Section A7.1.1.1.1 Hydrolysis as a function of pH and identification of breakdown products

Annex Point IIA7.6.2.1

			Official use only
		1 REFERENCE	
1.1	**Reference**	Hellpointner, E. (1996): Hydrolysis of [14C]KBR 3023 in sterile aqueous buffers. Bayer AG, Crop Protection Development, Institute for Metabolism Research and Residue Analysis, Leverkusen, Germany, Report No. MR 842/96 (PF No. 4185), unpublished, Date: 1996-09-12	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Lanxess Deutschland 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**	Yes, US-EPA Pesticide Assessment Guideline, Subdivision N, Section 161-1 (1982)	
1.1	**GLP**	Yes	
2.2	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Radio-labelled test substance: [hydroxyethyl-1-^{14}C] KBR 3023 (Icaridin)	
3.1.1	Lot/Batch number	Radio-labelled test substance: Lot No. 546/2, Synthesis No. KML 2169 (1996-05-22); Non-labelled test substance: Identity of test substance was confirmed by HPLC in comparison to reference batch (ID 941206ELB01)	
3.1.2	Specification	As given in section 2 of dossier	
3.1.3	Purity	Radio-labelled test substance: Radiochemical purity > 99 %, Specific radioactivity: 3.55 MBq (96 µCi/mg), Chemical purity > 98 % Non-labelled test substance: Purity: 98.2 %	
3.1.4	Further relevant properties	Water solubility of Icaridin: about 8.2 g/l (Krohn, 1996)	
3.2	**Reference substance**	No other substance was used as reference substance. The KBR 3023 (Icaridin) reference solution (solvent: methanol) was stored during the course of the study and used as reference for the 100 % and 10 % values of test concentration.	

Section A7.1.1.1.1 Hydrolysis as a function of pH and identification of breakdown products

Annex Point IIA7.6.2.1

3.2.1	Initial concentration of reference substance	-
3.3	**Test solution**	See Tables A7_1_1_1_1-1a and A7_1_1_1_1-1b for analytical results of the buffer solutions. The test solution is specified in Table A7_1_1_1_1-2. The study was carried out with buffer solutions at three pH levels: - pH 5: 0.01 M acetate buffer - pH 7: 0.01 M TRIS (tris(hydroxymethyl)aminomethane) buffer - pH 9: 0.01 M borate buffer The buffer stock solutions were diluted to the desired molarity of 0.01 with purified water (pH 6.8, electric conductivity 3 µS/cm) and sterilized.
3.4	**Testing procedure**	
3.4.1	Test system	See Table A7_1_1_1_1-3 for a description of the test system.
3.4.2	Temperature	Experiments were run at different temperatures: pH 5: 25 and 50 °C; pH 7: 25 and 50 °C; pH 9: 25 and 50 °C
3.4.3	pH	4 / 7 / 9
3.4.4	Duration of the test	The test durations were different depending on the test temperature: Pre-Test at 50°C (Test 1): 7 days Main Test at 25°C (Test 2): 30 days
3.4.5	Number of replicates	Test 1: One vessel was investigated at each sampling time for each temperature and each pH level Test 2: Two vessels for each incubation time and each pH level (plus 2 controls at day 0, plus pH/sterility controls at days 0, 7, 20 and 30).
3.4.6	Sampling	The sampling intervals were different depending on test temperature: Pre-Test at 50°C (Test 1): 0, 2.5, 6, 24, 54, 120 and 168 hours (7 days). Main Test at 25°C (Test 2): 0, 3, 7, 13, 20, 24 and 30 days.
3.4.7	Analytical methods	The concentrations of Icaridin were determined using reversed phase HPLC under the following conditions: Column: Merck LiChrosorb RP 18, 5 µm, 250 x 4 mm; Mobile phase: acetonitrile/ water; Flow rate: 1 ml/min.; Detector: Radioactivity flow-through detector with solid scintillator cell (Raytest Co.); Injection volume: 100 µl; For purity check of the radiolabeled test substance as well as for second independent analysis of the reference solutions (solvent: methanol; for

Section A7.1.1.1.1 Hydrolysis as a function of pH and identification of breakdown products

Annex Point IIA7.6.2.1

		the 100 % and 10 % values of test concentrations): AMD-TLC (Automated Multiple Development-Thin Layer Chromatography): Analysis with Bio-Imaging Analyser (Fuji, BAS 2000)
3.5	**Preliminary test**	No
		4 RESULTS
4.1	**Concentration and hydrolysis values**	See Table A7_1_1_1_1-4 and Table A7_1_1_1_1-5
4.2	**Hydrolysis rate constant (k_h)**	No degradation was observed
4.3	**Dissipation time**	No degradation was observed
4.4	**Concentration – time data**	Concentration of test substance expressed as percentage of initial concentrations is given A7_1_1_1_1-4 and Table A7_1_1_1_1-5 Concentration-time plots are provided in the report, but are not reasonable, since no degradation was observed
4.5	**Specification of the transformation products**	No degradation was observed
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	The hydrolytic stability of Euparen (dichlofluanid) was tested in accordance with the US-EPA Pesticide Assessment Guideline, Subdivision N, Section 161-1 (1982) at pH levels of 5, 7, and 9 at different temperatures (25 °C and 50 °C) in buffer solutions. The test duration and the sampling intervals were different depending on test temperature: Pre-Test at 50°C (Test 1): Duration: 7 days; Sampling after 0, 2.5, 6, 24, 54, 120 and 168 hours. Main Test at 25°C (Test 2): Duration: 30 days; Sampling after 0, 3, 7, 13, 20, 24 and 30 days
5.2	**Results and discussion**	The material balances were complete for the three test solutions during the incubation period of 7 days (Pre-Test at 50 °C) as well as during 30 days (Main-Test at 25 °C). KBR 3023 (Icaridin) was stable under acidic (pH 5), neutral (pH 7) and alkaline (pH 9) conditions and at both temperatures tested. Due to the HPLC analysis (confirmed by TLC) KBR 3023 (Icaridin) accounted for about 100 % of the radioactivity recovered in the solutions at termination of the experiments. Formation of hydrolysis products was not observed in the course of the study. Considering the high hydrolytic stability determined under environmental pH and temperature conditions it is not expected that hydrolytic processes will contribute to the degradation of KBR 3023 (Icaridin) in the environment.

Section A7.1.1.1.1 Hydrolysis as a function of pH and identification of breakdown products

Annex Point IIA7.6.2.1

5.2.1	k_h	No degradation was observed
5.2.2	DT_{50}	No degradation was observed
5.2.3	r^2	-
5.3	**Conclusion**	Validity criteria can be considered as fulfilled.
5.3.1	Reliability	1
5.3.2	Deficiencies	None

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	14 04 2007
Materials and Methods	The hydrolytic stability of Icaridin was tested in accordance with the US-EPA Pesticide Assessment Guideline, Subdivision N, Section 161-1 (1982) at pH levels of 5, 7, and 9 at different temperatures (25 °C and 50 °C) in buffer solutions. The test duration and the sampling intervals were different depending on test temperature: Pre-Test at 50°C (Test 1): Duration: 7 days; Sampling after 0, 2.5, 6, 24, 54, 120 and 168 hours. Main Test at 25°C (Test 2): Duration: 30 days; Sampling after 0, 3, 7, 13, 20, 24 and 30 days
Results and discussion	The material balances were complete for the three test solutions during the incubation period of 7 days (Pre-Test at 50 °C) as well as during 30 days (Main-Test at 25 °C). KBR 3023 (Icaridin) was stable under acidic (pH 5), neutral (pH 7) and alkaline (pH 9) conditions and at both temperatures tested. Due to the HPLC analysis (confirmed by TLC) KBR 3023 (Icaridin) accounted for about 100 % of the radioactivity recovered in the solutions at termination of the experiments. Formation of hydrolysis products was not observed in the course of the study. Considering the high hydrolytic stability determined under environmental pH and temperature conditions it is not expected that hydrolytic processes will contribute to the degradation of KBR 3023 (Icaridin) in the environment.
Conclusion	The guideline applied is an older guideline no longer included in the US. EPA. guideline program for testing of chemicals. It is concluded that abiotic hydrolysis does not contribute to the degradation of the active under environmental conditions and that the validity criteria of the study can be considered as fulfilled.
Reliability	Based on the assessment of the study a reliability indicator of 2 is considered appropriate for the study
Acceptability	The indications are that the study has been performed according to the guideline without major deviations and that the validity criteria of the study can be considered as fulfilled. The study is thus considered acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*

Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7_1_1_1_1-1a: Type and composition of the test solutions for the pre-test (Test 1)

Solution	[14C]-KBR 3023			Solvent	Total [kBq/50 ml]
	Bq/500 µl	kBq/ml	[µg/ml]		
Reference 100 %	4073.43	8.147	2.29	Methanol	407.34
pH 5	4127.50	8.255	2.33	0.01 M Citrat buffer	412.75
pH 7	4048.77	8.098	2.28	0.01 M Tris* buffer	404.88
pH 9	4219.26	8.439	2.38	0.01 M Borate buffer	421.93
Total					1646.90

* Tris = tris(hydroxymethyl)aminomethane

Table A7_1_1_1_1-1b: Type and composition of the test solutions for the main test (Test 2)

Solution	[14C]-KBR 3023			Solvent	Total [kBq/100 ml]
	Bq/100 µl	kBq/ml	[µg/ml]		
Reference 100 %	819.85	8.199	2.31	Methanol	819.85
pH 5	799.54	7.995	2.25	0.01 M Citrat buffer	799.54
pH 7	798.13	7.981	2.25	0.01 M Tris* buffer	798.13
pH 9	789.12	7.891	2.22	0.01 M Borate buffer	789.12
Total					3206.64

* Tris = tris(hydroxymethyl)aminomethane

Table A7_1_1_1_1-2: Description of test solution

Criteria	Details
Purity of water	Highly pure water purified in a Milli-Q unit (Millipore Co.)
Preparation of test medium	Stock solution: Radiolabelled KBR 3023 was dissolved in 10 ml methanol (865.2 kBq/ml). Application solution pre-test: Volumes of 50 ml application solution were prepared for each pH level on the sterile bench. About 2.3 mg [hydroxyethyl-1-^{14}C] KBR 3023 (Icaridin).
Test concentrations (mg a.i./L)	Start concentration for the experiments: About 2.2-2.3 mg [hydroxyethyl-1-^{14}C] KBR 3023 (Icaridin). The radiolabelled test substance was used without mixing with non-labelled KBR 3023.
Temperature (°C)	First test: 50 °C Main Test: 25 °C
Controls	The reference solution (solvent: methanol) was stored during the course of the study and used as reference for the 100 % and 10 % values of test concentration.
Identity and concentration of co-solvent	Methanol: used in stock and application solutions; Acetonitrile: used for the reference substance
Replicates	Test 1: One vessel was investigated at each sampling time for each temperature and each pH level Test 2: Two vessels for each incubation time and each pH level (plus 2 controls at day 0, plus pH/sterility controls at days 0, 7, 20 and 30).

