	–

Statistically significant difference to control ** = $p < 0.01$

[a] Value calculated for females with implantations

[b] Value calculated for females with viable foetuses

Section A6.8.1 Teratogenicity Study

Annex Point IIA VI.6.8.1 6.8.1 Oral teratogenicity study in the rabbit

Table A6_8_1-6: Foetal Malformations

Malformation	Historical control range 2000-2007	Dose (mg/kg bw/day) 0	100	300	1000	Dose response +/–
abdominal hernia with/without missing forelimbs		-	1	1	-	–
malposition of forelimb(s)	3(1)-5(3)	2 (2)	5 (3)	2 (2)	1	–
cleft palate	0	-	-	-	1	–
cardiac ventricular septal defect	1-4(4)	-	2 (2)	1	-	–
cardiac ventricular septal defect, ascending aorta and aortic arch smaller than normal, and pulmonary artery enlarged	1	-	-	1	-	–
fused ribs in the cartilaginous part with/without bifurcation or thickening	2(1)-3(2)	3 (2)	1	1	1	–
multiple skeletal malformations[#]	/	-	1	-	-	–
thickened ribs	/	1	-	-	-	–
missing presacral vertebra	5(3)	-	1	-	-	–
fusion of caudal vertebral bodies	/	1	1	1	1	–
number of foetuses per group	119-156	98	131	127	82	+
number of foetuses with malformations	0-18	7	10	6	3	–
malformed foetuses per group (%)	0.0-13.4	7.1	7.6	4.7	3.7	–
number of litters per group	18-21	19	22	19	12	+
number of litters with malformations	0-10	5	7	5	2	–
malformed litters per group (%)	0.0-47.6	26.3	31.8	26.3	16.7	–

() number of litters affected

[#] including missing scapulae and forelimbs, fused and bent ribs, spina bifida, fusion of vertebrae, and missing parts of frontal and parietal bones

Section A6.8.1 Teratogenicity Study

Annex Point IIA VI.6.8.1 6.8.1 Oral teratogenicity study in the rabbit

Table A6_8_1-7: Foetal External and Visceral Deviations

Deviation	Dose (mg/kg bw/day)				Dose response +/–
	0	100	300	1000	
retina slightly folded	-	2 (2)	2 (1)	2 (2)	–
slight dilation of lateral brain ventricle(s)	-	2 (1)	3 (2)	1	–
clear fluid in abdominal cavity	-	-	1	-	–
whitish discoloration of liver lobes	1	3 (3)	2 (2)	-	–
number of foetuses per group	98	131	127	82	+
number of foetuses with deviations	1	7	8	3	–
foetuses with deviat. per group (%)	1.0	5.3	6.3	3.7	–
number of litters per group	19	22	19	12	+
number of litters with deviations	1	6	5	2	–
litters with deviat. per group (%)	5.3	27.3	26.3	16.7	–

() number of litters affected

Table A6_8_1-8: Summary of significant Foetal skeletal Deviations

Deviation	Dose (mg/kg bw/day)							
	0		100		300		1000	
Number of foetuses	98		131		121		82	
	Number	%	Number	%	Number	%	Number	%
Cervical vertebral bodies Incompletely ossified, 1st	6	6.1	17	13.0	16	12.6	26**	31.7
Medial phalanx digits incompletely ossified								
5th right	1	1.0	2	1.5	5	3.9	13**	15.9
5th left	1	1.0	2	1.5	5	3.9	12**	14.6
Medial phalanx toes incompletely ossified								
5th right	1	1.0	1	0.8	2	1.6	12**	14.6
5th left	0	0.0	1	0.8	3	2.4	12**	14.6
Sternebrae unossified, 5th	12	12.2	17	13.0	34*	26.8	26**	31.7
Sternabrae fusion	3	3.1	12	9.2	10	7.9	19**	23.2
Hyoid body, incompletely ossified	78	79.6	102	77.9	120**	94.5	75	91.5

*P<0.05 ** P<0.01

Section A6.8.2 Multigeneration Reproduction Toxicity Study

Annex Point IIA VI.6.8.2 6.8.2 Dermal two-generation study in the rat

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1996c), A Two Generation Reproductive Toxicity Study with KBR 3023 Technical in the Sprague-Dawley Rat. [redacted] Report No. 107489, 1996-12-18 (unpublished)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Saltigo GmbH	
1.2.2	Company with letter of access	–	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes US-EPA FIFRA §83-4 (1984) EC Method B.35 (1987) OECD Guideline 416 (1983)	
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes, the following deviations to OECD 416 in its current version (January 2001) were noted: – Sperm parameters were not assessed – Uterus, epididymides, prostate, seminal vesicles, adrenals, spleen, brain, pituitary, and thyroids were not weighed	X
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in Section 2 of dossier.	
3.1.1	Lot/Batch number	030693	
3.1.2	Specification	As given in Section 2 of dossier.	
3.1.2.1	Description	Clear, colourless liquid	
3.1.2.2	Purity	96.7–97.7%	X
3.1.2.3	Stability	Verified by three analyses prior to, during and after the exposure period	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Sprague-Dawley	
3.2.3	Source	Sasco, Inc., Omaha, NE, USA	
3.2.4	Sex	♂+♀	

Section A6.8.2 Multigeneration Reproduction Toxicity Study

Annex Point IIA VI.6.8.2 6.8.2 Dermal two-generation study in the rat

3.2.5	Age/weight at study initiation	7-8 weeks of age, ♂: 197-276 g bw, ♀: 130-199 g bw
3.2.6	Number of animals per group	30/sex/group
3.2.7	Mating	See Table A6_8_2-1 below
3.2.8	Duration of mating	Up to 21 days
3.2.9	Deviations from standard protocol	Litters were culled to 8 pups/litter on day 4 of lactation
3.2.10	Control animals	Yes
3.3	**Administration/ Exposure**	Dermal
3.3.1	Animal assignment to dosage groups	See Table A6_8_2-1 below
3.3.2	Duration of exposure before mating	10 weeks
3.3.3	Duration of exposure in general P, F1, F2 males, females	P animals: from beginning of the study until sacrifice F1 animals: from weaning until sacrifice
		Dermal
3.3.4	Area covered	10% of body surface
3.3.5	Occlusion	Non-occlusive, without dressing. Elizabethan collars were used to prevent oral uptake of the test material.
3.3.6	Vehicle	Undiluted
3.3.7	Concentration in vehicle	–
3.3.8	Dose levels	50, 100, 200 mg Icaridin/kg bw/day
3.3.9	Duration of exposure	24 h, 5 days per week
3.3.10	Removal of test substance	No
3.3.11	Controls	Yes, shaved but untreated
3.4	**Examinations**	
3.4.1	Clinical signs	Yes, twice daily
3.4.2	Body weight	Yes, once weekly during; F1 pups every 3 days; on days 0, 6, 13, and 20 of gestation, and on days 0, 7, 14, and 21 of lactation

