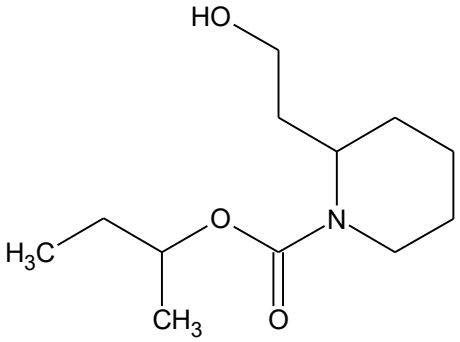
Section A2 Identity of Active Substance

Annex point IIA, II 2

Subsection (Annex Point)				Official use only
2.1	**Common name (IIA, II)**	Common name:	Icaridin	√
		Synonym:	KBR 3023, Picaridin	
		Trade name:	Bayrepel™	
		INCI name:	Hydroxyethyl Isobutyl Piperidine Carboxylate	
2.2	**Chemical name (IIA, II 2.2)**	IUPAC name:	(RS)-sec-butyl-2-(2-hydroxyethyl)piperidine-1-carboxylate	√
		CAS name:	1-Piperidinecarboxylic acid, 2-(2-hydroxyethyl)-1-methylpropylester	
2.3	**Manufacturer´s development code number(s) (IIA, II 2.3)**	KBR 3023		√
2.4	**CAS No and EC numbers (IIA, II 2.4)**			
2.4.1	**CAS-No**	119515-38-7		√
	Isomer 1	Not allocated		
	Isomer n	Not allocated		
2.4.2	**EC-No**	ELINCS no.: 423-210-8		√
	Isomer 1	Not allocated		
	Isomer n	Not allocated		
2.4.3	**Other**	CIPAC no.: 740 (the name Hydroxyethyl Isobutyl Piperidine Carboxylate was allocated CIPAC no. 668, whereas CIPAC no. 740 refers to the common name, Icaridin)		√
2.5	**Molecular and structural formula, molecular mass (IIA, II 2.5)**			
2.5.1	**Molecular formula**	$C_{12}H_{23}NO_3$		√

Section A2 Identity of Active Substance

Annex point IIA, II 2

2.5.2 Structural formula		√
2.5.3 Molecular mass	229.3 g/mol	
2.6 Method of manufacture of the active substance (IIA, II 2.6)	The method of manufacture of the active substance is confidential. This information is provided separately in the confidential part of the dossier.	√
2.7 Specification of the purity of the active substance, as appropriate (IIA, II 2.7)	Icaridin has a specified minimal purity of 97.0%. (Reference: Anonymous, 2005a) Representative production batches of the active substance are analysed for their Icaridin content. This information is confidential and is provided separately in the confidential part of the dossier.	√
2.8 Identity of impurities and additives, as appropriate (IIA, II 2.8)	The identity of impurities and additives is confidential. This information is provided separately in the confidential part of the dossier.	√
2.8.1 Isomeric composition	Icaridin is prepared from [redacted]. Each of the precursors of Icaridin contains a center of asymmetry. The active ingredient therefore has two centers of asymmetry and consists of two pairs of enantiomers. During the industrial manufacturing process only the racemic form of [redacted] and [redacted] is used. Without presence of a stereospecific catalyst or a stereospecific solvent the result of such a reaction must be a racemat, a mixture of two pairs of diastereomers with the theoretical ratio of 1:1 resulting in a mixture of four enantiomers respectively In 1996 the ratio of two pairs of diastereomers was determined in 7 batches and was found to be constant 50 ± 0.3%. (Reference: Koch, 2006c) 5 batches from each SGO plant 1 and SGO plant 5 were analysed for	√

Section A2 Identity of Active Substance

Annex point IIA, II 2

		the enantiomeric ratio and optical rotation from Icaridin. The determination shows a racemic mixture of four enantiomers for Icaridin. The enantiomers are: KBR 5279 ((1S/2R)-Isomer) KBR 5280 ((1R/2R)-Isomer) KBR 5265 ((1S/2S)-Isomer) KBR 5264 ((1R/2S)-Isomer) The studies show that the ratio of the isomers is 25:25:25:25. No optical rotation was determined. (Reference: Menne and Boddenberg, 2016a, Report No.: 2016-00177 and Menne and Boddenberg, 2016b, Report No.: 2016-00178)	
2.9	**The origin of the natural active substance or the precursor(s) of the active substance (IIA, II 2.9)**	Not applicable as the active substance Icaridin has no natural origin.	√

Section A2 Identity of Active Substance

Annex point IIA, II 2

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*November 2016*
Materials and methods	*Acceptable*
Conclusion	*Acceptable*
Reliability	*1*
Acceptability	*Acceptable*
Remarks	2.2, IUPAC name amended to (RS)-sec-*butyl* (RS)-*2-(2-hydroxyethyl)piperidine-1-carboxylate.*

Section A2.8 Identity of impurities and additives (active substance)
Subsection A2.8

Annex Point IIA, II 2.8

Subsection			Official use only
2.8.1.1	**Common name**	The identity of impurities and additives in the active substance as manufactured is confidential. This information is provided separately in the confidential part of the dossier.	√
2.8.1.2	**Function**		
2.8.2	**IUPAC name**		
2.8.3	**CAS-No**		
2.8.4	**EC-No**		
2.8.5	**Other** CIPAC		
2.8.6	**Molecular formula**		
2.8.7	**Structural formula**		
2.8.8	**Molecular mass**		
2.8.9	**Concentration of the impurity or additive** typical and range of concentrations		

Section A2.8 **Subsection A2.8** **Annex Point IIA, II 2.8**	**Identity of impurities and additives (active substance)**
	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*January 2007*
Materials and methods	*Acceptable*
Conclusion	*Adopted*
Reliability	*0*
Acceptability	*Acceptable*
Remarks	*None*

Section A2 **Subsection A2.8** **Annex Point IIA, II 2.8**	**Identity of impurities and additives (active substance)** 2.8.5 OTHER NUMBERS	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [...]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [X]**	√
Detailed justification:	CAS and EC numbers, respectively, are given for the impurities of Icaridin where possible. Other numbers, for example CIPAC numbers, are not submitted for the different impurities since such numbers are not allocated for them.	√
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	*January 2007*	
Evaluation of applicant's justification	*No comments*	
Conclusion	*Acceptable*	
Remarks	*None*	

Section A2 **Subsection A2.9** **Annex Point IIA, II 2.9**	**Identity** THE ORIGIN OF THE NATURAL ACTIVE SUBSTANCE OR THE PRECURSOR(S) OF THE ACTIVE SUBSTANCE	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [...]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [X]**	√
Detailed justification:	Since Icaridin is neither a natural active substance itself nor any precursors of the molecule are natural products this point does not apply to Icaridin.	√
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	*January 207*	
Evaluation of applicant's justification	*No comments*	
Conclusion	*Acceptable*	
Remarks	*None*	