Table A7_1_1_1_1-3: Description of test system

Glassware	5 ml glass crimp-top vial filled on the sterile bench by 5 ml aliquots of the radiolabelled test solutions. The vials were closed by crimp caps with Teflon-faced septa.
Other equipment	HPLC (for purity check): Hewlett Packard 1090, AMD-TLC (Automated Multiple Development Thin Layer Chromatography, to check of the HPLC results): Analysis with Bio-Imaging Analyser (Fuji, BAS 2000) Autoclave, pH-meter, water bath, thermostat
Method of sterilisation	For the determination of the microorganisms in aqueous solutions a combined culture medium was used. Equal amounts of the three used agar media were mixed together and the resulting mixture was autoclaved. The hot medium (about 60°C) was filled into sterile Petri dishes on a sterile bench. Before taking the samples, the test vessels were wahed with Ethanol (70%) and were carefully flamed in addition. Incubation time in Test 1 was 7 days, whereas for Test 2, samples were incubated for 7, 20 and 30 days

Table A7_1_1_1_4: Results of the Pre-Test at 50 °C (Test 1): Radioactivity balance of the hydrolysis of KBR 3023 (Icaridin) at pH 5, 7 and 9, respectively after different incubation times (nominal initial concentration ca. 2.3 mg/l)

Incubation time (h)	pH 5			pH 7			pH 9		
	% Area	% Recovery	mg/l (Mean)	% Area	% Recovery	mg/l (Mean)	% Area	% Recovery	mg/l (Mean)
0	100	100.0	2.33	100	100.0	2.28	100	100.0	2.38
2.5	100	102.6	2.39	100	102.9	2.35	100	101.6	2.41
6	100	101.5	2.36	100	101.8	2.32	100	100.5	2.39
24	100	102.2	2.38	100	101.7	2.32	100	101.5	2.41
54	100	101.8	2.37	100	102.5	2.34	100	101.2	2.40
120	100	103.2	2.40	100	102.3	2.33	100	101.5	2.41
168	100	104.3	2.43	100	103.9	2.37	100	103.5	2.46

Table A7_1_1_1_5: Results of the Main Test at 25 °C (Test 2): Radioactivity balance of the hydrolysis of KBR 3023 (Icaridin) at pH 5, 7 and 9, respectively after different incubation times (nominal initial concentration ca. 2.2 mg/l)

Incubation time (days)	pH 5			pH 7			pH 9		
	% Area	% Recovery	mg/l (Mean)	% Area	% Recovery	mg/l (Mean)	% Area	% Recovery	mg/l (Mean)
0	100	100.0	2.25	100	100.0	2.25	100	100.0	2.23
3	100	104.1	2.34	100	101.5	2.28	100	101.4	2.25
7	100	100.4	2.26	100	99.7	2.23	100	99.5	2.21
13	100	100.8	2.27	100	100.0	2.25	100	100.0	2.22
20	100	101.2	2.28	100	100.8	2.27	100	100.9	2.24
26	100	102.0	2.30	100	101.8	2.29	100	101.3	2.25
30	100	102.0	2.30	100	101.1	2.27	100	100.8	2.24

Section 7.1.1.1.2 Annex Point IIA 7.6.2.2	Phototransformation in Water including identity of the products of transformation	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data […] **Limited exposure [...]**	**Technically not feasible [ ] Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	The phototransformation study required in annex IIA covers the direct photodegradation in water. Prerequisite for this process is a light absorption of the molecule, which than reacts e.g. with water or by photolytic cleavage. In the UBA guidelines this is taken into account and a phototransformation experiment is only required, if the molecule has sufficient light absorption in the sunlight range (epsilon > 10 at wavelengths $\lambda > 290$ nm). It was proven by an UV spectrum in water, that Icaridin shows no light absorption at wavelengths $\lambda > 290$ nm (see study summary A7.1.1.1.2). This approach is also sensible because experimental cut off of wavelengths $\lambda < 290$ nm is difficult and artefacts can be produced in the laboratory by effects of shorter wavelengths. These would cause phototransformation which will not occur under environmental conditions due to missing light absorption. Taking into account the above mentioned topics it is justified not to perform the phototransformation test with a compound which shows UV light absorption properties in water like Icaridin. The cut off criteria from the 1990 UBA guidelines were also incorporated in the rules for plant protection products e.g. in the Official Journal of the European Communities No L 172, 95/36/EC Placing of the Plant Protection Products on the Market; July 14, 1995. Indirect phototransformation for most chemicals is a slow and unspecific process, which should be required only in special cases.	
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	April 2007/December 2016
Evaluation of applicant's justification	Applicant's justification is OK. However, in the justification of non-submission there is a reference to study summary A7.1.1.1.2 which is missing from the CAR. We think it should state Doc IIIA, section A3 .4 Absorption spectra.
Conclusion	Applicant's justification is acceptable
Remarks	non
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A7.1.1.2.1 Biodegradability (ready) of

Annex Point IIA7.6.1.1 **ICARIDIN (KBR 3023)**

			Official use only
		1 REFERENCE	
1.1	**Reference**	Caspers, N & G. Mueller (1997): Investigation of the ecological properties of KBR 3023. Biodegradability. Bayer AG, Institute of Environmental Analysis, Leverkusen, Germany, Report No. 573 A/96 (unpublished), Date: 1997-01-23.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Lanxess Deutschland 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**	Yes, Council Directive 92/69/EEC, method C.4-B: Modified OECD Screening Test. This test method is in all essential parts identical with the OECD guideline 301 E.	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Icaridin (KBR 3023)	
3.1.1	Lot/Batch number	Batch No. 898446008	
3.1.2	Specification	As given in section 2 of dossier	
3.1.3	Purity	97.9 %	
3.1.4	Further relevant properties	Water solubility of Icaridin: about 8.2 g/l (Krohn, 1996)	
3.1.5	Composition of Product	-	
3.1.6	TS inhibitory to microorganisms	Result of the respiration inhibition test with activated sludge according to OECD Guideline 209: EC_{50} = 1110 mg/l; Reference: Mueller, G. (1997): Investigation of the Ecological Properties of KBR 3023, Bayer AG, Institute for Environmental Analyses, Leverkusen, Germany, Report No. 610 N/96 B, Date: 1997-01-23.	
3.1.7	Specific chemical analysis	There was no specific chemical analysis conducted in addition to the DOC measurement during the study.	

Section A7.1.1.2.1 Biodegradability (ready) of

Annex Point IIA7.6.1.1 **ICARIDIN (KBR 3023)**

3.2	**Reference substance**	Yes, Aniline (Purity ≥ 99.5 %)
3.2.1	Initial concentration of reference substance	19.1 mg/l DOC
3.3	**Testing procedure**	
3.3.1	Inoculum / test species	Test organism was a mixed population of aquatic micro-organisms (activated sludge). Origin: effluent of laboratory scale unit receiving sewage from the south Wupper area water authority. Coarse particles were separated by filtration. Without pre-treatment. (Proportion and nature of industrial waste present in sewage: predominantly domestic sewage; industrial effluent comes mainly from the metal-working industry).
3.3.2	Test system	The test was performed in test flasks. The DOC determination was performed using a Total organic carbon analyser (TOC 5050 A).
3.3.3	Test conditions	Flask 8, 9: Inoculum + Test substance; Flask 1, 2: Blank inoculum (without test substance); Flask 3, 4: Reference substance Aniline + Inoculum Flask 10: Toxicity control (Test substance + Aniline + Inoculum). Each test flask is inoculated with 0.5 ml effluent per litre medium. The mixtures are aerated in the dark at 22 ± 2 °C. Test temperature was 22 ± 2 °C
3.3.4	Method of preparation of test solution	The concentration of the stock solution was 1.0 g/l
3.3.5	Initial TS concentration	20.0 mg/l DOC
3.3.6	Duration of test	28 days
3.3.7	Analytical parameter	DOC (dissolved organic carbon)
3.3.8	Sampling	Degradation was followed by DOC determinations at 0 h and 7, 14, 21, 27 and 28 days
3.3.9	Intermediates/ degradation products	Not identified
3.3.10	Nitrate/nitrite measurement	No
3.3.11	Controls	Control without test substance and toxicity control
3.3.12	Statistics	The degree of biodegradation is calculated by expressing the concentration of DOC removed (corrected for that in the blank inoculum control) as a percentage of the concentration initially present.

Section A7.1.1.2.1 Biodegradability (ready) of
Annex Point IIA7.6.1.1 **ICARIDIN (KBR 3023)**

		4 RESULTS
4.1	**Degradation of test substance**	
4.1.1	Graph	Provided in the report
4.1.2	Degradation	1 % degradation after 28 days
4.1.3	Other observations	No
4.1.4	Degradation of TS in abiotic control	No abiotic degradation performed
4.1.5	Degradation of reference substance	A degradation of 98 % was achieved for Aniline within 14 days
4.1.6	Intermediates/ degradation products	Not applicable
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	The study was designed to assess the ready biodegradability of Icaridin (KBR 3023) and was conducted according to the Council Directive 92/69/EEC, method C.4-B. This test method was in all essential parts identical with the OECD guideline 301 E. A solution of the test substance in a mineral medium was inoculated and incubated under aerobic conditions in the dark at 22 ± 2 °C. Degradation was followed by DOC determinations at different intervals. The study shows no significant deviations from the guideline.
5.2	**Results and discussion**	Icaridin (KBR 3023) showed 1 % degradation after 28 days. A degradation of 98 % was achieved for the reference substance Aniline within 14 days. The used concentrations of the test substance did not show toxic effects to bacteria (toxicity control).
5.3	**Conclusion**	The validity criteria can be considered as fulfilled. Icaridin (KBR 3023) has to be classified as “Not Readily Biodegradable”.
5.3.1	Reliability	2
5.3.2	Deficiencies	The study shows no significant deviations from the guideline but: some reporting deficiencies: The composition of the mineral medium, pH and method of preparation of test solution are not described in detail

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 2007*
Materials and Methods	The study was designed to assess the ready biodegradability of Icaridin (KBR 3023) and was conducted according to the Council Directive 92/69/EEC, method C.4-B. This test method was in all essential parts identical with the OECD guideline 301 E. A solution of the test substance in a mineral medium was inoculated and incubated under aerobic conditions in the dark at 22 ± 2 °C. Degradation was followed by DOC determinations at different intervals. No significant deviations from the guideline were observed.
Results and discussion	Icaridin (KBR 3023) showed 1 % degradation after 28 days. A degradation of 98 % was achieved for the reference substance Aniline within 14 days. The used concentrations of the test substance did not show toxic effects to bacteria (toxicity control).
Conclusion	The validity criteria where considered as fulfilled. Icaridin (KBR 3023) is based on the test classified as “Not Readily Biodegradable”.
Reliability	Based on the assessment of the study a reliability indicator of 2 is considered appropriate for the study
Acceptability	The indications are that the study has been performed according to the guideline without major deviations and that the validity criteria of the study can be considered as fulfilled. The study is thus considered acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A7.1.1.2.2 Biodegradability (inherent)

Annex Point IIA7.6.1.1

			Official use only
		1 REFERENCE	
1.1	**Reference**	Mueller, G. (1999): Investigation of the Ecological Properties of KBR 3023. Bayer AG, Institute of Environmental Analysis, Leverkusen, Germany, Report No. 799 A/98 (unpublished), Date: 1999-08-25	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Lanxess Deutschland GmbH	
1.2.2	Companies with letter of access	-	
1.2.3	Criteria for data protection	Data submitted to the MS before 14 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. 302 B: Inherent Biodegradability: Zahn-Wellens/EMPA-Test (1992-07-17).	
2.2	**GLP**	Yes	
2.3	**Deviations**	Reference substance not clearly specified: Sodium benzoate (mentioned on p. 10) or aniline (mentioned on p. 11)? Detailed test conditions not mentioned in report	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Icaridin (KBR 3023)	
3.1.1	Lot/Batch number	Batch No.: 898711001 A	
3.1.2	Specification	As given in section 2 of dossier	
3.1.3	Purity	Purity: 99 %	
3.1.4	Further relevant properties	Water solubility of Icaridin: about 8.2 g/l (Krohn, 1996)	
3.1.5	Composition of Product	-	
3.1.6	TS inhibitory to micro-organisms	Result of the respiration inhibition test with activated sludge according to OECD Guideline 209: EC_{50} = 1110 mg/l; Reference: Mueller, G. (1997): Investigation of the Ecological Properties of KBR 3023, Bayer AG, Institute for Environmental Analyses, Leverkusen, Germany, Report No. 610 N/96 B, Date: 1997-01-23.	
3.1.7	Specific chemical	No	