Section A6.8.2 Multigeneration Reproduction Toxicity Study

Annex Point IIA VI.6.8.2 6.8.2 Dermal two-generation study in the rat

3.4.3	Food consumption	Yes, daily during pre-mating, on days 0, 6, 13, and 20 of gestation, and on days 0, 7, 14, and 21 of lactation
3.4.4	Oestrus cycle	Yes, daily for 10 animals/group (P+F1) for 3 weeks prior to mating
3.4.5	Sperm parameters	Testes weights, histopathological examination of testes and epididymides
3.4.6	Offspring	Number and sex of pups, stillbirths, live births, presence of gross anomalies, weight gain, physical or behavioural abnormalities
3.4.7	Organ weights P and F1	Ovaries, testes, liver, kidneys
3.4.8	Histopathology P and F1	Cervix, vagina, gross lesions, kidney, liver, uterus, ovaries, testes, epididymides, seminal vesicle, prostate, coagulating gland, treated skin
3.4.9	Histopathology F1 not selected for mating, F2	None
3.5	**Further remarks**	–

4 RESULTS AND DISCUSSION

4.1	**Effects**	
4.1.1	Parent males	All dose groups: topical skin lesions (scaling/sloughing, scab formation) No systemic effects. No effects on reproductive performance (see Table A6_8_2-2).
4.1.2	Parent females	All dose groups: topical skin lesions (scaling/sloughing, scab formation) No systemic effects. No effects on reproductive parameters (see Table A6_8_2-2).
4.1.3	F1 males	All dose groups: topical skin lesions (scaling/sloughing, scab formation) No systemic effects. No effects on reproductive performance (see Table A6_8_2-2).
4.1.4	F1 females	All dose groups: topical skin lesions (scaling/sloughing, scab formation) No systemic effects. No effects on reproductive parameters (see Table A6_8_2-2).
4.1.5	F2 males	No treatment-related necropsy findings
4.1.6	F2 females	No treatment-related necropsy findings
4.2	**Other**	–

5 APPLICANT'S SUMMARY AND CONCLUSION

Section A6.8.2 Multigeneration Reproduction Toxicity Study

Annex Point IIA VI.6.8.2 6.8.2 Dermal two-generation study in the rat

5.1	**Materials and methods**	A two-generation reproductive toxicity study was conducted with dermally administered Icaridin. The study was performed in accordance with OECD Guideline 416 in its original version (1983). The P generation was fitted with Elizabethan collars and treated with 0, 50, 100, or 200 mg undiluted Icaridin/kg bw throughout the study on a 5-days-per-week basis. Following mating, the males were sacrificed and subjected to gross necropsy. Pups culled on day 4 of lactation, pups not selected to be F1 generation parents, and all dams following weaning of their litters underwent gross necropsy. F1 pups that were selected to be parents of the F2 generation were fitted with collars and dosed following weaning. Histopathological examinations were performed on adult animals.	
5.2	**Results and discussion**	There were no compound-related clinical signs or effects on body weight or food consumption observed in either the adults or the pups during any phase of the study. There were no compound-related effects on any reproductive or litter parameter. Dermal findings at the dose site were noted in both generations. Although these dermal findings are considered to be a consequence of the dermal application methodology, a compound-related effect cannot be definitively discounted. Other than the dermal findings, no compound-related necropsy findings were seen in either the adults or the pups. No compound-related histopathologic findings were noted in the reproductive tissues of either the males or the females.	X
5.3	**Conclusion**		
5.3.1	LO(A)EL		
5.3.1.1	Parent males	No compound-related effects, LOAEL > 200 mg/kg bw/day	
5.3.1.2	Parent females	No compound-related effects, LOAEL > 200 mg/kg bw/day	
5.3.1.3	F1 males	No compound-related effects, LOAEL > 200 mg/kg bw/day	
5.3.1.4	F1 females	No compound-related effects, LOAEL > 200 mg/kg bw/day	
5.3.1.5	F2 males	No compound-related effects, LOAEL > 200 mg/kg bw/day	
5.3.1.6	F2 females	No compound-related effects, LOAEL > 200 mg/kg bw/day	
5.3.2	NO(A)EL		X

Section A6.8.2 Multigeneration Reproduction Toxicity Study

Annex Point IIA VI.6.8.2 6.8.2 Dermal two-generation study in the rat

5.3.2.1	Parent males	NOAEL = 200 mg/kg bw/day	
5.3.2.2	Parent females	NOAEL = 200 mg/kg bw/day	
5.3.2.3	F1 males	NOAEL = 200 mg/kg bw/day	
5.3.2.4	F1 females	NOAEL = 200 mg/kg bw/day	
5.3.2.5	F2 males	NOAEL = 200 mg/kg bw/day	
5.3.2.6	F2 females	NOAEL = 200 mg/kg bw/day	
5.3.3	Reliability	1	
5.3.4	Deficiencies	No	X
		The study fully complies with the version of OECD 416 that was in effect at the time the study was conducted.	
		Although the number of parameters stipulated by the updated version of OECD 416 has increased, the conclusion that Icaridin has no adverse effects on reproduction can be drawn based on the original data requirements.	