Section A3 Physical and Chemical Properties of Active Substance

Annex point IIA, III 3

Subsection (Annex point)	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.1 Melting point, boiling point, relative density (IIA, III 3.1)								
3.1.1 Melting point	EC method A.1 (Differential Scanning Calorimetry)	Purity: 98.9%,	No freezing, melting, crystallisation or glass transition was observed in a temperature range between -170 °C and 20 °C. A sample kept at -20 °C for 4 weeks did not solidify.	–	Y	1	Krohn, 1996 and Feldhues, 2006	√
3.1.2 Boiling point	The boiling point was calculated using the regression coefficients obtained during vapour pressure determination.	Purity: 98.9%	296 °C at 1013 hPa	RMS: The calculation is accepted. The value is close to literature value. The exact value is not relevant at these high temperatures.	Y	1	Krohn, 1996	√
3.1.3 Bulk density/ relative density								
Relative density	EC method A.3; OECD guideline 109 (bicapillary pycnometer)	Purity: 98.9%	Density at 20 °C: 1.07 g/ml	RMS: Be aware that the density is given – not the relative density. The result is accepted	Y	1	Krohn, 1996	√

Section A3 Physical and Chemical Properties of Active Substance

Annex point IIA, III 3

Subsection (Annex point)	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
Bulk density	–	–	–	The determination of the bulk density is not feasible because Icaridin is liquid.	–	–	–	√
3.2 Vapour pressure and Henry`s Law Constant (IIA, III 3.2)								√
Vapour pressure	EC method A.4; OECD guideline 104 (vapour pressure balance)	Purity: 98.9%	3.4×10^{-2} Pa at 20 °C, 5.9×10^{-2} Pa at 25 °C, 7.1×10^{-1} Pa at 50 °C	–	Y	1	Krohn, 1996	√
Henry´s Law Constant	Calculation (ratio between vapour pressure and water solubility)	Specification: min. 97%	9.1×10^{-4} Pa×m^3×mol^{-1} at 20 °C	Values used for calculation: Vapour pressure: 3.4×10^{-2} Pa at 20 C ; Water solubility, unbuffered: 8.6 g/L at 20 °C	Y	1	Krohn, 1996	√
3.3 Appearance (IIA, III 3.3)								√

Section A3 Physical and Chemical Properties of Active Substance

Annex point IIA, III 3

Subsection (Annex point)	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.3.1 Physical state	Visual and olfactory inspection	Purity: 98.9%	liquid	RMS: There is no reason to give information on both technical and purified material as the purity of typical technical material is above 98%.	Y	1	Krohn, 1996	√
3.3.2 Colour	Visual and olfactory inspection	Purity: 98.9%	colourless	RMS: There is no reason toe give information on both technical and purified material as the purity of typical technical material is above 98%.	Y	1	Krohn, 1996	√
3.3.3 Odour	Visual and olfactory inspection	Purity: 98.9%	odourless	RMS: There is no reason to give information on both technical and purified material as the purity of typical technical material is above 98%.	Y	1	Krohn, 1996	√
3.4 Absorption spectra (IIA, III 3.4)								√

Section A3 Physical and Chemical Properties of Active Substance

Annex point IIA, III 3

Subsection (Annex point)	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
UV/VIS	Since no official (OECD) guideline is available the test was performed according to internal standard operation procedures.	Purity: 98.8%	Icaridin was identified by UV/VIS spectrum; acetonitrile was used as solvent; No absorptivity was observed.	–	Y	1	Erstling, 2005	√
IR	Since no official (OECD) guideline is available the test was performed according to internal standard operation procedures.	Purity: 98.8%	Icaridin was identified by FTIR using a potassium bromide cell.	RMS: -OH: 3457 cm^{-1} methylen: 2938 cm^{-1} CH_3-: 2971 cm^{-1} , 2866 cm^{-1}, 1377 cm^{-1} C=O: 1691 cm^{-1}, 1666 cm^{-1} C-O: 1265 cm^{-1}, 1426 cm^{-1} C-N: 1174 cm^{-1}	Y	1	Erstling, 2005	√
NMR	Since no official (OECD) guideline is available the test was performed according to internal standard operation procedures.	Purity: 98.8%	Icaridin was identified by ^{1}H-NMR spectrum; $CDCl_3$ was used as solvent.	–	Y	1	Erstling, 2005	√

Section A3 Physical and Chemical Properties of Active Substance

Annex point IIA, III 3

Subsection (Annex point)	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
MS	Since no official (OECD) guideline is available the test was performed according to internal standard operation procedures.	Purity: 98.8%	Icaridin was identified by 70 eV electron impact ionaisation mass spectrum (EI-MS).	RMS: molecular ion 229. Fragments: 184, 156, 128, 84, 57.	Y	1	Erstling, 2005	√
3.5 Solubility in water (IIA, III 3.5)	EC method A.6; OECD guideline 105 (flask method) Analysed with HPLC	Purity: 98.2%	Results at 20 °C: 8.6 g/L in unbuffered aqueous solution; 8.2 g/L in buffered aqueous solutions in the range between pH 4 and pH 9.	The solubility in water is not influenced by the pH in the range between pH 4 and pH 9. The lower results for buffered solutions show an influence of salinity on the solubility.	Y	1	Krohn, 1996	√
	EC method A.6 (flask method) Analysed with HPLC	Purity: 98.8%	12.9 g/L at 10 °C, 10.6 g/L at 20 °C, 8.9 g/L at 30 °C	A temperature influence of the water solubility of Icaridin between 10 °C and 30 °C was observed.	Y	1	Jungheim, 2006a	√
3.6 Dissociation constant (-)	OECD guideline 112 (titration method)	Purity: 98.9%	Icaridin has no acidic or basic properties in aqueous solutions. It is not possible to specify dissociation constants for water.	–	Y	1	Krohn, 1996	√

Section A3 Physical and Chemical Properties of Active Substance

Annex point IIA, III 3

Subsection (Annex point)	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.7 Solubility in organic solvents, including the effect of temperature on solubility (IIIA, III 1)	Determination of a threshold value as a first approximation to the solubility. Portion of Icaridin is added with solvent and filled up to a fixed volume. After mechanical agitation visual inspection is followed whether a homogeneous solution is formed.	Purity: 97.9%	Results at 20 °C: Acetone, Acetonitrile, Dichlormethane, Dimethylsulfoxid, Ethylacetate, n-Heptane, 1-Octanol, Polyethylenglycol, 2-Propanol, Xylene: > 250 g/L	It resulted from the preliminary test that the solubility of Icaridin in representative organic solvents at 20 °C was in all cases > 250 g/L. It is not necessary to determine the exact value in the case of solubility > 250 g/L	Y	1	Krohn, 1996	√
	CIPAC MT 157; CIPAC MT 181	Purity: 98.8%	Results at 10 °C: Acetone, Acetonitrile, Dichloromethane, Ethylacetate, n-Heptane, 1-Octanol, Polyethyleneglycol 400, 2-Propanol, Xylene: > 250 g/L	The mixture of Icaridin in dimethylsulfoxide was frozen. Therefore a determination of the solubility of Icaridin in dimethylsulfoxide at 10 °C is not possible.	Y	1	Jungheim, 2006b	√

Section A3 Physical and Chemical Properties of Active Substance

Annex point IIA, III 3

Subsection (Annex point)	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.8 Stability in organic solvents used in b.p. and identity of relevant breakdown products (IIIA, III 2)	–	–	–	Icaridin as manufactured does not include an organic solvent. Therefore a study regarding stability in organic solvents is not applicable for Icaridin.	–	–	–	√
3.9 Partition coefficient n-octanol/water (IIA, III 3.6)	EC method A.8; OECD guideline 107 (shake-flask method)	Purity: 98.9%	Results of log Pow at 20 °C: unbuffered water: 2.11; buffered water, pH 4-9 (salt concentration = 0.1 mol/L): 2.23	The differences between the partition coefficients measured for unbuffered water and buffered water are due to an effect of salinity. RMS: Icaridin is surface active but the result is accepted as it is stated in the report that the concentration was analysed after phase separation	Y	1	Krohn, 1996	√