Section A7.1.1.2.2 Biodegradability (inherent)

Annex Point IIA7.6.1.1

	analysis	
3.2	**Reference substance**	Yes, Reference substance not clearly specified: Sodium benzoate (page 10) or aniline (mentioned on page 11) ?
3.2.1	Initial concentration of reference substance	96.1 mg/l DOC
3.3	**Testing procedure**	*Non-entry field*
3.3.1	Inoculum / test species	Test organism: Mixed population of aquatic microorganisms (activated sludge) Source of inoculum (sewage effluent): Aeration tank of sewage treatment work in Leverkusen-Buerrig, Germany; Type of inoculum: Predominantly domestic sewage; industrial effluent comes primarily from the metalworking industry. Treatment given: Activated sludge was washed twice; separation of the sludge by centrifugation Concentration of effluents in reaction mixture: 0.4 g sewage sludge/l
3.3.2	Test system	The test substance is suspended in a mineral medium, inoculated with a mixed population of aquatic microorganisms and incubated for 28 days under aerobic conditions in the dark at 20-25 °C. During this period, the biodegradation of the test substance is determined on the basis of the DOC reduction. <u>Test vessels:</u> No information given in report <u>Preparation of test vessels:</u> Flask 1 and 2: Blank inoculum, no test substance; Flask 3 and 4: Reference substance plus inoculum Flask 5 and 6: Test substance plus inoculum Flask 7: Toxicity control; Concentration of activated sludge in the test flasks: 0.4 mg ss/l; <u>DOC analysis:</u> DOC was determined with a method according to DIN 38409, Part 3. DOC analysis was performed in duplicate using a Total Organic Carbon Analyzer (Shimadzu TOC 500, TOC 5050 A).
3.3.3	Test conditions	See table A7_1_1_2_2-1 Detailed test conditions not mentioned in report
3.3.4	Method of preparation of test	Concentration of the stock solution: 1.0 g/l;

Section A7.1.1.2.2 Biodegradability (inherent)

Annex Point IIA7.6.1.1

	solution	No further information given in report
3.3.5	Initial TS concentration	101.5 mg /l DOC
3.3.6	Duration of test	28 days
3.3.7	Analytical parameter	DOC removal
3.3.8	Sampling	Frequency of sampling: DOC analysis was performed at the start of the test (0 h) and 3 h after the addition of the test substance in order to estimate any adsorption of Icaridin by the activated sludge. In addition samples were taken at day 1, 7, 14, 21, 27 and 28. pH and the oxygen concentration: No information given in report
3.3.9	Intermediates/ degradation products	Not identified
3.3.10	Nitrate/nitrite measurement	No
3.3.11	Controls	Blank control (composition see point 3.3.2)
3.3.12	Statistics	Not applicable; No information given in report
		4 RESULTS
4.1	**Degradation of test substance**	*Non-entry field*
4.1.1	Graph	Degradation curve of the test substance is given in the report on page 14 (Figure 1).
4.1.2	Degradation	Degradation values see Table A7_1_1_2_2-2
4.1.3	Other observations	None
4.1.4	Degradation of TS in abiotic control	No abiotic control
4.1.5	Degradation of reference substance	Degradation values see Table A7_1_1_2_2-2; The degradation curve is given in the report on page 14, Figure 2.
4.1.6	Intermediates/ degradation products	n.a.
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	Icaridin (KBR 3023) was tested for its inherent biodegradability using the `Zahn-Wellens /EMPA Test´ according to OECD Guideline No. 302 B. The degree of biodegradation was investigated by following the decrease of DOC. The study shows no significant deviations from OECD Guideline No.

Section A7.1.1.2.2 Biodegradability (inherent)

Annex Point IIA7.6.1.1

		302 B.
5.2	**Results and discussion**	Within the test period of 28 days, a degradation of 6 % was determined for KBR 3023 (Icaridin). For the reference substance sodium benzoate a degradation of 100 % was achieved within 28 days. Thus, Icaridin was not inherently biodegradable under the conditions of the Zahn-Wellens /EMPA Test. The degradation values for the test and reference substance are given in table A7_1_1_2_2-2.
5.3	**Conclusion**	Acceptability of the test: The reference substance sodium benzoate showed a removal by at least 70 % within 14 days. It was degraded by 98 % within 7 days. Hence, the study was considered to be valid. Within the test period of 28 days, a degradation of 6 % was determined for KBR 3023 (Icaridin). Thus, Icaridin was not inherently biodegradable under the conditions of the Zahn-Wellens /EMPA Test.
5.3.1	Reliability	2
5.3.2	Deficiencies	Yes, Reference substance not clearly specified: Sodium benzoate (page 10) or aniline (mentioned on page 11) ? Detailed test conditions not mentioned in report

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 2007*
Materials and Methods	Icaridin (KBR 3023) was tested for its inherent biodegradability using the `Zahn-Wellens /EMPA Test´ according to OECD Guideline No. 302 B. The degree of biodegradation was investigated by following the decrease of DOC. No major deviations from the OECD Guideline No. 302 where reported or identified.
Results and discussion	The reference substance sodium benzoate/aniline showed a removal by at least 70 % within 14 days. It was degraded by 98 % within 7 days. Within the test period of 28 days, a degradation of 6 % was determined for KBR 3023 (Icaridin).
Conclusion	Based on the test, Icaridin is thus categorized as not inherently biodegradable.
Reliability	Based on the assessment of the study a reliability indicator of 2 is considered appropriate for the study
Acceptability	The indications are that the study in general has been performed according to the guideline without major deviations and that the validity criteria of the study can be considered as fulfilled. The study is thus considered acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7_1_1_2_2-1: Test conditions

Criteria	Details
Composition of medium	No information given in report
Additional substrate	No information given in report
Test temperature	Between 20 and 25 °C
pH	No information given in report
Oxygen concentration [mg/l]	No information given in report
Aeration of dilution water	No information given in report
Suspended solids concentration	Concentration of effluents in reaction mixture: 0.4 g sewage sludge/l
Other relevant criteria	None

Table A7_1_1_2_2-2 Percentage degradation of KBR 3023 (Icaridin) and reference substance

Substance	Flask No.	Degradation (%) after different exposure periods						
		3 h	1 d	7 d	14 d	21 d	27 d	28 d
KBR 3023 (Icaridin)	**5**	0	1	0	5	3	6	6
	6	0	1	0	4	2	6	5
	Mean	**0**	**1**	**0**	**5**	**3**	**6**	**6**
Reference substance	**3**	0	66	98	99	99	99	100
	4	0	71	98	99	99	100	100
	Mean	**0**	**69**	**98**	**99**	**99**	**100**	**100**
Toxicity control	**7**	0	34	47	63	48	50	50

Section 7.1.1.2.3 Annex Point IIIA 12.2	Biodegradation in seawater	
	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 not used or released in the marine environment in considerable amounts. Therefore, a seawater biodegradation test 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	April 2007	
Evaluation of applicant's justification	Applicant's justification is OK	
Conclusion	Applicant's justification is acceptable	
Remarks	non	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A7.1.2.1 Biological sewage treatment (01)

Annex Point IIIA12.2 **ICARIDIN (KBR 3023)**

			Official use only
		1 REFERENCE	
1.1	**Reference**	Knepper, T.P. (2004): Analysis and fate of insect repellents Water Science and Technology, Vol. 50, No. 5, pp 301-308 (published), Date: 2004	
1.2	**Data protection**	No	
1.2.1	Data owner	Not applicable, publication	
1.2.2	Companies with letter of access	-	
1.2.3	Criteria for data protection	No data protection claimed	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No	
2.2	**GLP**	No	
2.3	**Deviations**	Not applicable	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Icaridin (KBR 3023, Bayrepel) Deet (N,N-diethyl-m-toluamide) Only the results of Icaridin will be summarized in this context	
3.1.1	Lot/Batch number	Not applicable	
3.1.2	Specification	Not applicable	
3.1.3	Purity	Not applicable	
3.1.4	Further relevant properties	No	
3.1.5	Composition of Product	Not applicable	
3.1.6	TS inhibitory to microorganisms	Result of the respiration inhibition test with activated sludge according to OECD Guideline 209: EC_{50} = 1087 mg/l; Reference: Mueller, G. (1997): Investigation of the Ecological Properties of KBR 3023, Bayer AG, Institute for Environmental Analyses, Leverkusen, Germany, Report No. 610 N/96 B, Date: 1997-01-23.	
3.1.7	Specific chemical analysis	Icaridin was analysed in the in-and effluent water of a wastewater treatment plant. After solid phase enrichment (SPE) of the surface water and effluent, 1 L and 0.5 L respectively at Rp-C18/LiChrolut EN material at pH 7 the quantification was done by GC/MS (HP gas chromatograph 5890 with a HP 5970 mass-selective detector (MSD)	

Section A7.1.2.1 Biological sewage treatment (01)

Annex Point IIIA12.2 **ICARIDIN (KBR 3023)**

		or a Varian Saturn II-Iontrap MS) in the single ion monitoring (SIM) mode utilising the qualifier ions m/z = 128 and 184. As limits of quantification (LOQ) values of 0.05 and 0.1 µg/l were calculated for surface water and WWTP effluent, respectively.
3.2	**Reference substance**	Not reported
3.2.1	Initial concentration of reference substance	Not applicable
3.3	**Testing procedure**	
3.3.1	Analytical parameter	Test substance (Icaridin) concentration
3.3.2	Sampling location	Influents and effluents of the WWTP Wiesbaden, Germany
3.3.3	Sampling dates	3., 4., 5., 6., 7., 8., and 9 of June 2000 and 5., 6., 7., 8., 9., 10., and 11. of August 2000
3.3.4	Sample quality	Daily mixed samples
3.3.5	Intermediates/ degradation products	Not identified
3.3.6	Statistics	None
		4 RESULTS
4.1	**Degradation of test substance**	
4.1.1	Graph	Provided in the publication
4.1.2	Concentration	Icaridin could be detected in the influents to the WWTP Wiesbaden at concentrations between 0.6 and 1.0 µg/L in June 2000 and 0.7 to 1.4 µg/L in August 2000. No Icaridin could be detected in the corresponding WWTP effluents.
4.1.3	Other observations	No
4.1.4	Intermediates/ degradation products	Not reported
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	In- and effluents of the wastewater treatment plant (WWTP) Wiesbaden, Germany have been analysed for Icaridin at each of seven days in June and in August, respectively. The test substance was quantified by GC/MS methods.
5.2	**Results and discussion**	Icaridin could be detected in the influents to the WWTP Wiesbaden at concentrations between 0.6 and 1.0 µg/L in June 2000 and 0.7 to 1.4 µg/L in August 2000. No Icaridin could be detected in the corresponding WWTP effluents. Hence, the results reveal a complete elimination of Icaridin during wastewater treatment in the WWTP Wiesbaden.
5.3	**Conclusion**	The results are an indication for a complete elimination of Icaridin in

Section A7.1.2.1 Biological sewage treatment (01)

Annex Point IIIA12.2 **ICARIDIN (KBR 3023)**

		sewage treatment plants
5.3.1	Reliability	2
5.3.2	Deficiencies	The publication shows no significant deviations but is brief regarding sampling and analytical measures and the reporting of the results.