Section A6.8.2 Multigeneration Reproduction Toxicity Study

Annex Point IIA VI.6.8.2 6.8.2 Dermal two-generation study in the rat

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	12 October 2006
Materials and Methods	Applicant's version is adopted
Results and discussion	Applicant's version is adopted. Even though no effects were seen in the highest tested dose in this study, the dermal penetration study in rats and the significant effects on the major excretions organs (indicating transport of foreign substance) liver and kidney at 400 and 500 mg/kg bw/day in the developmental and 90 days rat study reassures that the substance has been seen systemically absorbed.
Conclusion	Applicant's version is adopted with the addition of a NOAEL for local effects. LOAEL for local effects (skin lesions)= 50 mg/kg bw/day NOAEL for local effects (skin lesions) <50 mg/kg bw/day
Reliability	1
Acceptability	Acceptable
Remarks	Do not fully comply with current version of OECD guideline 416 (2001) – Sperm parameters were not assessed – Uterus, epididymides, prostate, seminal vesicles, adrenals, spleen, brain, pituitary, and thyroids were not weighed – No systemic toxicity at any dose level 3.1.2.2 - The lowest purity (96.7%) measured is below the limits of the test substance given in section 2 of the dossier.

Section A6.8.2 Multigeneration Reproduction Toxicity Study

Annex Point IIA VI.6.8.2 6.8.2 Dermal two-generation study in the rat

Table A6_8_2-1. Table for animal assignment for mating

		Number of animals			
		Controls	**50 mg/kg**	**100 mg/kg**	**200 mg/kg**
Parents	**m**	30	30	30	30
	f	30	30	30	30
F_1	**m**	30	30	30	30
	f	30	30	30	30

Section A6.8.2 Multigeneration Reproduction Toxicity Study

Annex Point IIA VI.6.8.2 6.8.2 Dermal two-generation study in the rat

Table A6_8_2-2. Table for reproductive toxicity study

Parameter		Generation	Controls		50 mg/kg		100 mg/kg		200 mg/kg		Dose-response	
			♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Clinical Observations	Incidence											
Nose, nasal stain		**P**	28/30	29/30	29/30	30/30	29/30	30/30	27/30	30/30	–	–
		F1	30/30	29/30	29/30	30/30	30/30	27/30	23/30	23/30	–	–
Histopathology	Incidence											
Treated skin, acanthosis		**P**	28/30	6/30	28/30	15/30	29/30	24/30	30/30	27/30	–	+
		F1	16/30	6/30	23/29	15/30	27/30	24/30	28/29	27/30	+	+
Treated skin, hyperkeratosis		**P**	30/30	24/30	30/30	24/30	30/30	29/30	30/30	30/30	–	–
		F1	22/30	24/30	26/29	24/30	30/30	29/30	29/29	30/30	+	+
Treated skin, chronic active inflammation		**P**	1/30	0/30	9/30	0/30	5/30	2/30	5/30	3/30	–	+
		F1	0/30	0/30	0/30	0/30	0/30	2/30	0/30	3/30	–	+

Reproductive Performance			Controls	50 mg/kg	100 mg/kg	200 mg/kg	Dose-response
Mating index	%	**P**	100.0	100.0	96.7	100.0	–
		F1	100.0	100.0	96.7	100.0	–
Fertility index	%	**P**	100.0	96.7	89.7	100.0	–
		F1	96.7	90.0	89.7	96.7	–
Number of implantation sites	Mean	**P**	12.6	12.8	12.9	12.1	–
		F1	12.0	12.3	12.3	11.4	–
Duration of pregnancy	Mean [days]	**P**	22.5	22.5	22.0	22.5	–
		F1	22.5	22.6	22.4	22.5	–
Birth index	%	**F1**	94.0	90.8	92.2	89.3	–
		F2	90.6	91.8	92.8	87.5	–
Live birth index	%	**F1**	97.5	98.1	98.4	98.1	–
		F2	99.1	96.6	95.9	94.4	–
Gestation index	%	**F1**	100.0	96.6	100.0	100.0	–
		F2	96.8	100.0	100.0	100.0	–
Litter size	Mean	**F1**	11.9	11.6	11.9	11.1	–
		F2	10.9	11.3	11.4	10.2	–
Pup weight	Mean [g]	**F1**	6.7	6.7	6.7	6.8	–
		F2	6.8	6.7	6.5	6.8	–
Sex ratio	% Males	**F1**	50.2	44.8	47.1	45.4	–
		F2	52.1	56.0	54.9	51.9	–
Viability index	%	**F1**	98.8	98.3	96.8	97.5	–
		F2	97.1	93.1	96.0	91.7	–

Section A6.8.2 Multigeneration Reproduction Toxicity Study

Annex Point IIA VI.6.8.2 6.8.2 Dermal two-generation study in the rat

Lactation index	%	**F1**	99.0	98.7	100.0	98.9	–
		F2	99.6	99.5	99.5	99.5	–
Sperm characterization		Not performed					

Section A6.9 Delayed Neurotoxicity

Annex Point IIIA VI.1 6.9 Acute dermal neurotoxicity study in rats

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1 REFERENCE

1.1 Reference [redacted] (1996a), An Acute Dermal Neurotoxicity Screening Study with Technical Grade KBR 3023 in Fischer 344 Rats. [redacted], Report No. 107467, 1996-10-14 (unpublished)

1.2 Data protection Yes

1.2.1 Data owner Saltigo GmbH

1.2.2 Company with letter of access –

1.2.3 Criteria for data protection Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study Yes

US-EPA FIFRA 81-8 (SS), ≅ OECD Guideline 424(1997)