Section A3 Physical and Chemical Properties of Active Substance

Annex point IIA, III 3

Subsection (Annex point)	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
	EC method A.8; OECD guideline 117 (HPLC)	Purity: 98.8%	Results of log Pow at pH 7 and different temperatures: 30 °C: 2.3 40 °C: 2.4 50 °C: 2.5	The log Pow-values showed slight temperature dependence. RMS: The result is accepted as no problem due to the surface activity is reported.	Y	1	Jungheim, 2005	√
3.10 Thermal stability, identity of relevant breakdown products (IIA, III 3.7)	OECD guideline 113 (differential thermal analysis (DTA) and thermogravimetric analysis (TGA))	Purity: 98.9%	DTA: no exothermic reaction in sealed glass and in open containers until 400 °C; TGA: weight loss starting above 120 °C under air and under nitrogen.	Icaridin was classified as thermally stable at ambient temperature under air.	Y	1	Krohn, 1996	√
3.11 Flammability, including auto-flammability and identity of combustion products (IIA, III 3.8)								

Section A3 Physical and Chemical Properties of Active Substance

Annex point IIA, III 3

Subsection (Annex point)	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
Evolution of flammable gases when contact with water	EC method A.12	Purity: 98.3%	Icaridin does not liberate gases in hazardous amounts as defined in EC method A.12.	–	Y	1	Mix, 1996	√
Pyrophoric properties	EC method A.13	Purity: 98.3%	Icaridin has no pyrophoric properties in the sense of EC method A.13.	–	Y	1	Mix, 1996	√
Auto-flammability	EC method A.15	Purity: 98.3%	Icaridin exhibits an ignition temperature of 375 °C.	–	Y	1	Mix, 1996	√
	EC method A.15	Purity: 98.8%	Icaridin exhibits an auto ignition temperature of 375 °C.	–	Y	1	Heitkamp, 2001	√
3.12 Flash-point (IIA, III 3.9)	EC method A.9	Purity: 98.3%	151 °C at 1027 hPa	–	Y	1	Mix, 1996	√
	EC method A.9	Purity: 98.8%	142 °C at 1007 hPa	–	Y	1	Heitkamp, 2001	√

Section A3 Physical and Chemical Properties of Active Substance

Annex point IIA, III 3

Subsection (Annex point)	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.13 Surface tension (IIA, III 3.10)	EC method A.5; OECD guideline 115 (ring method)	Purity: 98.9%	The surface tension of aqueous solutions of Icaridin with a concentration of approximately 1 g/L was determined to be 49 mN/m at 20 °C.	Icaridin has to be classified as surface active according to EC method A.5.	Y	1	Krohn, 1996	√
3.14 Viscosity (-)	OECD guideline 114 Rotational viscometer	Purity: 98.8%	0.104 Pa×s at 23 °C and a shear rate from 0 to 100 s^{-1}.	–	Y	1	Jungheim, 2006c	√
3.15 Explosive properties (IIA, III 3.11)	EC method A.14	Purity: 98.3%	Icaridin is not explosive in the sense of EC method A.14.	Thermal stability and mechanical sensitivity are tested	Y	1	Mix, 1996	√
3.16 Oxidizing properties (IIA, III 3.12)	–	–	Based on scientific judgement it is certified that due to the chemical structural formula Icaridin does not contain oxidising groups in its molecular backbone and thus may not react exothermically with a combustible material. Therefore Icaridin does not have oxidising properties.		N	2	Koch, 2006	√

Section A3 Physical and Chemical Properties of Active Substance

Annex point IIA, III 3

Subsection (Annex point)	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.17 Reactivity towards container material (IIA, III 3.13)	–	–	Based on experience by the packaging of Icaridin, following packaging material is recommended for the direct contact with the active substance: - Polyethylene Inliner Based on experience by storing of Icaridin in a tank, following material is suitable for the direct contact with the active substance: - 1.4571 VA = 17/12/2 CrNiMo-steel, stabilised with Ti, C max. 0.08 Based on trials on corrosion of Icaridin towards different metals, following materials are also suitable for the direct contact with the active substance: - 1.4401 = 18/12/2 CrNiMo-steel, C max. 0.06 - 1.4313 = 13/4 CrNi-steel		N	2	Lindel, 2005	√

Section A3 Physical and Chemical Properties of Active Substance
Annex point IIA, III 3

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*January 2007*
Materials and methods	*Acceptable*
Conclusion	*Adopted*
Reliability	*1-2*
Acceptability	*acceptable*
Remarks	*The remarks are inserted in the column "remarks/justification".*

Section A3 **Subsection A3.1.3** **Annex Point IIA, III 3.1**	**Physical and chemical properties of the active substance** BULK DENSITY	
	Justification for non-submission of data	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [X]** **Scientifically unjustified [ ]** **Other justification [ ]**	√
Detailed justification:	The determination of the bulk density is only applicable to solid substances. Since the active substance Icaridin is a colourless liquid the determination of the bulk density does not apply.	√
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	*January 2007*	
Evaluation of applicant's justification	*No comments*	
Conclusion	*Acceptable*	
Remarks	*None*	

Section A3 **Subsection A3.8** **Annex Point IIIA, III 2**	**Physical and chemical properties of the active substance** STABILITY IN ORGANIC SOLVENTS	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ] Scientifically unjustified [ ]** **Other justification [X]**	 √
Detailed justification:	Since the active substance Icaridin as manufactured does not include an organic solvent a study regarding stability in organic solvents does not apply. For data regarding storage stability of the Icaridin containing formulation (ethanol/water-mixture) please refer to Document III B, Section 3.7.	√
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	*January 2007*	
Evaluation of applicant's justification	*No comments*	
Conclusion	*Acceptable*	
Remarks	*None*	

Section A4.1 Analytical Methods for Detection and Identification

Annex Point IIA, IV 4.1 ANALYTICAL METHOD FOR THE DETERMINATION OF PURE ACTIVE SUBSTANCE IN THE ACTIVE SUBSTANCE AS MANUFACTURED

Official use only

1 REFERENCE

1.1	**Reference**	Jungheim, R., 2009, Validation of a GC-method for the determination of KBR 3023 and significant impurities in KBR 3023. CURRENTA GmbH & CO. OHG, Services Analytik, Leverkusen, Germany, Study No. 2009/0004/01 (unpublished), 2009-05-04.
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

2.1	**Guideline study**	SANCO guidance 3030/99 rev. 4 of 11/07/00
2.2	**GLP**	Yes
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Preliminary treatment**	
3.1.1	Enrichment	An amount of the active substance as manufactured is dissolved in dichloromethane by manual shaking after it was weighed to the nearest 0.01 mg into a measuring flask. After filling the mark with dichloromethane an aliquot of the solution is analysed according to the indicated conditions.
3.1.2	Cleanup	
3.2	**Detection**	
3.2.1	Separation method	The active substance is determined by means of gas chromatography according to the following conditions:

Separation column:
Fused silica capillary
Length: 25 m
Internal diameter: 0.32 mm
Film thickness: 1.2 µm
Stationary phase: CP-Sil 5 CB
Carrier gas: Helium
Split: 70 mL/min
Sample injection: Split injection
Pressure: 120 kPa
Injection volume: 1 µL
Injector temperature: 250 °C

		Temperature program: Temp. [°C] Time [min] Ramp [K/min] 60 2 10 190 5 5 260 5 -- Retention time: Icaridin approx. 18.8 min
3.2.2	Detector	Flame ionisation detector Detector temperature: 300 °C
3.2.3	Standard(s)	External standard: Icaridin (purity: 98.2%)
3.2.4	Interfering substance(s)	Due to the analytical procedure there are no interfering substances expected.
3.3	**Linearity**	
3.3.1	Calibration range	To determine the linearity of the detector response six concentrations ranging from 80 – 120% of the typical initial weight (where 100% = 115 mg / 5 mL) were measured.
3.3.2	Number of measurements	One measurement per standard concentration was performed.
3.3.3	Linearity	Correlation coefficient: 0.9976
3.4	**Specificity: interfering substances**	No interferences were observed.
3.5	**Recovery rates at different levels**	The determination of the recovery rates is not necessary for the active substance content in the active substance as manufactured.
3.5.1	Relative standard deviation	Relative standard deviation was determined for the precision of the method. Please refer to Section 3.7 (Precision) below.
3.6	**Limit of determination**	No limit of determination is given since the method is only used for testing of specification limits.
3.7	**Precision**	
3.7.1	Repeatability	For the determination of method precision six replicate sample determinations with an initial weight of approx. 115 mg / 5 mL were performed. The values ranged from 99.180 to 99.197%. The mean value obtained was 99.2% and the relative standard deviation 0.01%.
3.7.2	Independent laboratory validation	No independent laboratory validation is available.

Section A4.1 Analytical Methods for Detection and Identification

Annex Point IIA, IV 4.1 ANALYTICAL METHOD FOR THE DETERMINATION OF PURE ACTIVE SUBSTANCE IN THE ACTIVE SUBSTANCE AS MANUFACTURED

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1	**Materials and methods**	The content of Icaridin in the active substance as manufactured is determined by means of gas chromatography using flame ionisation detection after dissolving samples in dichloromethane. The quantitative evaluation is done by area normalisation. For confirmation purposes GC-MS measurements are used.
4.2	**Conclusion**	The analytical method was successfully validated with respect to linearity, specificity, accuracy, and precision. The method is suitable for the determination of the Icaridin content in the active substance as manufactured in the range of 80-120%.
4.2.1	Reliability	Reliability indicator: 1
4.2.2	Deficiencies	No

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*November 2016*
Materials and methods	*Acceptable*
Conclusion	*The analytical method is validated according to SANCO/0303/99 rev. 4*
Reliability	*1*
Acceptability	*The analytical method is accepted*
Remarks	*The normalisation method is used to avoid several sources of measurements uncertainties (e.g. weighing and dilution errors) and has been shown to have a much higher precision and accuracy based on standard deviation, than internal or external standard methods.*

Section A4.1 Analytical Methods for Detection and Identification

Annex Point IIA, IV 4.1 ANALYTICAL METHOD FOR THE DETERMINATION OF IMPURITIES IN THE ACTIVE SUBSTANCE AS MANUFACTURED

		5 REFERENCE	Official use only
5.1	**Reference**	The analytical methods for the determination of impurities in the active substance as manufactured are confidential. This information is provided separately in the confidential part of the dossier.	√
5.2	**Data protection**		
5.2.1	Data owner		
5.2.2	Companies with letter of access		
5.2.3	Criteria for data protection		

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*January 2007*
Materials and methods	-
Conclusion	-
Reliability	-
Acceptability	-
Remarks	-

Section A4.2 Analytical Methods for Detection and Identification

Annex Point IIA, IV 4.2 ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN AIR

Official use only

6 REFERENCE

6.1	**Reference**	Eben, A., 1989, KBR 3023 Concentration determination in the test atmosphere after spraying, Bayer AG, Toxicology Department, Wuppertal, Germany, Report No. 18510 (unpublished), 1989-11-13.
6.2	**Data protection**	Yes
6.2.1	Data owner	LANXESS Deutschland GmbH
6.2.2	Companies with letter of access	-
6.2.3	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.

7 GUIDELINES AND QUALITY ASSURANCE

7.1	**Guideline study**	No guidelines available
7.2	**GLP**	Yes
7.3	**Deviations**	No

8 MATERIALS AND METHODS

8.1	**Preliminary treatment**	
8.1.1	Enrichment	Three adsorption tubes, each filled with 600 mg wadding, are connected in series to the sampling apparatus. For sampling air is sucked through the adsorption tubes (air throughput 2.0 L/min). The total volume of air (10 L) passing through, the temperature of the gas meter, the temperature of the inhalation chamber and the barometric pressure are read off. After sampling the adsorption tubes connected in series are mounted on a 100 mL volumetric flask, in the reverse order from that of the original direction of gas flow. A funnel is attached and 100 mL carbon tetrachloride is allowed to flow slowly through the tubes. The flask is then made up to the mark with carbon tetrachloride. The determination of the active substance is performed after gas chromatographic separation by means of a flame ionisation detector according to the indicated conditions.
8.1.2	Cleanup	
8.2	**Detection**	
8.2.1	Separation method	Icaridin residues in air are analysed by means of gas chromatography using flame ionisation detection under the following conditions: Method: "Splitless" (run time 0.7; valve 5 off) Column: Fused silica column (series 530 µ); length: 10m; film thickness: 2.0 µm Column packaging: HP-17 (crosslinked 50% phenyl methyl silicone) (from Hewlett-Packard) Gas settings: Carrier gas: Helium, 10 mL/min Make-up gas: Helium. 20 mL/min

		Fuel gas: Hydrogen: 30 mL/min Synthetic air: 450 mL/min Temperatures: Injector: 200 °C Detector: 300 °C Column: 1.5 min at 65 °C, then to 210 °C at 30 °C/min finally 10 min at 210 °C Volume injected: 1.0 µL Retention time: Solvent: 0.58 min Active substance: 7.32 min The evaluation is performed using an integrator.
8.2.2	Detector	Icaridin residues in air are detected by means of flame ionisation detection.
8.2.3	Standard(s)	External standard (Icaridin, purity: 99.1%)
8.2.4	Interfering substance(s)	Substances of sample matrix or adsorption material may interfere with Icaridin.
8.3	**Linearity**	
8.3.1	Calibration range	At regular intervals and alternating with the analysis samples, comparison standards of the active substance, at comparable concentrations, are injected for calibration purposes. Thus no extrapolation was performed. The determination of a calibration curve is therefore not necessary.
8.3.2	Number of measurements	
8.3.3	Linearity	
8.4	**Specificity: interfering substances**	No significant interferences were detected at the retention time corresponding to Icaridin.
8.5	**Recovery rates at different levels**	To determine recovery rates, solutions which contained 7635.0 (solution 1) or 600.0 (solution 2) µg active substance per mL ethanol / polyethyleneglycol 400 (1:1) were produced. 1.0 mL of solution 1 (7635.0 µg absolute) and 0.2 mL solution 2 (120.0 µg absolute) were each placed in the wadding in the first of three adsorption tubes arranged in series. Fortified adsorption tubes were analysed in quintuplet for each fortification level. In one test of each fortification level the three tubes were eluted separately. The overall mean recovery was 99.8% (n = 10) and the corresponding relative standard deviation 3.6%. Recoveries determined at each fortification level are compiled in Table A4_2-1. The accuracy of the method is acceptable as the recovery results are in the range of 70 to 110%. The total amount of Icaridin was contained in the first adsorption layer. No active substance could be detected in the eluates of the second and third adsorption tube.
8.5.1	Relative standard deviation	The overall relative standard deviation was 3.6%. The relative standard deviations determined at each fortification level are compiled in Table A4_2-1.