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	29/4-2010
Materials and Methods	Applicants version is acceptable.
Results and discussion	Adopt applicant's version.
Conclusion	Adopt applicant's version. Icaridin could be detected in the influents to the WWTP Wiesbaden at concentrations between 0.6 and 1.4 µg/l in June and August 2000. No Icaridin could be detected in the corresponding effluents (LOQ=0.1 µg/l).
Reliability	2
Acceptability	acceptable
Remarks	The publication is published in a scientific peer reviewed journal. No information regarding the size or type of WWTP is given.
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A7.1.2.1 Biological sewage treatment (02)

Annex Point IIIA12.2 **ICARIDIN (KBR 3023)**

			Official use only
		1 REFERENCE	
1.1	**Reference**	Knepper, T.P. and Mueller, J. (2005): Monitoring of Bayrepel and its metabolite Bayrepel-acid in wastewater influents and effluents, ground and tap water. Europe University of Applied Sciences Fresenius, Idstein, Germany (published), Date: 2005-10-10	
1.2	**Data protection**	No	
1.2.1	Data owner	Not applicable, publication	
1.2.2	Companies with letter of access	-	
1.2.3	Criteria for data protection	No data protection claimed	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No	
2.2	**GLP**	No	
2.3	**Deviations**	Not applicable	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Icaridin (KBR 3023, Bayrepel) Icaridin-acid (Bayrepel-acid, metabolite of Icaridin)	
3.1.1	Lot/Batch number	Not applicable	
3.1.2	Specification	Not applicable	
3.1.3	Purity	Not applicable	
3.1.4	Further relevant properties	No	
3.1.5	Composition of Product	Not applicable	
3.1.6	TS inhibitory to microorganisms	Result of the respiration inhibition test with activated sludge according to OECD Guideline 209: EC_{50} = 1087 mg/l; Reference: Mueller, G. (1997): Investigation of the Ecological Properties of KBR 3023, Bayer AG, Institute for Environmental Analyses, Leverkusen, Germany, Report No. 610 N/96 B, Date: 1997-01-23.	
3.1.7	Specific chemical analysis	The enrichment was conducted with 1 L tap- and groundwater, 0.2 L wastewater effluent and 0.1 L wastewater influent. Ground- and wastewater samples were filtered through a glass fibre filter (0.45 µm, prewashed with methanol and milli-Q-water).	

Section A7.1.2.1 Biological sewage treatment (02)

Annex Point IIIA12.2 **ICARIDIN (KBR 3023)**

Neutral enrichment of Icaridin

Prior to enrichment, 110 ng (10 µL of a solution of 11 ng·µL^{-1}) of internal standard atrazine D5 were spiked to all samples. The SPE (solid phase extraction) was carried out in the neutral pH-range. The samples were passed through the Oasis® HLB 3cc cartridges under vacuum at a flow rate of approx. 20 mL·min^{-1}. Prior to extraction, the cartridges were conditioned with 2 mL of n-hexane, 6 mL of methanol and 10 mL of groundwater. After enrichment, the cartridges were dried under a gentle stream of nitrogen gas for 45 min. Afterwards, the enriched compounds were eluted with 3 × 1.5 mL of acetone/ethyl acetate (1:1; v:v) in 10 mL glass vials with stretched tip. All extracts were evaporated to approx. 150 µL in a gentle nitrogen flow and 100 ng (10 µL of a solution of 10 ng·µL^{-1}) of external standard fluazifop-buthyl were added. The extract was made up with acetone/ethyl acetate (1:1; v:v) to 200 µL final volume. After all, the extracts were stirred, filled in micro glass vials and stored at -20 °C in a freezer until analysis by gas chromatography – mass spectrometry (GC–MS).

Acidic enrichment and derivatisation of Icaridin-acid

Prior to enrichment, the samples were adjusted to pH 2 by adding sulphuric acid 3.5 M and 220 ng (20 µL of a solution of 11 ng·µL^{-1}) of internal standard MCPP D3 were spiked to all samples. The SPE was carried out in the acidic pH-range. The samples were passed through the Oasis® MCX 3cc cartridges under vacuum at a flow rate of approx. 20 mL·min^{-1}. Prior to extraction, the cartridges were conditioned with 2 mL of n-hexane, 6 mL of methanol and 10 mL of groundwater adjusted to pH 2. After enrichment, the cartridges were dried under a gentle stream of nitrogen gas for 45 min. Afterwards, the enriched compounds were eluted with 4 × 1.5 mL of acetone in 10 mL glass vials with stretched tip. All extracts were evaporated to dryness under a gentle nitrogen flow. The samples were redissolved in 600 µL of n-hexane.

The GC–MS derivatisation was performed using 100 µL diazomethane in diethylether (in excess) at ambient temperature. The reaction mixture was stirred and reaction was stopped after 60 min by addition of one - two droplets of acetic acid in acetone (10:90, v:v). 500 ng (10 µL of a solution of 50 ng·µL^{-1}) of external standard PCB 30 were added. The extract was made up with n-hexane to 1 mL final volume. After all, the extracts were stirred, filled in amber glass vials and stored at -20 °C in a freezer until analysis by GC–MS.

Set-up of the GC-MS

Equipment:	GC-MS system 6890-GC/5973inert-MSD
Capillary column:	HP-5 MS (30 m, 0.25 mm i.d., 0.25 µm film thickness (Agilent, Palo Alto, CA, USA)
Carrier gas:	Helium, flow of 1.1 mL/min
Injection temperature:	250°C
Interface temperature:	280°C
Injection volume:	1 µL
Solvent delay:	7.00 min

Section A7.1.2.1 Biological sewage treatment (02)

Annex Point IIIA12.2 **ICARIDIN (KBR 3023)**

		Oven temperature program: Initial temperature: 50°C 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 temperature: 290°C Post time: 10.0 min Detector parameter: Temperature: 250°C Mode: SIM, EM+ Dwell time: 100 ms
3.2	**Reference substance**	Not reported
3.2.1	Initial concentration of reference substance	Not applicable
3.3	**Testing procedure**	
3.3.1	Analytical parameter	Test substance (Icaridin) and metabolite (Icaridin-acid) concentrations
3.3.2	Sampling location	Influents and effluents of the wastewater treatment plants (WWTPs) Wiesbaden and Stockstadt, Germany. WWTP Stockstadt: located close to a nature protection area. Abatement against mosquitoes is constricted and therefore a higher application of insect repellents could be expected. WWTP Wiesbaden: influent samples were taken after primary treatment. The WWTP was selected due to a broad data base. The treatment steps of both WWTPs are summarized in Table A7_1_2_1-1. Groundwater samples were taken from the cities Biblis, Dornheim, Hähnlein and Niedernhausen, all cities are located in Germany. Tapwater samples were taken from the cities Frankfurt, Mainz, Seligenstadt, Stockstdt and Wiesbaden, all cities are located in Germany.
3.3.3	Sampling dates	The sampling was done between July 2004 to August 2005 (WWTP Wiesbaden) and June 2005 to August 2005 (WWTP Stockstadt). The exact dates can be gathered from Table A7_1_2_1-2 and Table A7_1_2_1-3. Groundwater samples were taken on 08.07.2005 (Niedernhausen) and 05.09.2005 (Biblis, Dornheim and Hähnlein) Tapwater samples were collected on 15.8.2005 (Wiesbaden), 19.8.2005 (Mainz and Stockstadt), 10.8.2005 (Frankfurt) and 21.8.2005 (Seligenstadt).
3.3.4	Sample quality	WWTP samples: 24-h- mixed samples

Section A7.1.2.1 Biological sewage treatment (02)

Annex Point IIIA12.2 **ICARIDIN (KBR 3023)**

		Tapwater and groundwater: random sampling
3.3.5	Intermediates/ degradation products	Yes. Icaridin-acid
3.3.6	Statistics	None

4 RESULTS

4.1	**Degradation of test substance**	
4.1.1	Graph	The results are presented in tabular form.
4.1.2	Concentration	The Icaridin concentrations in the in- and effluents of the WWTPs Wiesbaden (data from 2004 and 2005) and Stockstadt (data from 2005) are presented in Table A7_1_2_1-2 and Table A7_1_2_1-3, respectively. Both Tables also include the calculation of elimination rates for the parent compound. Icaridin concentrations in ground- and tapwater are presented in Table A7_1_2_1-4.
4.1.3	Other observations	No
4.1.4	Intermediates/ degradation products	The Icaridin-acid concentrations in the in- and effluents of the WWTPs Wiesbaden (data from 2004 and 2005) and Stockstadt (data from 2005) are presented in Table A7_1_2_1-2 and Table A7_1_2_1-3, respectively. Icaridin-acid concentrations in ground- and tapwater are presented in Table A7_1_2_1-4.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	In- and effluents of two wastewater treatment plants (WWTP) in Germany have been analysed for Icaridin and its metabolite Icaridin-acid at several occasions in 2004 (only WWTP Wiesbaden) and 2005 (WWTPS Wiesbaden and Stockstadt). In addition, randomly taken tapwater and groundwater samples were analysed for the parent and its metabolite.

Section A7.1.2.1 Biological sewage treatment (02)

Annex Point IIIA12.2 **ICARIDIN (KBR 3023)**

5.2 Results and discussion

WWTP Wiesbaden

Icaridin influent residues measured in 2004 revealed a peak concentration in August (2.5 µg/L), whereas contents measured in July, October, November and December were equal or less 1.0 µg/L. Comparable, in 2005 maximum Icaridin contents in the Wiesbaden WWTP influents could also be found during the summer months June to August (max. 2.9 µg/L), whereas from January to March residues were below 0.4 µg/L. In none of the effluent samples (2004 and 2005) Icaridin could be detected.

The metabolite Icaridin-acid could be detected in most influents and all effluent samples. Maximum Icaridin-acid effluent concentrations amounted to 0.55 µg/L in samples taken in October 2004 and 0.95 to 2.1 µg/L in the samples analysed between June and August.

Elimination rates of Icaridin were calculated to vary between 3 and 84%.

WWTP Stockhausen

Icaridin residues in the Stockhausen influent were in general higher (0.4 to 6.4 µg/L), compared to the remains measured in the Wiesbaden WWTP influent. The peak Icaridin concentration (6.4 µg/L) was found in June 2005. None of the effluents contained traces of the parent compound.

Icaridin-acid was found at a peak influent concentration of 0.60 µg/L, whereas the effluents contained metabolite amounts of 0.35 µg/L at maximum.

Elimination rates for Icaridin were calculated to vary between 75 and 100%.

Groundwater and tapwater

None of the samples contained residues of Icaridin or Icaridin-acid above the limit of determination (0.01 µg/L).

5.3 Conclusion

The monitoring measurements of Icaridin concentrations in WWTP influents reveal a seasonal course with higher concentrations occurring during the summer month.

Icaridin, when entering a WWTP undergoes an almost complete primary degradation, yielding the more stable metabolite Icaridin-acid. The metabolite could be detected in WWTP influents and effluents. Icaridin-acid could be found in the influents, because samples have been taken following a mechanical pre-treatment of the wastewater, indicating the quick transformation of Icaridin when entering the WWTP.

There are indications that the elimination rate of Icaridin in a WWTP might be dependent in its concentration in the WWTP influent, i.e., the elimination is higher at elevated influent residues.

Neither ground- nor tapwater contained residues of Icaridin or Icaridin-acid, indicating the complete degradation and/or removal of the substances by sewage treatment plants and groundwater

Section A7.1.2.1 Biological sewage treatment (02)

Annex Point IIIA12.2 **ICARIDIN (KBR 3023)**

		conditioning systems.
5.3.1	Reliability	2
5.3.2	Deficiencies	The publication shows no significant deficiencies.