2.2 GLP Yes

2.3 Deviations None

3 MATERIALS AND METHODS

3.1 Test material As given in Section 2 of dossier

3.1.1 Lot/Batch number 030693

3.1.2 Specification Technical grade Icaridin

3.1.2.1 Description Clear, colourless liquid

3.1.2.2 Purity 97.4-97.7%

3.1.2.3 Stability Confirmed by analyses conducted before and after the exposure period.

3.2 Reference Substance (positive control) Historical positive controls: acrylamide and carbaryl.

3.3 Test Animals

Section A6.9 Delayed Neurotoxicity

Annex Point IIIA VI.1 6.9 Acute dermal neurotoxicity study in rats

3.3.1	Species	Rat
3.3.2	Strain	Fischer 344 CDF (F-344)/BR Rats
3.3.3	Source	Sasco, Inc., Madison, WI, USA
3.3.4	Sex	♂ + ♀
3.3.5	Age/weight at study initiation	9 weeks Mean weight: ♂: 185 g; ♀: 145 g
3.3.6	Number of animals per group	12 per sex per dose group
3.3.7	Control animals	Yes
3.4	**Administration**	Dermal
3.4.1	Exposure	Single administration onto ~10% of the body surface, undiluted test material. All animals wore rodent jackets to prevent access to the dosing site until 24 h after dosing.
3.4.2	Dose Levels	0, 200, 600 and 2000 mg/kg bw
3.4.3	Vehicle	None
3.4.4	Total volume applied	Depends on dose and body weight
3.4.5	Post exposure period	None
3.4.6	Anticholinergic substances used	None
3.4.7	Controls	Untreated
3.5	**Examinations**	
3.5.1	Body Weight	Weighing before application and during study in weekly intervals Perfused animals were also weighed on the day sacrificed for terminal body weight measurement
3.5.2	Signs of Toxicity	Examination for mortality and moribundity: at least once daily Detailed physical examination for clinical signs of toxicity: once daily
3.5.3	Observation schedule	Functional Observational Battery and figure-eight maze: One week prior to treatment, 4 h, 7 d, and 14 d after treatment.
3.5.4	Clinical Chemistry	No
3.5.5	Pathology	Yes, all animals.

Section A6.9 Delayed Neurotoxicity

Annex Point IIIA VI.1 6.9 Acute dermal neurotoxicity study in rats

	Organs:	All organs (including the brain), body cavities, cut surfaces, external orifices and surfaces.
3.5.6	Histopathology	Yes. Six animals per sex and dose group were perfused and tissues were collected and fixed for histopathology. Tissues from high-dose and control animals were selected for microscopical examination.
	Organs:	8 sequential levels of the brain(coronal section 1-8)
		4 levels of the spinal cord (cervical, thoracic, lumbar)
		Left and right gasserian ganglia
		Spinal nerve roots and dorsal root ganglia
		Left and right sciatic nerves, tibial nerves, sural nerves, optic nerves
		Left and right eyes
		Gastrocnemius muscle
3.6	**Further remarks**	–
		4 RESULTS AND DISCUSSION
4.1	**Body Weight**	No effects
4.2	**Clinical signs of toxicity**	No effects
4.3	**Clinical Chemistry**	Not performed
4.4	**Pathology**	No compound-related gross lesions occurred at any dose level.
4.5	**Histopathology**	No compound-related microscopic lesions occurred in neural or skeletal tissues from high dose males and females.
4.6	**Other**	The brain weight was not affected.
		Also, there were no effects on food consumption, FOB (functional observational battery) and activity.
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	Undiluted technical grade Icaridin was administered as a single dermal dose to young adult Fischer 344 rats (12/sex/dose/level). Dosages of 0, 200, 600 and 2000 mg/kg were applied.
		The following observations and measurements were included in the study: clinical observations, mortality, body weight, food consumption, automated measurements of activity (figure-eight maze), functional observational battery, and a gross necropsy. Skeletal muscle, peripheral nerves, eyes (with optic nerves) and tissues from the central nervous system were also examined for microscopic lesions.
5.2	**Results and discussion**	No compound-related deaths or clinical signs occurred at any dose level. Body weight, brain weight and food consumption were also not affected.

Section A6.9 Delayed Neurotoxicity

Annex Point IIIA VI.1	6.9 Acute dermal neurotoxicity study in rats
	In the functional observational battery, there were no compound-related effects at any dose level. Parameters of motor and locomotor activity were not affected by treatment at any dose. Likewise, there was no effect on habituation at any dose. There were no compound-related ophthalmic findings or gross lesions. In addition, there were no compound-related microscopic lesions in neural or muscle tissues from high dose males and females.
5.3 Conclusion	Icaridin is not considered to have delayed neurotoxic effects under the conditions of this study.
5.3.1 LOAEL	> 2000 mg/kg/day
5.3.2 NOAEL	2000 mg/kg/day
5.3.3 Reliability	1
5.3.4 Deficiencies	No

Section A6.9 Delayed Neurotoxicity

Annex Point IIIA VI.1 6.9 Acute dermal neurotoxicity study in rats

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	1 November 2006
Materials and Methods	Applicant's version is adopted
Results and discussion	Applicant's version is adopted
Conclusion	Applicant's version is adopted.
Reliability	1
Acceptability	Acceptable
Remarks	No systemic toxic effects obtained at any dose level.

Section A6.9 Delayed Neurotoxicity

Annex Point IIIA VI.1 6.9 Subchronic dermal neurotoxicity study in rats

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1996b), Subchronic Dermal Neurotoxicity Screening Study with Technical Grade KBR 3023 in Fischer 344 Rats. [redacted], Report No. 107466, 1996-10-09 (unpublished)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Saltigo GmbH	
1.2.2	Company with letter of access	–	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes US-EPA-FIFRA 82-5 (b), (now replaced by OPPTS 870.6100) ≅ OECD Guideline 424(1997) The study was conducted according to US-EPA-FIFRA 82-5(b), which was replaced by OPPTS 870.6100. This new guideline does not cover 90-day rodent neurotoxicity studies. However, the study fulfils the requirements of OECD Guideline 424.	
2.2	**GLP**	Yes	
2.3	**Deviations**	Range of ambient temperature of animal room was slightly greater than laid down in OECD Guideline 424.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in Section 2 of dossier	
3.1.1	Lot/Batch number	030693	
3.1.2	Specification	Technical grade Icaridin	
3.1.2.1	Description	Clear, colourless liquid	
3.1.2.2	Purity	97.4-98.1%	
3.1.2.3	Stability	Confirmed by analyses conducted before, during and after the exposure period.	
3.2	**Reference Substance (positive control)**	Historical positive controls: acrylamide and carbaryl.	
3.3	**Test Animals**		
3.3.1	Species	Rat	