8.6	**Limit of determination**	Under the cited conditions, the limit of detection was 9.0 mg active substance / m^3 air when taking a sample volume of 10 L air and a final volume of 100 mL solution.
8.7	**Precision**	
8.7.1	Repeatability	The precision was determined from the recovery rates. The overall relative standard deviation was 3.6%. The precision of the method is acceptable as the relative standard deviation was < 20%.
8.7.2	Independent laboratory validation	No independent laboratory validation available.

9 APPLICANT'S SUMMARY AND CONCLUSION

9.1	**Materials and methods**	An analytical method for the determination of Icaridin in air after spraying was developed and validated. For sampling air is sucked through wadding adsorption tubes (air throughput 2.0 L/min, sample volume 10 L). The adsorbed active ingredient is extracted with carbon tetrachloride and determined after gas chromatographic separation by means of a flame ionisation detector.
9.2	**Conclusion**	The results show that the analytical method permits reliable determination of residues of Icaridin in air with good accuracy and precision.
9.2.1	Reliability	Reliability indicator: 1
9.2.2	Deficiencies	No

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*January 2007*
Materials and methods	*Carbon tetrachloride is used in the clean-up process. Carbon tetrachloride is carcionogenic and not acceptable. The method must be validated at ambient temperature and normal humidity as well as at 35C and at least 80% humidity. (The last is sufficient in case of sufficient recovery) Temperature and humidity is not reported in the study submitted.*
Conclusion	*Not adopted – method is not acceptable*
Reliability	*4*
Acceptability	*Not acceptable (See Material and methods)*
Remarks	*None*

Table A4_2-1: Results of recoveries

Fortification level [µg absolute]	Recoveries [%]	Mean recovery (n=5) [%]	Relative standard deviation [%]	Overall mean recovery (n=10) [%]	Overall relative standard deviation [%]
7635.0	102.9 (*), 100.5, 104.4, 102.3, 100.9	102.2	1.5	99.8	3.6
120.0	96.3 (*), 103.8, 95.7, 96.9, 94.7	97.5	3.7		

(*) The three adsorption tubes were eluted separately

Section A4.2	**Analytical Methods for Detection and Identification**	
Annex Point IIA, IV 4.2	ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN ANIMAL AND HUMAN BODY FLUIDS AND TISSUES	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [X]**	√
Detailed justification:	Since Icaridin is not classified as toxic or highly toxic no analytical method for its determination in animal and human body fluids and tissues must be submitted.	√
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	*January 2007*	
Evaluation of applicant's justification	*No comments.*	
Conclusion	*Acceptable*	
Remarks	*None*	

Section A4.2 Analytical Methods for Detection and Identification

Annex Point IIA, IV 4.2 ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN WATER

Method for the determination of Icaridin (Bayrepel®) and its degradation product Bayrepel acid in ground and tap water

			Official use only
		10 REFERENCE	
10.1	**Reference**	Knepper, T.P., 2005, Monitoring of Bayrepel and Bayrepel-acid in wastewater influents and effluents, ground and tap water, Europa Fachhochschule Fresenius, Idstein, Germany, 2005-10-10	√
10.2	**Data protection**	No	√
10.2.1	Data owner	LANXESS Deutschland GmbH	√
10.2.2	Companies with letter of access	–	
10.2.3	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.	√
		11 GUIDELINES AND QUALITY ASSURANCE	
11.1	**Guideline study**	No	√
		No guideline available	
11.2	**GLP**	No, but method was developed and validated in an accredited laboratory.	√
11.3	**Deviations**	No	√
		12 MATERIALS AND METHODS	
12.1	**Preliminary treatment**		
12.1.1	Enrichment	**Neutral enrichment of Bayrepel**	√
12.1.2	Cleanup	1 L samples of ground or tap water are filtered through a glass fiber filter (0.45 µm, prewashed with methanol and milli-Q-water). Prior to enrichment, 110 ng (10 µL of a solution of 11 ng/µL) of internal standard atrazine D5 are spiked to the samples. The solid phase extraction (SPE) is carried out in the neutral pH-range. The samples are passed through the Oasis® HLB 3cc cartridges under vacuum at a flow rate of approximately 20 mL/min. Prior to extraction, the cartridges are conditioned with 2 mL of n-hexane, 6 mL of methanol and 10 mL of ground water. After enrichment, the cartridges are dried under a gentle stream of nitrogen gas for 45 min. Afterwards, the enriched compounds are eluted with 3 x 1.5 mL of acetone/ethyl acetate (1:1, v:v) in 10 mL glass vials with stretched tip. All extracts are evaporated to approximately 150 µL in a gentle nitrogen flow and 100 ng (10 µL of a solution of 10 ng/µL) of external standard fluazifop-buthyl are added. The extract was made up with acetone/ethyl acetate (1:1, v:v) to 200 µL final volume. After all, the extracts are stirred and filled into micro glass vials. 1 µL of the final solution is analysed by GC-MS under the indicated conditions.	

Acidic enrichment and derivatisation of Bayrepel-acid

1 L samples of ground or tap water are filtered through a glass fiber filter (0.45 µm, prewashed with methanol and milli-Q-water). Prior to enrichment, the samples are adjusted to pH 2 by adding sulphuric acid 3.5 M and 220 ng (20 µL of a solution of 11 ng/µL) of internal standard MCPP D3 are spiked to the samples. The SPE is carried out in the acidic pH-range. The samples are passed through the Oasis® MCX 3cc cartridges under vacuum at a flow rate of approximately 20 mL/min. Prior to extraction, the cartridges are conditioned with 2 mL of n-hexane, 6 mL methanol and 10 mL of ground water adjusted to pH 2. After enrichment, the cartridges are dried under a gentle stream of nitrogen gas for 45 min. Afterwards, the enriched compounds are eluted with 4 x 1.5 mL of acetone in 10 mL glass vials with stretched tip. All extracts are evaporated to dryness under a gentle nitrogen flow. The samples are redissolved in 600 µL of n-hexane. The GC-MS derivatisation is performed using 100 µL diazomethane in diethylether (in excess) at ambient temperature. The reaction mixture is stirred and reaction is stopped after 60 min by addition of one-two droplets of acetic acid in acetone (10:90, v:v). 500 ng (10 µL solution of 50 ng/µL) of external standard PCB 30 are added. The extract is made up with n-hexane to 1 mL final volume. After all, the extracts are stirred and filled into amber glass vials. 1 µL of the final solution is analysed by GC-MS under the indicated conditions.