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	29/4-2010
Materials and Methods	No information is given regarding the sampling procedures of groundwater samples (e.g. depth, how the sampling was done, is groundwater potentially influenced by agriculture/sludge?).
Results and discussion	Adopt applicant's version.
Conclusion	5.3: "There are indications that the elimination rate of Icaridin in a WWTP might be dependent in its concentration in the WWTP influent, i.e., the elimination is higher at elevated influent residues". I disagree, since there is not demonstrated a correlation between concentration and elimination. The difference could just as well be due to other differences between the two STPs. "Neither ground- nor tapwater contained residues of Icaridin or Icaridin-acid, indicating the complete degradation and/or removal of the substances by sewage treatment plants and groundwater conditioning systems." This statement is not acceptable. Icaridin or Icaridin-acid could potentially reach the groundwater at a later stage. This depends among other things on the type of soil above the ground water and the depth at which the groundwater is located. Icaridin was first used in 1998 and these samples were taken in 2005.
Reliability	2
Acceptability	acceptable
Remarks	No information is given regarding where the data have been published?
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7_1_2_1-1: Treatment steps of the investigated wastewater treatment plants (WWTPs) Wiesbaden and Stockstadt, Germany

WWTP	Mechanical treatment	Biological treatment	Others	Hydraulic retention time
Wiesbaden	Rack, grit chamber, preliminary sedimentation	Anaerobic, anoxic/denitrification, anoxic/denitrification	Chemical phosphate-removal microscreen	Mechanical treatment: 48 h Biological treatment and others: 24 h
Stockstadt	Rack, grit chamber	Anaerobic, anoxic/denitrification, anoxic/denitrification	Chemical phosphate-removal	24 h

Table A7_1_2_1-2: Concentrations of Icaridin and Icaridin-acid in the WWTP Wiesbaden as well as elimination rates of Icaridin

WWTP water	Sampling date	Icaridin concentration (µg/L)	Icaridin-acid concentration (µg/L)	Elimination of Icaridin (%)
Influent	19.-20.07.2004	0.60	0.65	70
Effluent	20.-21.07.2004	< LOD*	0.38	
Influent	23.-24.08.2004	2.50	0.50	84
Effluent	24.-25.08.2004	< LOD	0.48	
Influent	18.-19.10.2004	1.00	0.30	58
Effluent	19.-20.10.2004	< LOD	0.55	
Influent	08.-09.11.2004	0.50	0.30	48
Effluent	09.-10.11.2004	< LOD	0.42	
Influent	29.-30.11.2004	0.50	< LOQ**	24
Effluent	30.-01.12.2004	< LOD	0.38	
Influent	27.-28.12.2004	< LOD	< LOQ	n.a.
Effluent	28.-29.12.2004	< LOD	0.23	
Influent	31.01.-01.02.2005	< LOD	0.00	n.a.
Effluent	01.-02.02.2005	< LOD	0.23	
Influent	15.-16.02.2005	0.22	0.00	9
Effluent	16.-17.02.2005	< LOD	0.20	
Influent	28.02.-01.03.2005	0.35	< LOD	34
Effluent	01.-02.03.2005	< LOD	0.23	
Influent	14.-15.03.2005	0.39	< LOD	31
Effluent	15.-16.03.2005	< LOD	0.27	
Influent	29.-30.03.2005	0.34	< LOD	3
Effluent	30.-31.03.2005	< LOD	0.32	
Influent	20.-21.06.2006	2.00	0.40	48
Effluent	21.-22.06.2005	< LOD	1.3	
Influent	04.-05.07.2005	1.00	0.30	23
Effluent	05.-06.07.2005	< LOD	1.0	
Influent	18.-19.07.2005	2.90	0.50	40
Effluent	19.-20.07.2007	< LOD	2.1	
Influent	02.-03.08.2005	1.30	0.30	41
Effluent	03.-04.08.2005	< LOD	0.95	
Influent	17.-18.08.2005	1.90	0.30	48
Effluent	18.-19.08.2005	< LOD	1.2	

* LOD = limit of detection; n.a. = not analysable
** LOQ = limit of quantification (0.10 µg/L for the influent and 0.05 µg/L for the effluent)

Table A7_1_2_1-3: Concentrations of Icaridin and Icaridin-acid in the WWTP Stockstadt as well as elimination rates of Icaridin

WWTP water	Sampling date	Icaridin concentration (µg/L)	Icaridin-acid concentration (µg/L)	Elimination of Icaridin (%)
Influent	20.-21.06.2005	6.4	0.60	95
Effluent	21.-22.06.2005	< LOD*	0.35	
Influent	22.-23.06.2005	5.4	0.60	94
Effluent	23.-24.06.2005	< LOD	0.35	
Influent	04.-05.07.2005	0.40	0.20	75
Effluent	05.-06.07.2005	< LOD	0.15	
Influent	06.-07.07.2005	2.80	0.40	95
Effluent	07.-08.07.2005	< LOD	0.15	
Influent	18.-19.07.2005	6.10	0.50	96
Effluent	19.-20.07.2005	< LOD	0.25	
Influent	20.-21.07.2005	3.60	0.40	95
Effluent	21.-22.07.2005	< LOD	0.20	
Influent	01.-02.08.2005	3.00	0.30	95
Effluent	02.-03.08.2005	< LOD	0.15	
Influent	03.-04.08.2005	5.30	0.50	97
Effluent	04.-05.08.2005	< LOD	0.15	
Influent	15.-16.08.2005	1.10	< LOD	91
Effluent	16.-17.08.2005	< LOD	0.10	
Influent	17.-18.08.2005	1.20	< LOD	100
Effluent	18.-19.08.2005	< LOD	< LOQ**	

* LOD = limit of detection
** LOQ = limit of quantification (0.10 µg/L for the influent and 0.05 µg/L for the effluent)

Table A7_1_2_1-4: Concentrations of Icaridin and Icaridin-acid in groundwater and tapwater samples

Type of water	Location/date of sampling	Icaridin concentration (µg/L)	Icaridin-acid concentration (µg/L)
Groundwater	Biblis 05.09.2005	< LOD*	< LOD
	Dornheim 05.09.2005	< LOD	< LOD
	Hähnlein 05.09.2005	< LOD	< LOD
	Niedernhausen 08.07.2005	< LOD	< LOD
Tapwater	Frankfurt 20.08.2005	< LOD	< LOD
	Mainz 19.08.2005	< LOD	< LOD
	Seligenstadt 21.08.2005	< LOD	< LOD
	Stockstadt 19.08.2005	< LOD	< LOD
	Wiesbaden 15.08.2005	< LOD	< LOD

* LOD (groundwater and tapwater) = 0.01 µg/L

Section A7.1.2.1 Biological sewage treatment (03)

Annex Point IIIA12.2 **ICARIDIN (KBR 3023)**

			Official use only
		1 REFERENCE	
1.1	**Reference**	Knepper, T.P., Maes, A. and Mueller, J. (2005): Occurrence and fate of insect repellents in the aquatic environment Europe University of Applied Sciences Fresenius, Idstein, Germany (in preparation for being published in Environ. Sci. & Technol.) Knepper, T.P., Mueller, J. and Maes, A. (2005): Occurrence and fate of insect repellents in the aquatic environment Europe University of Applied Sciences Fresenius, Idstein, Germany (poster presentation)	
1.2	**Data protection**	No	
1.2.1	Data owner	Not applicable, publication	
1.2.2	Companies with letter of access	-	
1.2.3	Criteria for data protection	No data protection claimed	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No	
2.2	**GLP**	No	
2.3	**Deviations**	Not applicable	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Icaridin (KBR 3023, Bayrepel) Icaridin-acid (Bayrepel-acid, metabolite of Icaridin)	
3.1.1	Lot/Batch number	Not applicable or not reported	
3.1.2	Specification	Not applicable or not reported	
3.1.3	Purity	Not applicable or not reported	
3.1.4	Further relevant properties	No	
3.1.5	Composition of Product	Not applicable	
3.1.6	TS inhibitory to microorganisms	Result of the respiration inhibition test with activated sludge according to OECD Guideline 209: EC_{50} = 1087 mg/l; Reference: Mueller, G. (1997): Investigation of the Ecological Properties of KBR 3023, Bayer AG, Institute for Environmental Analyses, Leverkusen, Germany, Report No. 610 N/96 B, Date: 1997-01-23.	
3.1.7	Specific chemical	The sample preparation for analysis of Icaridin and Icaridin-acid	

Section A7.1.2.1 Biological sewage treatment (03)

Annex Point IIIA12.2 **ICARIDIN (KBR 3023)**

	analysis	involved solid phase extraction (SPE) and esterification in the case of Icaridin-acid. Analysis was done by gas chromatography-mass-spectrometry (GC/MS). Details of the sample preparation and analysis are described in Document IIIA, 7.1.2.1(02).
3.2	**Reference substance**	Not reported
3.2.1	Initial concentration of reference substance	Not applicable
3.3	**Testing procedure**	
3.3.1	Testing items	1. Fixed-bed bioreactor test (FBBR) to investigate the biodegradation of Icaridin and the formation and biodegradation of Icaridin-acid. The tests were conducted either with Rhine water or with effluent water of the WWTP Wiesbaden. 2. Measurement of Icaridin and Icaridin-acid concentrations in water samples from German rivers (only Icaridin-acid) and lakes. 3. Measurement of the Icaridin concentration in the wastewater influent and the Icaridin-acid concentration in the wastewater effluent of the WWTP Wiesbaden, Germany, over the years 2001, 2002 and 2003.
3.3.2	Analytical parameter	Test substance (Icaridin) and metabolite (Icaridin-acid) concentrations
3.3.3	Experimental set-up	1. Fixed-bed bioreactor test (FBBR): River water or effluent wastewater is running in circuit under aerobic conditions through a test vial containing porous glass beads. The glass beads serve as constituents for immobilisation of the microorganisms being present in the corresponding waters. The FBBR test was conducted either with Rhine water or with effluent wastewater of the WWTP Wiesbaden.
3.3.4	Test substance concentration	1. Fixed-bed bioreactor test (FBBR): 10 µg/L Icaridin and 100 µg/L Icaridin (results are not reported). Spiking was done one and three times in the test with Rhine river water and one and two times (25/04/2002 and 22/08/2002) in the test with WWTP effluent water.
3.3.5	Sampling location	2. Measurement of Icaridin and Icaridin-acid concentrations in water samples from German rivers and lakes: *cf.* Table A7_1_2_1-1 and Table A7_1_2_1-2 3. Measurement of Icaridin and Icaridin-acid concentrations in in-and effluents of a WWTP Wastewater treatment plant Wiesbaden, Germany
3.3.6	Sampling dates	1. Fixed-bed bioreactor test (FBBR): The sampling was done continuously over a period of 4 weeks up to 3 months 2. Measurement of Icaridin and Icaridin-acid concentrations in water samples from German rivers and lakes: *cf.* Table A7_1_2_1-1 and Table A7_1_2_1-2

Section A7.1.2.1 Biological sewage treatment (03)

Annex Point IIIA12.2 **ICARIDIN (KBR 3023)**

		3. Measurement of Icaridin and Icaridin-acid concentrations in in- and effluents of a WWTP *cf.* Figure A7_1_2_1-5
3.3.7	Sample quality	2. Measurement of Icaridin and Icaridin-acid concentrations in water samples from German rivers and lakes: Rivers: weekly mixed samples Lakes: randomly taken samples 3. Measurement of Icaridin and Icaridin-acid concentrations in in- and effluents of a WWTP Weekly mixed samples
3.3.8	Intermediates/ degradation products	Yes. Icaridin-acid
3.3.9	Statistics	None
		4 RESULTS
4.1	**Degradation of test substance**	
4.1.1	Graph	Figure A7_1_2_1-1 presents the results of the Fixed-bed bioreactor (FBBR) test conducted with Rhine water and one or three spikings of the water with 10 µg Icaridin/L. It resumes the primary degradation of the parent compound. Figure A7_1_2_1-3 presents the results of the Fixed-bed bioreactor (FBBR) test conducted with the effluent of the WWTP Wiesbaden and one or two spikings of the water with 10 µg Icaridin/L. It resumes the primary degradation of the parent compound. Figure A7_1_2_1-5 shows the Icaridin influent concentrations into the WWTP Wiesbaden from 2001 to 2003.
4.1.2	Concentration	Icaridin and Icaridin-acid concentrations in different German lakes sampled in 2003 are summarised in Table A7_1_2_1-2.
4.1.3	Other observations	No
4.1.4	Intermediates/ degradation products	Figure A7_1_2_1-2 demonstrates the formation and primary degradation of the metabolite Icaridin-acid in the bioreactor test conducted with Rhine water and one or three spikings of the water with 10 µg Icaridin/L. Figure A7_1_2_1-4 demonstrates the formation and primary degradation of the metabolite Icaridin-acid in the bioreactor test conducted with the effluent of the WWTP Wiesbaden and one or two spikings of the water with 10 µg Icaridin/L. **Table A7_1_2_1-1:summarises the Icaridin-acid concentrations in different German rivers sampled in 2003.** Icaridin-acid concentrations in different German lakes sampled in 2003 are summarised in Table A7_1_2_1-2.