Section A6.9 Delayed Neurotoxicity

Annex Point IIIA VI.1 6.9 Subchronic dermal neurotoxicity study in rats

3.3.2	Strain	Fischer 344 CDF (F-344)/BR Rats
3.3.3	Source	Sasco, Inc., Madison, WI, USA
3.3.4	Sex	♂ + ♀
3.3.5	Age/weight at study initiation	8 weeks Mean weight: ♂: 119.1-127.2 g; ♀: 110.6-119.2 g
3.3.6	Number of animals per group	12 per sex per dose group
3.3.7	Control animals	Yes
3.4	**Administration**	Dermal
3.4.1	Exposure	Administration once daily, five days a week for 13 weeks. All animals wore Elizabethan collars throughout the study to prevent ingestion of the test material. The collars were removed just prior to FOB and motor activity testing to prevent interference of the collars with the tests.
3.4.2	Dose Levels	0, 50, 100 and 200 mg/kg bw
3.4.3	Vehicle	None
3.4.4	Total volume applied	Depends on body weight
3.4.5	Post exposure period	None
3.4.6	Anticholinergic substances used	None
3.4.7	Controls	Water
3.5	**Examinations**	
3.5.1	Body Weight	Weighing before application and during study in weekly intervals Perfused animals were also weighed on the day sacrificed for terminal body weight measurement
3.5.2	Signs of Toxicity	Examination for mortality and moribundity: twice daily (once daily on weekends and holidays) Detailed physical examination for clinical signs of toxicity: once a week
3.5.3	Observation schedule	Functional Observational Battery and motor activity: Once during the week prior to initiating the exposure and again during the weeks 4, 8 and 13. Ophthalmology examinations: once pre exposure and once pre-terminal (week 12).
3.5.4	Clinical Chemistry	No
3.5.5	Pathology	Yes

Section A6.9 Delayed Neurotoxicity

Annex Point IIIA VI.1 6.9 Subchronic dermal neurotoxicity study in rats

	Organs:	All organs (including the brain), body cavities, cut surfaces, external orifices and surfaces
3.5.6	Histopathology	Yes
	Organs:	8 sequential levels of the brain(coronal section 1-8)
		4 levels of the spinal cord (cervical, thoracic, lumbar)
		Left and right gasserian ganglia
		Spinal nerve roots and dorsal root ganglia
		Left and right sciatic nerves, tibial nerves, sural nerves, optic nerves
		Left and right eyes
		Gastrocnemius muscle
3.6	**Further remarks**	–
		4 RESULTS AND DISCUSSION
4.1	**Body Weight**	No effects
4.2	**Clinical signs of toxicity**	No effects
4.3	**Clinical Chemistry**	Not performed
4.4	**Pathology**	No compound-related ophthalmic findings or gross lesions occurred at any dose level.
4.5	**Histopathology**	No compound-related microscopic lesions occurred in neural or skeletal tissues from high dose males and females.
4.6	**Other**	The brain weight was not affected. Also, there were no effects on food consumption, FOB (functional observational battery) and activity.
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	Undiluted technical grade Icaridin was administered by the dermal route to young adult Fischer 344 rats (12/sex/dose/level) for 13 weeks. Dosages of 0, 50, 100 and 200 mg/kg/day (based on mean group body weight) were applied. All rats were evaluated for neurobehavioural abnormalities; half (6/sex/level) of them were used for neuropathology. The following observations and measurements were included in the study: clinical observations, mortality, body weight, food consumption, automated measurements of activity (figure-eight maze), functional observational battery, and a gross necropsy. Skeletal muscle, peripheral nerves, eyes (with optic nerves) and tissues from the central nervous system were also examined for microscopic lesions.
5.2	**Results and**	No compound-related deaths or clinical signs occurred at any dose level.

Section A6.9 Delayed Neurotoxicity

Annex Point IIIA VI.1		6.9 Subchronic dermal neurotoxicity study in rats
	discussion	Body weight, brain weight and food consumption were also not affected. In the functional observational battery, there were no compound-related effects at any dose level. Parameters of motor and locomotor activity were not affected by treatment at any dose. Likewise, there was no effect on habituation at any dose. There were no compound-related ophthalmic findings or gross lesions. In addition, there were no compound-related microscopic lesions in neural or muscle tissues from high dose males and females.
5.3	**Conclusion**	Icaridin is not considered to have delayed neurotoxic effects under the conditions of this study.
5.3.1	LOAEL	> 200 mg/kg/day
5.3.2	NOAEL	200 mg/kg/day
5.3.3	Reliability	1
5.3.4	Deficiencies	No

Section A6.9 Delayed Neurotoxicity

Annex Point IIIA VI.1 6.9 Subchronic dermal neurotoxicity study in rats

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	1 November 2006
Materials and Methods	Applicant's version is adopted
Results and discussion	Applicant's version is adopted
Conclusion	Applicant's version is adopted.
Reliability	1
Acceptability	Acceptable
Remarks	The dose selection did not give any systemic toxicity.

Section A6.9 Delayed Neurotoxicity

Annex Point IIIA VI.1 6.9 Subchronic dermal neurotoxicity study in rats

Table A6_9-1. Table for delayed neurotoxicity

		0 mg/kg/day		50 mg/kg/day		100 mg/kg/day		200 mg/kg/day	
Number of male animals at the start		12		12		12		12	
		Number of animals affected | % of animals affected							
Deaths		2	17%	0	0%	0	0%	0	0%
Showing lesions		0	0%	0	0%	0	0%	0	0%
Showing effects in	**behaviour**	0	0%	0	0%	0	0%	0	0%
	motor and locomotor activity	0	0%	0	0%	0	0%	0	0%
Pathology findings		3	25%	0	0%	0	0%	1	8%
Number of female animals at the start		12		12		12		12	
		Number of animals affected | % of animals affected							
Deaths		1	8%	0	0%	1	8%	0	0%
Showing lesions		0	0%	0	0%	0	0%	0	0%
Showing effects in	**behaviour**	0	0%	0	0%	0	0%	0	0%
	motor and locomotor activity	0	0%	0	0%	0	0%	0	0%
Pathology findings		1	8%	0	0%	1	8%	0	0%