12.2 Detection

12.2.1 Separation method

Icaridin and Bayrepel acid are determined by means of gas chromatography according to the following conditions: √

GC-MS system:	6890-GC/5973inert-MSD
Separation column:	capillary column
Length:	30 m
Internal diameter:	0.25 mm
Film thickness:	0.25 µm
Stationary phase:	HP-5 MS
Supplier:	Agilent, PaloAlto, CA, USA
Temperatures:	
Injector:	250 °C
Interface	280 °C
Oven:	
Initial temperature:	50 °C (On)
Initial time:	0.75 min
Ramps:	50-120 °C with 20 °C/min in 3.5 min 120-230 °C with 2 °C/min in 55 min 230-290 °C with 20 °C/min in 3 min
Post temp:	290 °C
Post time:	10.0 min
Carrier gas:	
Type:	helium
Flow:	1.1 mL/min

Section A4.2 Analytical Methods for Detection and Identification

Annex Point IIA, IV 4.2

ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN WATER

Method for the determination of Icaridin (Bayrepel®) and its degradation product Bayrepel acid in ground and tap water

		Injection volume: 1 µL (split/splitless mode) Solvent delay: 7.00 min Retention time: Icaridin: 22.1 min; Bayrepel-acid: 24.7 min	
12.2.2	Detector	Mass spectrometric detector Detector parameters: Temperature: 250 °C Mode: SIM, EI+ Dwell time: 100 ms Characteristic ions are used during GC/MS analysis for identification and quantification of each analyte. Selected masses: Icaridin: m/z 128 and m/z 184 Bayrepel acid: m/z 128, m/z 156, m/z 184 and m/z 257	√
12.2.3	Standard(s)	The quantitative evaluation of Icaridin is carried out using Atrazine D5 as internal and Fluazifop-buthyl as external standard. For quantification of the degradation product MCPP D3 is used as internal and PCB 30 as external standard.	√
12.2.4	Interfering substance(s)	Substances of sample matrix may interfere.	√
12.3	**Linearity**		
12.3.1	Calibration range	The calibration was done for Icaridin and Bayrepel-acid in ground water at the following concentrations: 0.01, 0.02, 0.03, 0.05, 0.10, 0.30, 0.50 and 1.00 µg/L.	√
12.3.2	Number of measurements	One measurement per concentration was performed.	√
12.3.3	Linearity	The correlation coefficients obtained for the different characteristic ions are compiled in Table 4.2-1.	√

Section A4.2 Analytical Methods for Detection and Identification

Annex Point IIA, IV 4.2

ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN WATER

Method for the determination of Icaridin (Bayrepel®) and its degradation product Bayrepel acid in ground and tap water

12.4	**Specificity: interfering substances**	Two significant ions are used for identification and quantification of Icaridin (a third ion m/z 229 is present in the chromatogram too) and four significant ions for Bayrepel acid during the mass spectrometric detection. Therefore no interferences were observed.	√
12.5	**Recovery rates at different levels**	Five ground water samples are spiked with 300 ng/L Icaridin and Bayrepel-acid, respectively. Each sample was measured once. Recovery rates: Icaridin: Range of recoveries: 96 - 105%; Mean value: 101% Bayrepel acid: Range of recoveries: 94 - 103%; Mean value: 99%	√
12.5.1	Relative standard deviation	The relative standard deviation for Icaridin and its metabolite was 3.7%.	√
12.6	**Limit of determination**	The limit of detection for Icaridin and Bayrepel-acid in ground water / tap water is 0.01 µg/L.	See remark
12.7	**Precision**		
12.7.1	Repeatability	The fivefold determination of samples enriched with Icaridin and Bayrepel-acid, respectively resulted in a relative standard deviation of 3.7% for both analytes (please refer to point 3.5, recovery rates).	√
12.7.2	Independent laboratory validation	No independent laboratory validation is available.	√
		13 APPLICANT'S SUMMARY AND CONCLUSION	
13.1	**Materials and methods**	An analytical method for the determination of Icaridin and its biodegradation product Bayrepel acid in ground and tap water was developed and validated. The compounds are separated by means of gas chromatography using mass spectrometric detection after solid-phase extraction of water samples. Before gaschromatographic analysis a derivatisation of the metabolite has to be performed. The quantitative evaluation is carried out according to the method of the internal and external standard.	√
13.2	**Conclusion**	The analytical method is valid with respect to specificity, linearity, rates of recovery, precision and limits of quantification and detection. The method is suitable for the determination of Icaridin and Bayrepel acid residues in ground and tap water.	See remark
13.2.1	Reliability	Reliability indicator: 2	√
13.2.2	Deficiencies	No	√

Section A4.2 Analytical Methods for Detection and Identification

Annex Point IIA, IV 4.2

ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN WATER

Method for the determination of Icaridin (Bayrepel®) and its degradation product Bayrepel acid in ground and tap water

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*January 2007*
Materials and methods	*Acceptable*
Conclusion	*Not adopted. It is concluded that LOQ is 0.01 µg/L. LOQ is defined as the lowest fortified level validated with RSD <20 % and a recovery between 70 and 110 %. Lowest fortified level in this study is 0.3 µg/L. The method does not fulfil the required LOQ at 0.1 µg/L for drinking water.*
Reliability	*2 (not GLP)*
Acceptability	*Not acceptable. The LOQ does not fulfil the requirement for drinking water.*
Remarks	*2013: 2013: Analytical method for Bayrepel acid is not acceptable due to derivatisation with diazomethane.*

Table 4.2-1: Linearity

Compound	Characteristic Ion (m/z)	Correlation coefficient
Icaridin	128	0.999
	184	0.999
Bayrepel acid	128	1.000
	156	0.999
	184	1.000
	257	0.999

Table 4.2-2: Recovery

Compound	Icaridin	Icaridin-acid
Groundwater Spiked conc. 0.3 µg/L	Mean value: 101 % (104, 96, 102, 105, 99) RSD: 3.7%	Mean value: 99 % (94, 96, 103, 99, 104) RSD: 3.7%
Wastewater (effluent/influent) Spiked conc. 0.3 µg/L	Mean value: 98 % (95, 96, 104) RSD: 5.0%	Mean value: 110 % (113, 105, 113, 107) RSD: 3.8%

Section A4.2 Analytical Methods for Detection and Identification

Annex Point IIA, IV 4.2 ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN SOIL

			Official use only
		14 REFERENCE	
14.1	**Reference**	Weber, H. and Anspach, Th., 2001, Enforcement method for the determination of the residues of KBR 3023 in soil – Validation of DFG Method S 19 (extended revision) combined with a detection by LC-MS/MS, Dr. Specht & Partner, Chemische Laboratorien GmbH, Hamburg, Germany, Report No. BAY-0105V, Az. G01-0008 (unpublished), 2001-05-22.	√
14.2	**Data protection**	Yes	√
14.2.1	Data owner	LANXESS Deutschland GmbH	√
14.2.2	Companies with letter of access	–	
14.2.3	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.	√
		15 GUIDELINES AND QUALITY ASSURANCE	
15.1	**Guideline study**	The applicability of the DFG Method S 19 (extended revision) for the determination of Icaridin in soil was examined according to the guidance document SANCO/825/00 rev. 6 of 20/06/00 of the European Commission, the BBA-guideline: Residue analytical methods for post-registration control purposes of July 21, 1998 and Directive 91/414/EEC, Annex VI, Part C, No. 2.6.2.	√
15.2	**GLP**	Yes	√
15.3	**Deviations**	No	√
		16 MATERIALS AND METHODS	
16.1	**Preliminary treatment**		
16.1.1	Enrichment	Before analysis, the soil (LUFA Speyer standard soil 2.2) was mixed thoroughly without further preparation.	√
16.1.2	Cleanup	<u>Outline of the method:</u> Specimen material (25 g per analysis) is extracted with acetone. Water is added before-hand in an amount that takes full account of the natural water content of the specimen so that during extraction the acetone:water ratio remains constant at 2+1 (v+v). For liquid-liquid partition ethyl acetate / cyclohexane (1+1, v+v) and sodium chloride are added and after repeated mixing excess water is separated. The evaporated residue of an aliquot of the organic phase is cleaned by gel permeation chromatography (GPC) on Bio Beads S-X3 polystyrene gel using a mixture of ethyl acetate and cyclohexane (1+1, v+v) as eluent. The residue containing fraction is concentrated and analysed for residues of Icaridin by liquid chromatography using tandem mass spectrometric detection according to the indicated conditions.	