Section A7.1.2.1 Biological sewage treatment (03)

Annex Point IIIA12.2 **ICARIDIN (KBR 3023)**

Figure A7_1_2_1-5 shows the Icaridin-acid effluent concentrations of the WWTP Wiesbaden from 2001 to 2003.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The biodegradation of Icaridin and the formation and biodegradation of the metabolite Icaridin-acid were investigated by using a Fixed-bed bioreactor (FBBR) and either surface Rhine water or the effluent of a WWTP.

Samples of three German rivers taken in spring and summer 2003 have been analysed for Icaridin-acid. In addition, randomly taken samples from German lakes were analysed for Icaridin and its metabolite.

Furthermore, Icaridin and Icaridin-acid concentrations in the in- and effluents of the WWTP Wiesbaden have been determined. The samples were taken in 2001, 2002 and 2003.

5.2 Results and discussion

Results of the FBBR tests

In the bioreactor test conducted with Rhine water it could be demonstrated, that biodegradation of Icaridin took place after the first spiking, however, it started after a lag phase. A significant increase in the rate and extent of conversion could be seen with the third spiking, demonstrating that an adaptation period is required for initiation of the microbial metabolisation. The rate of Icaridin-acid production following the third spiking was going hand-in-hand with the decline of the parent and thus significantly more rapid and reaching a higher concentration after the third spiking. Subsequent conversion of the metabolite can also be seen to be greater following the third spiking, where a lower final concentration was determined in this case.

Analogous to the surface water experiment, the conversion of Icaridin in the WWTP effluent was significantly enhanced after the second spiking compared to the first one.

The formation and conversion of the Icaridin-acid metabolite however showed a different pattern in the sewage treatment plant effluent by comparison with the surface water. Once again the formation following the second spiking was significantly more rapid as compared with the spiking before, with the maximum concentrations being reached after 5 days and 20 days, respectively. The rate of conversion of the metabolite thereafter however, was much more rapid for both spikings in the sewage effluent, as compared to the rates observed in the surface water experiment. Much higher peak concentrations and rates of formation of Icaridin-acid, and enhanced subsequent primary reduction were observed for the sewage effluent.

Icaridin acid concentrations in German rivers

The compound could not be detected above the detection limit in the rivers Rhine and Main. However, water samples taken from the river Nidda between March and July contained the metabolite at amounts between 0.07 µg/L and 0.36 µg/L.

Section A7.1.2.1 Biological sewage treatment (03)

Annex Point IIIA12.2 **ICARIDIN (KBR 3023)**

		Icaridin and Icaridin acid concentrations in German lakes Icaridin and Icaridin-acid could be identified in each 14 of 21 lake water samples. Their measured concentrations were in general < 0.1 µg/L (Icaridin) and ≤0.25 µg/L (Icardin-acid). Only in one lake (Neu-Malsch) remarkable high residues (up to 6.26 µg/L Icaridin and 0.41 µg/L Icaridin-acid) could be found. Icaridin and Icaridin acid concentrations in the in- and effluent of the WWTP Wiesbaden The Icaridin influent concentrations in 2001 were in general below 1.5 µg/L. Only one measurement conducted at the end of July/beginning of August of this year revealed a maximum amount of 2.6 µg/L. The concentrations of the metabolite were also higher during the summer months, reaching a maximum value of approximately 1.6 µg/L at the end of August/beginning of September. In 2002 influent concentrations of Icaridin increased compared to 2001, reaching a maximum value of approximately 2.5 µg/L in August. Even higher concentrations were observed during the extreme hot and dry summer 2003, where Icaridin concentrations in the influent reached values up to 4.5 µf/L. However, Icaridin-acid concentrations in the years 2002 and 2003 were lower compared to 2001, reaching maximum amounts of approximately 0.25 µg/L. In none of the years under consideration Icaridin or Icaridin-acid could be detected during the winter and early spring months (November to April).
5.3	**Conclusion**	The bioreactor tests reveal Icaridin to be quantitatively transferred into Icaridin acid. This process is forced by the adaptation of the microorganisms to the compound. Icaridin-acid in turn is further degraded after an adaptation of the microorganisms has taken place. The whole biodegradation process, especially the degradation of Icaridin-acid proceeds faster in a medium with a higher microoganism density (faster degradation (up to 90%) in effluent water compared to surface water). In lakes for bathing Icaridin and Icaridin-acid residues could be found occasionally and if detected at all, amounts were generally low. Only one lake (Neu-Malsch) exhibited high residues of the compounds, which might be the result of spillage. The occurrence of the metabolite Icardin-acid in bathing lakes is an indication for a degradation of the parent in natural surface water systems. The increase in the removal of Icaridin-acid following wastewater processing in the years 2002 and 2003 compared with 2001 can possibly be attributed to the adaptation of the microorganisms responsible for the degradation during wastewater treatment.
5.3.1	Reliability	2
5.3.2	Deficiencies	The publications show no significant deviations but they are brief or incomplete regarding sampling, test system description and the reporting of the results. They have to be seen in the context with the Documents A7.1.2.1(01), A7.1.2.1(02), and A7.1.2.1(04)

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	29/4-2010
Materials and Methods	The applicants version is acceptable.
Results and discussion	Adopt applicant's version.
Conclusion	Include revised version: 5.3. Conclusion: the high concentrations found in lake Neu-Malsch is not attributed to a spillage in the article itself. This is speculative and should thus be removed from the study summary. The increase in removal of Icaridin-acid from 2001 to 2002 is in the article mentioned to might have been caused by a increase in hydraulic residence time in the STP from week 37 of 2001. Thus, there is no proof that the increased removal is due to adaptation of the microorganisms as mentioned in the study summary.
Reliability	3
Acceptability	not acceptable The data are in the form of a poster and an article in preparation for being published and the article is only half finished with a lot of questions not answered. The article is therefore not acceptable in the current form.
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

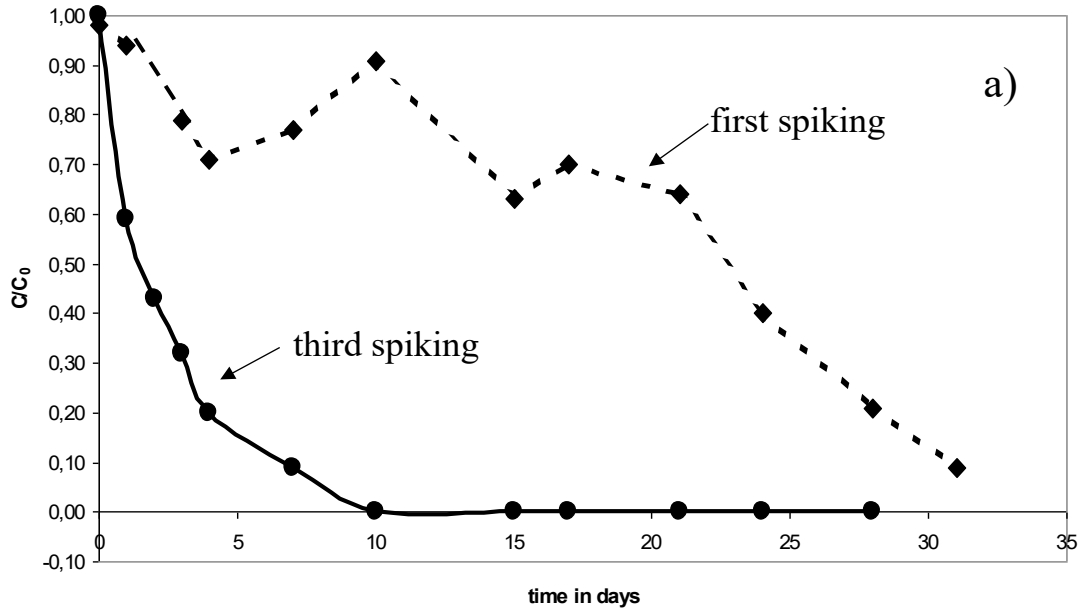


Figure A7_1_2_1-1: Fixed-bed bioreactor (FBBR) primary degradation of Icaridin in surface water (river Rhine) spiked one or three times with 10 µg/L Icaridin (♦= first spiking; ● = third spiking)

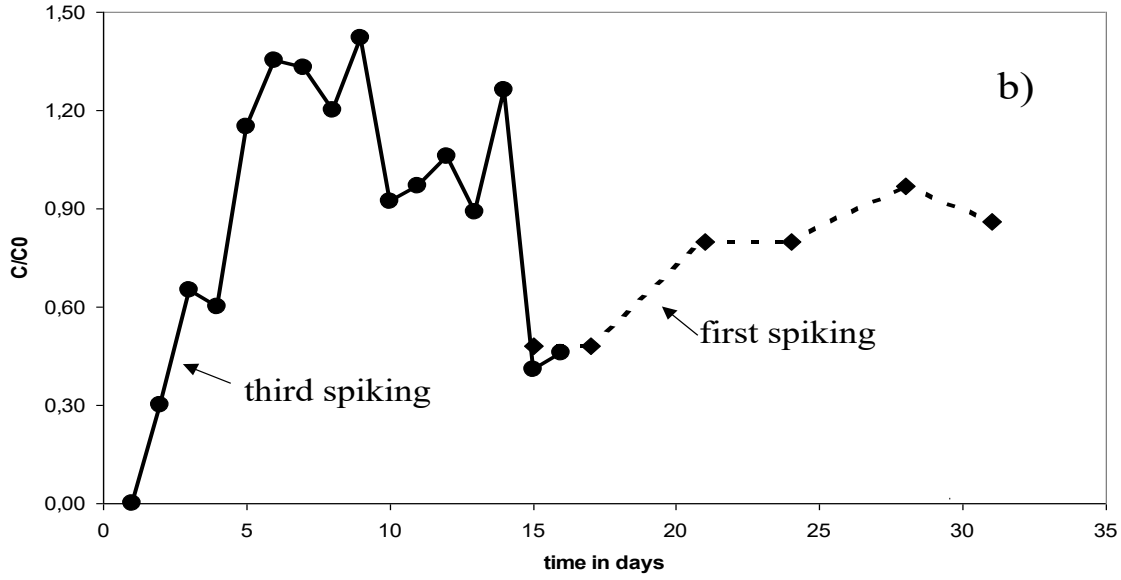


Figure A_7_1_2_1-2: Formation and primary degradation of Icaridin-acid in a FBBR test with surface water (river Rhine) spiked one or three times with 10 µg/L Icaridin (♦= first spiking; ● = third spiking)