Section 6.10 Annex Point IIIA VI.7	Mechanistic study - any studies necessary to clarify effects reported in toxicity studies	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	The core data set generated for toxicity testing of Icaridin did not reveal any specific effects that required mechanistic investigation. No such tests have been performed.	
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	30 November 2006	
Evaluation of applicant's justification	Applicant's version is adopted	
Conclusion	Applicant's version is adopted	
Remarks		

Section A6.12 Human Case Report

Annex Point IIA VI.6.9.1 6.12.1 Medical surveillance data on manufacturing plant personnel

Official use only

1 REFERENCE

1.1	**Reference**	Kehrig, B. and Bischof, H. (2006): Occupational Medical Experiences with Icaridin in the [redacted], 2006-02-14, unpublished.
1.2	**Data protection**	Yes
1.2.1	Data owner	Saltigo GmbH
1.2.2	Companies with letter of access	–
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA.

2 GUIDELINES AND QUALITY ASSURANCE (NOT APPLICABLE)

3 MATERIALS AND METHODS

3.1	**Substance**	Icaridin
3.2	**Persons exposed**	
3.2.1	Sex	–
3.2.2	Age/weight	–
3.2.3	Known Diseases	–
3.2.4	Number of persons	~70
3.2.5	Other information	–
3.3	**Exposure**	–
3.3.1	Reason of exposure	Occupational
3.3.2	Frequency of exposure	Daily
3.3.3	Overall time period of exposure	–
3.3.4	Duration of single exposure	–
3.3.5	Exposure concentration/ dose	–
3.3.6	Other information	–
3.4	**Examinations**	
3.4.1	Medical examinations	History, full physical examination with orientating neurological status (reflexes, sensibility, coordination) and skin status.

Section A6.12 Human Case Report

Annex Point IIA VI.6.9.1 6.12.1 Medical surveillance data on manufacturing plant personnel

3.4.2	Laboratory examinations	Blood sedimentation rate, full blood count, AST, ALT, γ-GT, glucose, creatinine, cholesterol, urine status	
3.4.3	Technical examinations	Lung function, ECG/ergometry, vision testing, audiometry, chest X-ray, sonography (if necessary)	
3.5	**Treatment**	–	
3.6	**Remarks**	–	
		4 RESULTS	
4.1	**Results of examinations**	No adverse health effects observed.	
4.2	**Effectivity of medical treatment**	–	
4.3	**Outcome**	–	
4.4	**Other**	–	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	About [redacted] of Icaridin are produced in the [redacted]. Lanxess standard protective garment is worn: a full mask with filter ABEK-P3, protective gloves for chemicals, and a chemical-resistant suit. Occupational medical surveillance of workers potentially exposed to Icaridin is performed yearly on a routine basis.	
5.2	**Results and discussion**	No adverse health effects observed.	X
5.3	**Conclusion**	No problems related to handling / production of Icaridin were reported.	X

Evaluation by Competent Authorities

Use separate "evaluation boxes" to provide transparency as to the comments and views submitted

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	12 October 2006
Materials and Methods	Adopt applicant's version
Results and discussion	Revised version: No accidental exposure to Icaridin was reported. No adverse health effects observed.
Conclusion	Revised version: The routine medical examinations of plant personnel that have been performed since 1997 did not indicate any specific adverse effects on the health of employees.
Remarks	

Section 6.12.2 Annex Point IIA VI.6.9.2	Direct observation, e.g. clinical cases, poisoning incidents if available	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	No poisoning incidents involving Icaridin or Icaridin-containing products have been reported directly to the manufacturers or in the published medical literature. The Icaridin-containing formulations of the Autan family are formulated with the bittering agent Bitrex. This makes accidental ingestion of harmful amounts of the repellent product highly unlikely.	X
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	22 November 2006	
Evaluation of applicant's justification	The addition of Bitrex does not necessarily stop small children from potential ingestion of product, since it is not expected that they will be able to interpret from where the ugly taste comes. The addition of Bitrex is not included in the formulations sold in Denmark.	
Conclusion	Applicant's justification is not acceptable.	
Remarks		

Section A6.12 Medical data in anonymous form

Annex Point IIA VI.6.9.4 6.12.4 Phototoxicity study in human volunteers

Official use only

		1 REFERENCE
1.1	**Reference**	[redacted] (1996), Controlled Intra-Individual Comparative Study of the Phototoxicity of the Repellent KBR 3023. [redacted] Report No. 107792, 1996-06-31 (unpublished)
1.2	**Data protection**	Yes
1.2.1	Data owner	Saltigo GmbH
1.2.2	Company with letter of access	–
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	No. No particular guideline for phototoxicity studies on human volunteers.
2.2	**GLP**	No. This study was conducted in an academic hospital where no GLP certification exists.
2.3	**Deviations**	Not applicable
		3 MATERIALS AND METHODS
3.1	**Test material**	KBR 3023 (Icaridin) in ethanolic solution
3.2	**Persons exposed**	
3.2.1	Sex	♂ + ♀
3.2.2	Age/weight	Between 18 and 50 years
3.2.3	Known Diseases	All subjects were free of acute, chronic or recurring disease
3.2.4	Number of persons	50
3.2.5	Other information	Skin types II or III (Fitzpatrick classification)
3.3	**Exposure**	Dermal
3.3.1	Reason of exposure	Controlled study
3.3.2	Frequency of exposure	Single
3.3.3	Overall time period of exposure	Day 0: application of the test and control series Day 1: irradiation of the test areas Day 2 to 4: assessment of the test areas