Section A4.2 Analytical Methods for Detection and Identification

Annex Point IIA, IV 4.2 ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN SOIL

16.2 Detection

16.2.1 Separation method

Icaridin residues in soil are analysed by means of liquid chromatography using tandem mass selective detection under the following conditions: √

Pre-column: Phenomenex Security Guard C18(2), 5 µm, 4 x 2 mm, No. AJ0-4286

Column: Phenomenex LUNA C18(2), 5 µm, 150 x 2 mm, No. 00F-4252-B0

Eluent: A: Methanol containing 10 mM ammonium acetate
B: HPLC-water containing 10 mM ammonium acetate

	Time [min]	A [%]	B [%]
Equilibration:	2.0	70	30
Injection:	0.0	70	30
Isocratic:	4.0	70	30

Flow rate: 0.4 mL/min

Injection volume: 10µL

Retention time Icaridin: about 2.8 min

For evaluation concentrations in specimen extracts are determined by comparing the detector responses (peak area of the specimen) with the pertinent detector responses obtained from the neighbouring external standard.

16.2.2 Detector

Icaridin residues in soil are detected by means of tandem mass spectrometric detection under the following conditions: √

Type of interface: Turbo-Ion Spray (Electrospray ionisation)

Ionisation mode: positive

Acquisition time: 4.0 min

Mass transition: 230.4→130.3 amu (Quantifier)
230.4→174.2 amu (Qualifier)

16.2.3 Standard(s)

External standard (Icaridin, purity: 98.1%); √

External standard solutions: 0.06 and 0.006 µg/mL (in methanol/water (7+3, v/v))

16.2.4 Interfering substance(s)

Substances of specimen matrix may interfere with Icaridin. √

16.3 Linearity

16.3.1 Calibration range

The linearity of the detector response was confirmed by injecting seven standard solutions in the range of 1.25 to 100 ng/mL Icaridin, covering the working range. √

The single concentrations used for the linearity determination as well as the corresponding peak areas are compiled in Table 4_2-1.

16.3.2	Number of measurements	Each standard concentration was measured once.	√
16.3.3	Linearity	Coefficient of correlation: 1.0000 Linear regression: Y = 8827 * X + 2127	√
16.4	**Specificity: interfering substances**	No significant interferences from the specimen matrix were detected at the retention time corresponding to Icaridin in any of the control specimens.	√
16.5	**Recovery rates at different levels**	A series of recovery experiments was performed by fortifying control (untreated) specimens of LUFA Speyer standard soil 2.2. Fortification experiments were performed at the limit of quantification (0.005 mg/kg) and ten times that level (0.05 mg/kg). Fortified specimens of soil were analysed in quintuplet for each fortification level. Control specimens were analysed in duplicate. The overall mean recovery was 84% (n = 10) and the corresponding coefficient of variation was 3.9%. Recoveries determined at each fortification level are compiled in Table A4_2-2. The accuracy of the method is acceptable as the recovery results are in the range of 70 to 110%.	√
16.5.1	Relative standard deviation	The overall coefficient of variation was 3.9%. The coefficients of variation determined at each fortification level are compiled in Table A4_2-2.	√
16.6	**Limit of determination**	The limit of quantification was 0.005 mg/kg and the limit of detection was 0.001 mg/kg. The chromatographic peaks were greater than the signal equivalent to three times the background noise.	√
16.7	**Precision**		√
16.7.1	Repeatability	The overall coefficient of variation was 3.9%. The precision of the method is acceptable as the coefficient of variation was < 20%.	√
16.7.2	Independent laboratory validation	No independent laboratory validation available.	√

Section A4.2 Analytical Methods for Detection and Identification

Annex Point IIA, IV 4.2 ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN SOIL

		17 APPLICANT'S SUMMARY AND CONCLUSION	
17.1	**Materials and methods**	The applicability of the DFG Method S 19 (extended revision) for the determination of Icaridin in soil was examined. The extraction of Icaridin from soil was performed according to extraction module E 2 followed by a clean-up procedure according to module GPC. Because Icaridin could not be analysed by gas chromatography as a part of the DFG Method S 19, it was determined by high pressure liquid chromatography with tandem mass spectrometric detection (LC-MS/MS).	√
17.2	**Conclusion**	The results show that the DFG S 19 Method (extended revision), combined with the chromatographic determination by high pressure liquid chromatography and the high specific MS/MS detection, permits reliable determination of residues of Icaridin in soil with excellent accuracy and precision from 0.005 to 0.050 mg/kg. The method was therefore considered valid for the determination of Icaridin residues in soil.	v
17.2.1	Reliability	Reliability indicator: 1	√
17.2.2	Deficiencies	No	√

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*January 2007*
Materials and methods	*Acceptable*
Conclusion	*Adopted*
Reliability	*1*
Acceptability	*Acceptable*
Remarks	*The highest fortification level is outside the range of calibration. But the method is accepted as the lowest level is inside the range and as the validation data for the high and low level do not differ significant from each other.* *Feb 2017: The study is in compliance with the guidance available at the time of performance. However for compliance with current guidance (SANCO/820/00 ver.8.1,) a request for validation of a 2nd mass transition will be done at renewal stage.*

TableA4_2-1: Linearity of detector response

External standard X [ng/mL]	Peak area Y [counts]
1.25	10800
2.50	21600
6.00	54700
12.5	112000
25.0	228000
50.0	447000
100	882000

Table A4_2-2: Results of recoveries

Fortification level [mg/kg]	Recoveries [%]	Mean recovery (n=5) [%]	Coefficient of variation [%]	Overall mean recovery (n=10) [%]	Overall Coefficient of variation [%]
0.005	89, 87, 81, 82, 82	84	4.3	84	3.9
0.05	81, 83, 84, 90, 84	84	4.0		

Section A4.2 Analytical Methods for Detection and Identification

Annex Point IIA, IV 4.2

ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN WATER

Method for the determination of Icaridin and its degradation product Bayrepel acid in surface water