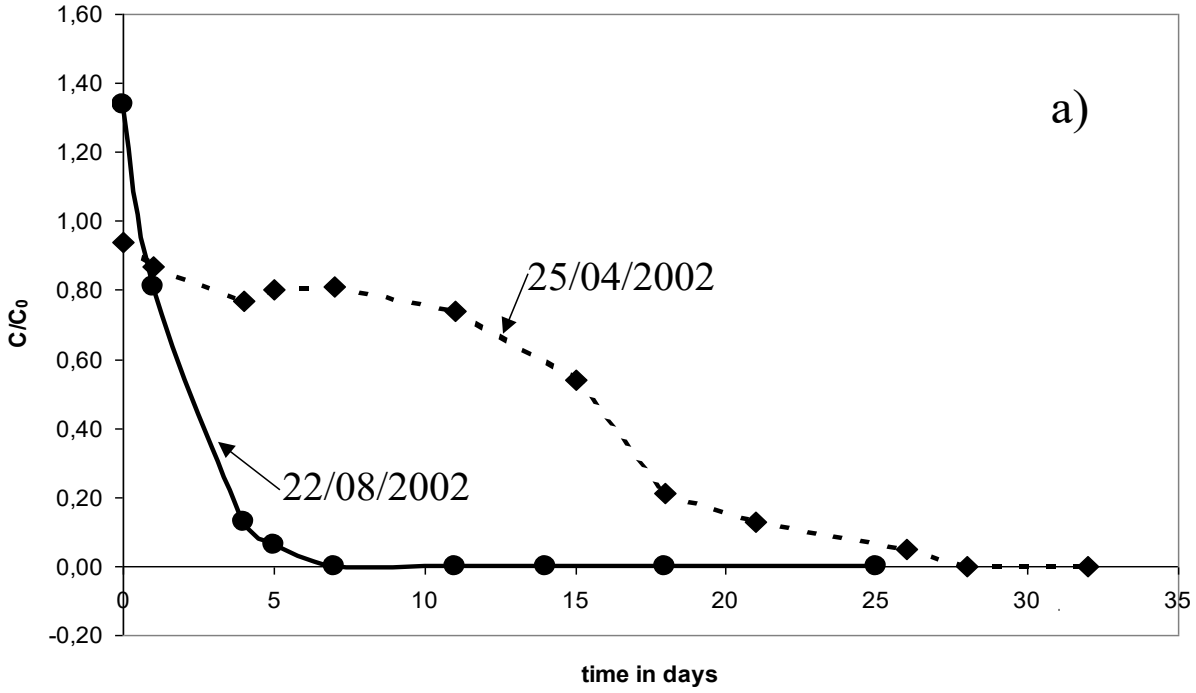


Figure A_7_1_2_1-3: Fixed-bed bioreactor (FBBR) primary degradation of Icaridin in the effluent of a sewage treatment plant (Wiesbaden) spiked one or two times with 10 µg/L Icaridin (♦= 25/04/2002, first spiking; ● = 22/08/2002, second spiking)

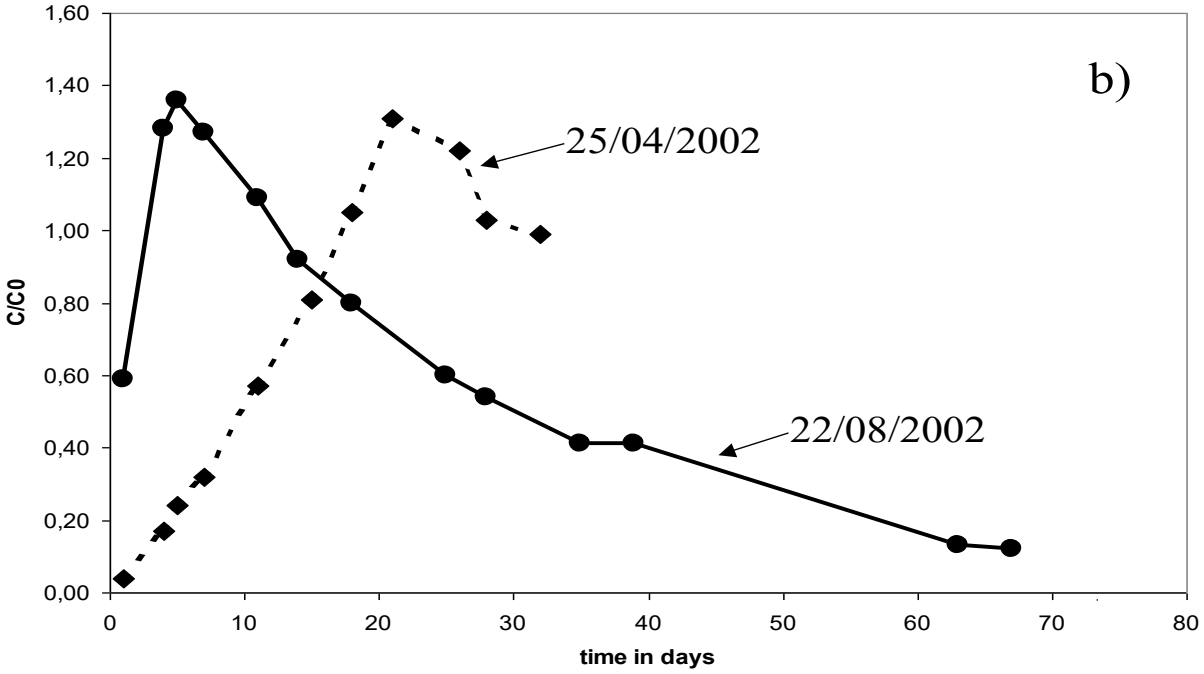


Figure A_7_1_2_1-4: Formation and primary degradation of Icaridin-acid in a FBBR test with the effluent of a sewage treatment plant (Wiesbaden) spiked one or two times with 10 µg/L Icaridin (♦= 25/04/2002, first spiking; ● = 22/08/2002, second spiking)

Table A7_1_2_1-1: Icaridin-acid concentrations in different rivers sampled in 2003 (weekly mixed samples)

River	Sampling period	Number of samples	Minimum concentration (µg/L)*	Maximum concentration (µg/L)*	Mean concentration (µg/L)
Rhine	05 - 09 2003	10	n.d.	n.d.	n.d.
Main	05 - 09 2003	17	n.d.*	n.d.	n.d.
Nidda	03 - 07 2003	17	0.07	0.36	0.18

* LOQ = 0.03 µg/L, n.d. = not detectable

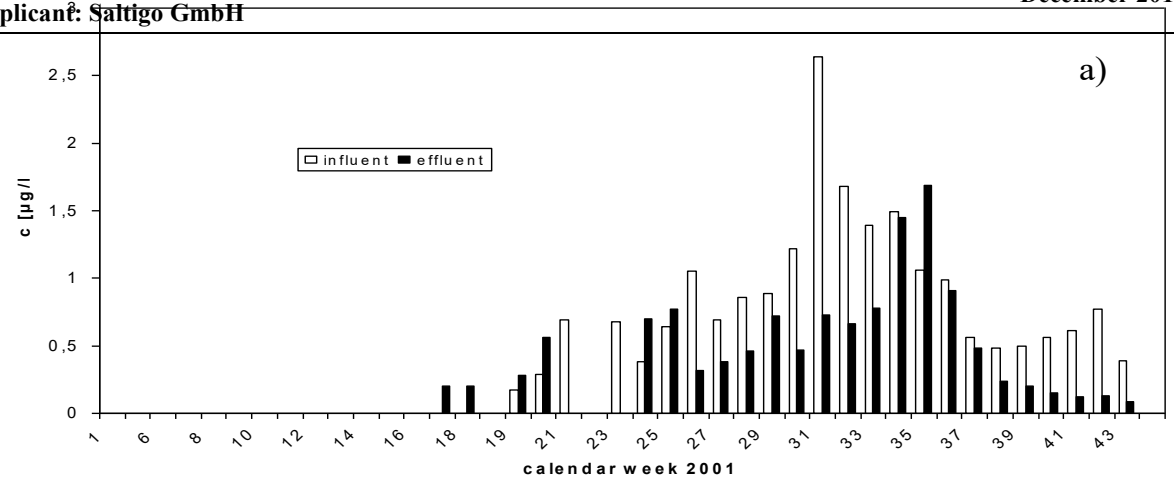
Table A7_1_2_1-2: Icaridin and Icaridin-acid concentrations in different lakes sampled in 2003 (randomly taken samples)

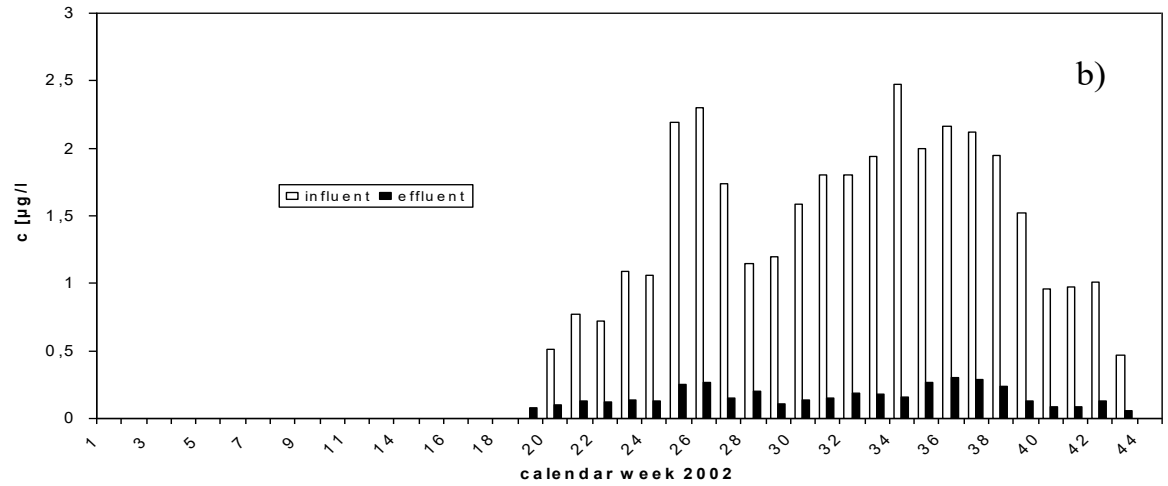
Lake	Sampling date	Icaridin concentration (µg/L)*	Icaridin-acid concentration (µg/L)*
Mariannenaue/Rhein	14-06-2003	n.d.	n.d.
HLUG-See 117	17-06-2003	n.d.	n.d.
Neu-Malsch	17-06-2003	0.76	0.31
Neu-Malsch	06-08-2003	6.26	0.41
Maroth (Westerwald)	22-06-2003	0.04	0.18
Strandbad Rodenbach	16-07-2003	0.07	0.07
Strandbad Rodenbach	01-08-2003	0.07	0.12
Schultheisweiher	16-07-2007	0.05	0.07
Schultheisweiher	01-08-2003	n.d.	0.09
Bärensee	16-07-2007	0.04	0.23
Bärensee	01-08-2003	0.07	0.25
Klein-Krotzenburg	11-06-2003	0.03	< LOQ
Klein-Krotzenburg	16-07-2007	0.05	0.03
Klein-Krotzenburg	01-08-2003	< LOQ	0.09
Waldschwimmbad Rüsselsheim	16-07-2007	0.08	0.07
Waldschwimmbad Rüsselsheim	01-08-2003	< LOQ	0.21
Birkensee	16-07-2007	0.04	< LOQ
Birkensee	01-08-2003	n.d.	0.08
Mainflingen	16-07-2007	0.04	< LOQ
Kärchersee Biblis	03-08-2003	n.d.	n.d.