Section A6.12 Medical data in anonymous form

Annex Point IIA VI.6.9.4 6.12.4 Phototoxicity study in human volunteers

3.3.4	Duration of single exposure	24 h	
3.3.5	Exposure concentration/dose	Vehicle (ethanol), 0.2, 0.4 mg/cm², 20% Icaridin solution, untreated skin (extended negative control). The series was applied in duplicate Finn chambers (irradiated test series and non-irradiated control series)	3.3.6
3.3.7	Other information	The test series were applied to the dorsum of the subjects. One of the two test series was irradiated with 10 J/cm² UV-A radiation (320-400 nm) from a distance of 30 cm.	
3.4	**Examinations**	After 10-20 min, and 24, 48 and 72 h after the irradiation, the test series fields and the non-radiated control series fields were evaluated using an erythema score as follows: – no erythema ± barely visible, fuzzy erythema + erythema ++ erythema, infiltrate +++ erythema, infiltrate, papula vesicles ++++ erythema, infiltrate, blisters or erosions	
3.5	**Remarks**	–	

4 RESULTS

4.1 Clinical Signs Not reported

4.2 Results of examinations

Reaction	Incidence	Non-irradiated	Irradiated
No reactions	18 test persons		
± reactions	26 test persons	20	23
+ reactions	6 test persons	5	3
++ reactions	1 test person	1	1
+++ reactions	none		
++++ reactions	none		

The single individual showing a ++ reaction also revealed a ++ reaction towards the vehicle control.

4.3 Outcome None of the test subjects exhibited a clinically relevant photo-toxic reaction.

4.4 Other –

Section A6.12 Medical data in anonymous form

Annex Point IIA VI.6.9.4 6.12.4 Phototoxicity study in human volunteers

		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	A photo-patch test was conducted to identify potential photosensitising properties of Icaridin. The method was developed by Epstein and Rowe (*J. Invest. Dermatol.* **2**, 43-51, 1939; *Ann. Allergy* **22**, 1-11, 1964) and represents the most important procedure for diagnosis of photo-sensitisation. Fifty healthy volunteers of both sexes participated in this study. The test and control series comprised Finn chambers loaded with several concentrations of the test substance and a vehicle control. After 24 h of exposure, the test series was irradiated with UV-A light. The control series was left non-irradiated. Cutaneous reactions were assessed at several time points following irradiation.	X
5.2	**Results and discussion**	A slight irritant potential is present, which was rarely and irregularly increased by UV-A irradiation. It must be borne in mind that the test substance was occlusively applied for 24 h in order to detect even very slight irritant potentials of test substances. None of the test subjects exhibited a clinically relevant photo-toxic reaction.	
5.3	**Conclusion**	Icaridin can be classified as harmless with regard to its photo-toxic properties.	X

Section A6.12 Medical data in anonymous form

Annex Point IIA VI.6.9.4 6.12.4 Phototoxicity study in human volunteers

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	22 November 2006
Materials and Methods	Revised version: 5.1 A photo-patch test was conducted to identify potential phototoxic properties of Icaridin. The method was developed by Epstein and Rowe (*J. Invest. Dermatol.* **2**, 43-51, 1939; *Ann. Allergy* **22**, 1-11, 1964). Photoallergic potential of Icaridin was not investigated.
Results and discussion	Applicant's version is adopted
Conclusion	Revised version: There was no evidence of phototoxicity after Icaridin was applied to human skin under the conditions of the study
Remarks	

Section A6.12 Medical data in anonymous form

Annex Point IIA VI.6.9.6 6.12.6 Sensitisation/allergenicity observations

			Official use only
		1 REFERENCE	
1.1	**Reference**	Corazza, M. *et al.* (2005), Allergic Contact Dermatitis due to an Insect Repellent: Double Sensitization to Picaridin and Methyl Glucose Dioleate. Dept. of Dermatology, University of Ferrara, Italy, *Acta Derm. Venereol.*; **85**(3):264-5 (published)	
1.2	**Data protection**	No	
		2 GUIDELINES AND QUALITY ASSURANCE (NOT APPLICABLE)	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Icaridin, 2.5% in petrolatum	
3.2	**Persons exposed**		
3.2.1	Sex	♂	
3.2.2	Age/weight	39 years	
3.2.3	Known Diseases	Not reported	
3.2.4	Number of persons	1	
3.3	**Exposure**	Dermal	
3.3.1	Reason of exposure	A series of tests was performed on a patient with an allergic contact dermatitis suspected to be caused by the use of an Icaridin-containing repellent product. An open epicutaneous test using the commercial repellent product (Autan Family® Spray) was positive. A first confirmatory patch test was performed with the Italian standard (SIDAPA) series. A second patch test was undertaken with all the constituents of Autan Family® spray (see point 4.2).	
3.3.2	Frequency of exposure	Not reported	
3.3.3	Overall time period of exposure	Not reported	
3.3.4	Duration of single exposure	Not reported	
3.3.5	Exposure concentration/dose	The patient used a 10% Autan Family® spray. The first (open) test used an unspecified amount of the commercial Autan formulation. The confirmatory patch test on the single Autan constituents was performed, inter alia, with 2.5% Icaridin in petrolatum.	3.3.6

Section A6.12 Medical data in anonymous form

Annex Point IIA VI.6.9.6 6.12.6 Sensitisation/allergenicity observations

3.4 Examinations

An open epicutaneous test was performed using Autan Familiy® spray.

A patch test was performed with the Italian standard (SIDAPA) series.

A second patch test was undertaken with all the constituents of Autan Family® spray.

3.5 Remarks

–

4 RESULTS

4.1 Clinical Signs

Open epicutaneous exposure to Autan Family® spray resulted in an itching erythematous reaction within a few hours.

4.2 Results of examinations

Two constituents of Autan Family® spray resulted in strong dermal reactions.

The skin reactions on day 2 (D2) and 3 (D3) towards the components of Autan Family spray were scored as follows:

Constituents	D2	D3
Methyl glucose dioleate 10% pet.	+++	+++
Picaridin 2.5% pet.	++	+++
Denatured ethanol	–	–
Citric acid 1% aqua	–	–
Glycerin	–	–
Aloe vera 10% pet.	–	–
Perfume 10% pet.	–	–
Di.N.octyl ether 10% pet.	–	–
Polydimethylsiloxane 10% pet.	–	–

4.3 Outcome

A strong reaction to the excipient methyl glucose dioleate and to the active substance, Picaridin (=Icaridin) was observed in the confirmatory patch test.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

A 39-year-old man presented with a widespread persistent itching erythematous-oedematous dermatitis involving the limbs. An allergic contact dermatitis was suspected and therapy with antihistamines and topical and systemic corticosteroids healed the lesions in about 10 days with transient pigmentary lesions.