			Official use only
		18 REFERENCE	
18.1	**Reference**	Knepper, T.P., 2004, Analysis and mass spectrometric characterization of the insect repellent Bayrepel and its main metabolite Bayrepel-acid, Europe University for Applied Science Fresenius, Idstein, Germany *Journal of Chromatography* A 1046, pp. 159-166 (published)	See remark
18.2	**Data protection**	No	
18.2.1	Data owner	–	
18.2.2	Companies with letter of access	–	
18.2.3	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.	
		19 GUIDELINES AND QUALITY ASSURANCE	
19.1	**Guideline study**	No	
		No guideline available	
19.2	**GLP**	No, but method was developed and validated in an accredited laboratory.	
19.3	**Deviations**	No	
		20 MATERIALS AND METHODS	
20.1	**Preliminary treatment**		
20.1.1	Enrichment	Surface water samples are filtered through glass fiber filters (0.45 µm), prewashed with methanol and Milli-Q water. Solid phase extraction (SPE) is performed with 1 L samples. The SPE of Icaridin is performed in the neutral pH-range, whilst when analysing for acidic metabolites, samples are adjusted to pH 2 by adding 3.5 M sulphuric acid prior to enrichment. The samples are filtered under vacuum (20 mL/min) through glass cartridges filled with 0.1 g LiChrolute EN and 0.25 g Isolute C_{18}ec (encapped C_{18}). Prior to extraction, the cartridges are conditioned with 6 mL n-hexane, 6 mL methanol and 10 mL ground water, respectively for Icaridin analyses, and with 10 mL ground water adjusted to pH 2 for the screening of acidic metabolites. After enrichment and drying under a gentle stream of nitrogen gas for 60 min, the enriched compounds are eluted and prepared for analysis by the following methods:	
20.1.2	Cleanup		

Section A4.2 Analytical Methods for Detection and Identification

Annex Point IIA, IV 4.2 ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN WATER

Method for the determination of Icaridin and its degradation product Bayrepel acid in surface water

Icaridin:
After eluting with 3 X acetone-ethyl acetate (1:1, v/v), the extracts are evaporated under gentle nitrogen flow to 100 µL, internal standard is added (fluazifop-butyl) and the extract is made up to 200 µL final volume.

Bayrepel acid:
After eluting with 2 X 1.5 mL methanol, the extracts are evaporated under nitrogen to dryness. The samples are then derivatised using 700 µL n-hexane and 150 µL diazomethane in diethyl ether (in excess) at 20 °C, with the reaction terminated after 60 min by addition of two droplets of acetic acid in acetone. (10%, v/v). The internal standard heptadecanoic nitrilo acid is added and the extract made up to a final volume of 1 mL with n-hexane.

20.2 Detection

20.2.1 Separation method

Icaridin and Bayrepel acid are determined by means of gas chromatography according to the following conditions:

Separation column:

Length:	30 m
Internal diameter:	0.25 mm
Film thickness:	0.25 µm
Stationary phase:	XTI-5
Supplier:	Restek, Bellefonte, PA, USA

Temperatures:

Injector:	230 °C	
Transfer line:	250 °C	
Ion source:	200 °C	
Oven:	initial temperature:	50 °C for 1 min
	heating rate:	12 °C/min
	final temperature:	300 °C for 10 min

Carrier gas:
Type: helium

Injection volume: 2 µL (splitless)

20.2.2 Detector

Mass spectrometric detection;

Two characteristic ions are used during GC/MS analysis for identification and quantification of each analyte.

Selected ions:
Icaridin: m/z 128, m/z 184
Bayrepel acid: m/z 128, m/z 156

20.2.3 Standard(s)

All chemicals were of analytical grade.

Purity of the reference compound Icaridin: > 99%.

Bayrepel acid (1-piperidine carboxylic acid, 1-methylpropylester, 2-acetic acid) was synthesised and characterised (purity: approx. 95%).

The quantitative evaluation of Icaridin is carried out using fluazifop-

		butyl (the certified pesticide standard) as internal standard. For quantification of the degradation product heptadecanoic nitrilo acid is used as internal standard.
20.2.4	Interfering substance(s)	Substances of sample matrix may interfere.
20.3	**Linearity**	
20.3.1	Calibration range	To determine the linearity of the detector response an eight-point calibration was performed in the range of 0.03 to 2 µg/L in ground water for each compound. The concentrations of the calibration line were: 0.03 (graphically determined), 0.05, 0,1, 0,25, 0,5, 0,75, 1, 1,5 and 2 µg/L.
20.3.2	Number of measurements	One measurement per standard concentration was performed.
20.3.3	Linearity	The calibration curves were linear in the tested range.
20.4	**Specificity: interfering substances**	Two characteristic ions are used for identification and quantification of Icaridin and its metabolite Bayrepel acid during the mass spectrometric detection. No interferences from the matrix were observed.
20.5	**Recovery rates at different levels**	The recovery rates were checked for Icaridin and Bayrepel acid by three standard additions. A mean recovery rate of 98% was determined for Icaridin and a mean recovery rate of 97% was obtained for Bayrepel acid.
20.5.1	Relative standard deviation	Relative standard deviations were not stated in the report.
20.6	**Limit of determination**	The limit of quantification (LOQ) was determined from the calibration curve and the limit of detection (LOD) was defined as 2/1 signal/noise. The LOQ of Icaridin and its degradation product Bayrepel acid in surface water is 0.03 µg/L. The LOD of both analytes is 0.01 µg/L.
20.7	**Precision**	
20.7.1	Repeatability	The precision of the method is established based on the findings for recovery rates which were performed under repeatability conditions.
20.7.2	Independent laboratory validation	No independent laboratory validation is available.

Section A4.2 Analytical Methods for Detection and Identification

Annex Point IIA, IV 4.2 ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN WATER

Method for the determination of Icaridin and its degradation product Bayrepel acid in surface water

21 APPLICANT'S SUMMARY AND CONCLUSION

21.1	**Materials and methods**	An analytical method for the determination of Icaridin and its biodegradation product Bayrepel acid in surface water was developed and validated. The compounds are separated by means of gas chromatography using mass spectrometric detection after solid-phase extraction of water samples and derivatisation (Bayrepel acid). The quantitative evaluation is carried out according to the method of the internal standard.
21.2	**Conclusion**	A method for the determination of Icaridin and Bayrepel acid residues in surface water was developed and validated successfully. The method has been shown to give acceptable specificity, linearity, recovery and limits of determination and detection. The method is suitable for the determination of Icaridin and Bayrepel acid residues in surface water.
21.2.1	Reliability	Reliability indicator: 2
21.2.2	Deficiencies	Relative standard deviations were not stated in the report. This has no influence on the validity of the analytical method.

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*January 2007*
Materials and methods	*The materials and the method may be acceptable, but it is not possible to evaluate it. No validation data is submitted. The reference is a scientific paper without any background data e.g. correlation coefficent of the linearity, recovery data for the individual samples or RSD of recovery. Recovery is tested on 3 samples – not at 5 samples as required.*
Conclusion	*Not adopted. Not possible to evaluate the method.*
Reliability	*4*
Acceptability	*Not acceptable*
Remarks	*2013: Analytical method for Bayrepel acid is not acceptable due to derivatisation with diazomethane.*

Section A4.3	**Analytical Methods for Detection and Identification**	
Annex Point IIIA, IV 1	ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN/ON FOOD OR FEEDSTUFFS	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [X]**	√
Detailed justification:	Analytical method for the determination of active substance residues in/on food or feedstuffs is required if the active substance or the material treated with it is to be used in a manner which may cause contact with food or feedstuffs, or intended to be placed on, in or near soils in agricultural or horticultural use. Icaridin is intended to be used as insect repellent against biting arthropods (biting midges, flies, ticks). Since an exposure to food and feedstuffs can be excluded when applied according to the recommended use, it is justified not to submit an analytical method for the determination of Icaridin in/on food or feedstuffs.	√
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	*January 2007*	
Evaluation of applicant's justification	*No comments*	
Conclusion	*Acceptable*	
Remarks	*None*	