The patient stated that he had used an insect repellent (Autan Family Spray) the day before the onset of the dermatitis. He had an open test with the commercial product. After the first application an itching erythematous reaction appeared only a few hours later.

A patch test performed with the Italian standard (SIDAPA) series revealed only a weak (–D2/+D3) reaction to *Myroxylon Pereirae* 25% pet. Further patch tests were therefore carried out with all the constituents of Autan Family Spray, supplied by SC Johnson Wax.

Section A6.12 Medical data in anonymous form

Annex Point IIA VI.6.9.6 6.12.6 Sensitisation/allergenicity observations

5.2	**Results and discussion**	An initial open epicutaneous test confirmed the suspicion that Autan Family spray contained an ingredient causing contact dermatitis in the patient. A further confirmatory patch test with the individual constituents of Autan Family Spray identified both the active substance Icaridin and methyl glucose dioleate as creating strong reactions.
5.3	**Conclusion**	One case of contact allergy caused by Icaridin has been reported in medical literature.

Section A6.12 Medical data in anonymous form

Annex Point IIA VI.6.9.6 6.12.6 Sensitisation/allergenicity observations

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	01 November 2006
Materials and Methods	Applicant's version is adopted.
Results and discussion	Applicant's version is adopted.
Conclusion	Applicant's version is adopted.
Remarks	-

Section A6.12 Medical data in anonymous form

Annex Point IIA VI.6.9.7 6.12.7 Specific treatment in case of an accident or poisoning: first aid measures, antidotes and medical treatment

			Official use only
		1 REFERENCE	
1.1	**Reference**	Röder, K. (2000), Bayrepel (KBR 3023) containing products – Human poisoning, first aid, medical treatment and antidot. Bayer AG, Leverkusen, Germany, 2000-05-26 (unpublished)	
		2 GUIDELINES AND QUALITY ASSURANCE (NOT APPLICABLE)	
		3 MATERIALS AND METHODS	
3.1	**Substance**	Icaridin-containing products	
3.2	**Persons exposed**	Consumers (general public)	
3.3	**Exposure**	Probably oral	
3.3.1	Reason of exposure	Accidental, attempted suicide	
3.3.2	Frequency of exposure	Single	
3.4	**Examinations**	–	
3.5	**Treatment**	Induce vomiting and apply charcoal. Apart from that: symptomatic treatment. A specific antidote is not available.	
3.6	**Remarks**	All Icaridin-containing formulations can be considered non-toxic (LD_{50} > 5000 mg/kg bw). Treatment applies when the substance was ingested in very high amounts.	X
		4 RESULTS	
4.1	**Clinical Signs**	Not reported	
4.2	**Results of examinations**	–	
4.3	**Effectivity of medical treatment**	Not reported	
4.4	**Outcome**	Not reported	
4.5	**Other**	–	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	–	
5.2	**Results and discussion**	–	
5.3	**Conclusion**	–	

Section A6.12 Medical data in anonymous form

Annex Point IIA VI.6.9.7	6.12.7 Specific treatment in case of an accident or poisoning: first aid measures, antidotes and medical treatment

Section A6.12 Medical data in anonymous form

Annex Point IIA VI.6.9.7 6.12.7 Specific treatment in case of an accident or poisoning: first aid measures, antidotes and medical treatment

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	22 November 2006
Materials and Methods	3.6. Revised version: All Icaridin-containing formulations can be considered non-toxic when applied onto skin (Icaridin dermal LD_{50} male rats > 5000 mg/kg bw). Treatment applies when the substance has been ingested in very high amounts. Icaridin oral LD_{50} in fasted male rats is 2236 mg /kg bw.
Results and discussion	
Conclusion	
Remarks	Comment: This section concerns active substance. It is not relevant to refer to product LD_{50} here but to oral LD_{50} for active substance. It would be appropriate to mention target organ and the type of symptoms that could be expected in case of accidental oral ingestion.

Section 6.13 Annex Point IIIA VI.6.2	Toxic effects on livestock and pets	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	For several years, icaridin-containing products have been authorised for use on horses and their riders. No adverse effects have been reported. Based on the absence of adverse effects in a chronic dermal study on dogs, it is concluded that the use of icaridin is safe for pets. The low oral toxicity of icaridin in the rat allows the conclusion that accidental oral intake of the pet will have no adverse health effects. .	
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	30 October 2006	
Evaluation of applicant's justification	Applicant’s version is adopted	
Conclusion	Applicant’s version is adopted	
Remarks		

Section 6.14 Annex Point IIIA XI.2	Other test(s) related to the exposure of humans	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	Exposure towards Icaridin from repellent products applied directly onto skin has been estimated using sufficiently conservative assumptions on the use pattern and subsequent exposure-related behaviour (e.g., hand-to-mouth contact). Dermal penetration of Icaridin has been tested in human volunteers. This eliminates a great deal of uncertainty that typically arises from animal studies on this endpoint. Icaridin-containing repellents are on the worldwide market since 1998. Since its introduction, equivalents of 190 million 100-mL bottles have been sold. Besides a single allergy report (likely to have been caused by a now-eliminated impurity), no adverse effects were reported Risk assessment indicated that the use of Icaridin in these repellent products is safe. Additional tests related to the exposure of humans were therefore not considered necessary.	
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	30 November 2006	
Evaluation of applicant's justification	Exposure scenario and risk assessment has been revised by CA, however it is agreed that further human studies at this moment is not necessary.	
Conclusion	Applicant’s justification is acceptable	
Remarks		

Section 6.15 Annex Point IIIA VI.6.4	Food and feedingstuffs	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [X]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	Icaridin is used in repellent products that are applied directly onto skin. Contamination of food and feedingstuff can be excluded. The contamination of finger food by contact with treated skin is accounted for in the human exposure scenario as hand-to-mouth contact.	
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	30 October 2006	
Evaluation of applicant's justification	Applicant’s version is adopted	
Conclusion	Applicant’s version is adopted	
Remarks		