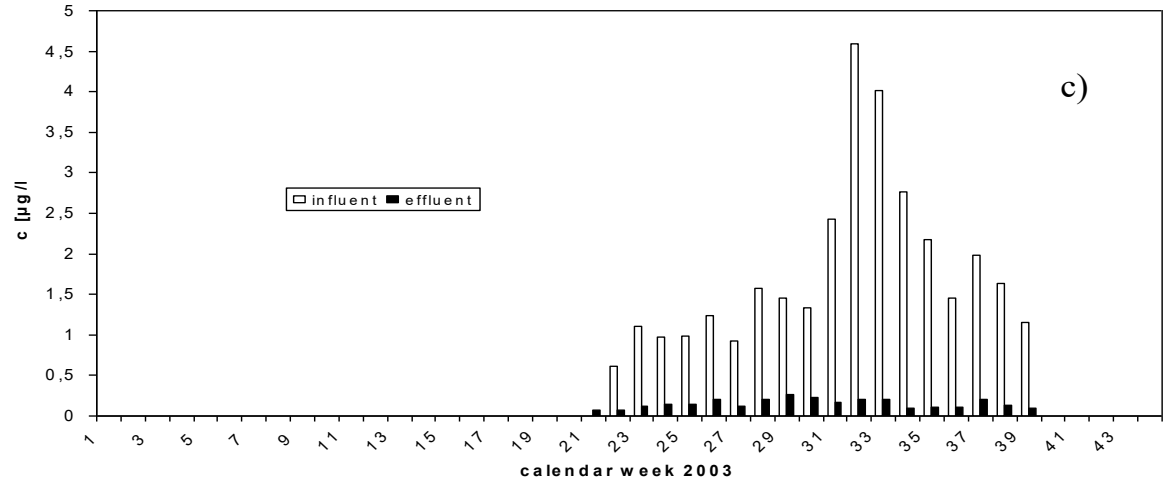
Surfschule Biblis	03-08-2003	0.06	n.d.

* LOQ = 0.03 µg/L, n.d. = not detectable

Figure A7_1_2_1-5: Icaridin in the influent and Icaridin-acid in the effluent of the WWTP Wiesbaden plant in weekly mixed samples of the years 2001, 2002 and 2003


a)
influent
effluent
c [µg/l]
calendar week 2001


b)
influent
effluent
c [µg/l]
calendar week 2002


c)
influent
effluent
c [µg/l]
calendar week 2003

Section A7.1.2.1 Biological sewage treatment (04)

Annex Point IIIA12.2 **ICARIDIN (KBR 3023)**

			Official use only
		1 REFERENCE	
1.1	**Reference**	Knepper, T.P. and Maes, A (2003): The presence of the insect repellents DEET and Bayrepel in aquatic environments Europa Fachhochschule Fresenius, Idstein, Germany and ESWE-Institut für Wasserforschung und Wassertechnologie GmbH, Wiesbaden, Germany, published in 60. Jahresbericht ARW, Köln, 71-86, Date: 2003	
1.2	**Data protection**	No	
1.2.1	Data owner	Not applicable, publication	
1.2.2	Companies with letter of access	-	
1.2.3	Criteria for data protection	No data protection claimed	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No	
2.2	**GLP**	No	
2.3	**Deviations**	Not applicable	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Icaridin (KBR 3023, Bayrepel)	
3.1.1	Lot/Batch number	Not applicable	
3.1.2	Specification	Not applicable	
3.1.3	Purity	Not applicable	
3.1.4	Further relevant properties	No	
3.1.5	Composition of Product	Not applicable	
3.1.6	TS inhibitory to microorganisms	Result of the respiration inhibition test with activated sludge according to OECD Guideline 209: EC_{50} = 1087 mg/l; Reference: Mueller, G. (1997): Investigation of the Ecological Properties of KBR 3023, Bayer AG, Institute for Environmental Analyses, Leverkusen, Germany, Report No. 610 N/96 B, Date: 1997-01-23.	
3.1.7	Specific chemical analysis	The analysis of Icaridin is not described in this publication. However, the procedure is presumably the same as described in Document IVA, 7.1.2.1(02).	
3.2	**Reference substance**	Not reported	

Section A7.1.2.1 Biological sewage treatment (04)

Annex Point IIIA12.2 **ICARIDIN (KBR 3023)**

3.2.1	Initial concentration of reference substance	Not applicable
3.3	**Testing procedure**	
3.3.1	Analytical parameter	Test substance (Icaridin) concentrations
3.3.2	Sampling location	Influents and effluents of the wastewater treatment plant (WWTP) Wiesbaden, Germany.
3.3.3	Sampling dates	2001: Start: approximately calendar week 7 End: approximately calendar week 44 2002: Start: approximately calendar week 2 End: approximately calendar week 44 2003: Start: approximately calendar week 20 End: approximately calendar week 44
3.3.4	Sample quality	Weekly mixed samples
3.3.5	Intermediates/ degradation products	No
3.3.6	Statistics	None
		4 RESULTS
4.1	**Degradation of test substance**	
4.1.1	Graph	The Icaridin concentrations in the influent streams to the WWTP Wiesbaden in the years 2001, 2002 and 2003 are shown in Figure A7_1_2_1-1
4.1.2	Other observations	Icaridin could not be detected in the WWTP effluent streams.
4.1.3	Intermediates/ degradation products	No matter of this publication
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	In- and effluents of the wastewater treatment plant (WWTP) Wiesbaden, Germany, have been analysed for Icaridin during the years 2001 to 2003.

Section A7.1.2.1 Biological sewage treatment (04)

Annex Point IIIA12.2 **ICARIDIN (KBR 3023)**

5.2	**Results and discussion**	Icaridin was detectable in the influent streams to the wastewater treatment plant in Wiesbaden from May onwards. Peak concentrations were observed during the summer months. It could neither be found in the effluent streams nor in the influent streams of the winter months (approximately November until Mai). The maximum Icaridin concentrations in 2001 and 2002 were 2.6 µg/L and 2.5 µg/L, respectively, both peaks occurring in August. In the year 2003, the maximum Icaridin concentration was higher compared to the previous years (4.6 µg/L in August), which is presumably attributable to the very high temperatures in that summer.
5.3	**Conclusion**	The monitoring measurements of Icaridin concentrations in WWTP influents reveal a seasonal course with higher concentrations occuring during the summer month and no residues being detectable during winter. Since Icaridin when detectable in the influent streams could not be found in the effluent streams, a (primary) degradation must have been occurred. The formation of Icaridin-acid during wastewater treatment is proposed.
5.3.1	Reliability	2-3
5.3.2	Deficiencies	The publication does not give information on the analysis of the test substance and is brief regarding sampling and the reporting of the results. It has to be seen in the context to be seen in the context with the Documents A7.1.2.1(01), A7.1.2.1(02), and A7.1.2.1(03).

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	29/4-2010
Materials and Methods	The applicants version is acceptable.
Results and discussion	Adopt applicant's version.
Conclusion	Adopt applicant's version.
Reliability	3
Acceptability	not acceptable No information is given on the analysis. The article is very brief in general.
Remarks	The article is not published in a scientific peer reviewed journal.
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

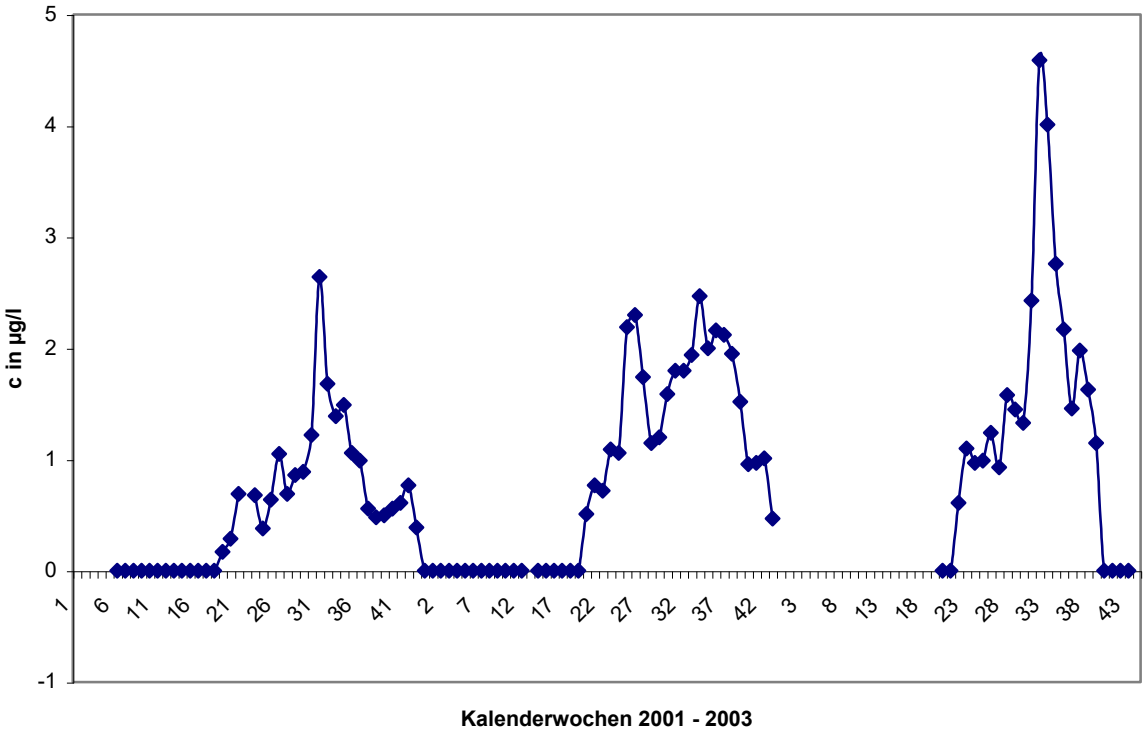


Figure A7_1_2_1-1: **Concentration of Icaridin in the influent streams to the wastewater treatment plant Wiesbaden, Germany in the years 2001, 2002 and 2003 (weekly mixed samples, Kalenderwoche = calendar week)**

Section A7.1.2.2 Biodegradation in freshwater (01)

Annex Point IIIA12.2 **ICARIDIN (KBR 3023)**

			Official use only
		1 REFERENCE	
1.1	**Reference**	Knepper, T.P., Maes, A. and Mueller, J. (2005): Occurrence and fate of insect repellents in the aquatic environment Europe University of Applied Sciences Fresenius, Idstein, Germany (in preparation for being published in Environ. Sci. & Technol.) Knepper, T.P., Mueller, J. and Maes, A. (2005): Occurrence and fate of insect repellents in the aquatic environment Europe University of Applied Sciences Fresenius, Idstein, Germany (poster presentation)	
1.2	**Data protection**	No	
1.2.1	Data owner	Not applicable, publication	
1.2.2	Companies with letter of access	-	
1.2.3	Criteria for data protection	No data protection claimed	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No	
2.2	**GLP**	No	
2.3	**Deviations**	Not applicable	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Icaridin (KBR 3023, Bayrepel) Icaridin-acid (Bayrepel-acid, metabolite of Icaridin)	
3.1.1	Lot/Batch number	Not applicable or not reported	
3.1.2	Specification	Not applicable or not reported	
3.1.3	Purity	Not applicable or not reported	
3.1.4	Further relevant properties	No	
3.1.5	Composition of Product	Not applicable	
3.1.6	TS inhibitory to microorganisms	Result of the respiration inhibition test with activated sludge according to OECD Guideline 209: EC_{50} = 1087 mg/l; Reference: Mueller, G. (1997): Investigation of the Ecological Properties of KBR 3023, Bayer AG, Institute for Environmental Analyses, Leverkusen, Germany, Report No. 610 N/96 B, Date: 1997-01-23.	
3.1.7	Specific chemical	The sample preparation for analysis of Icaridin and Icaridin-acid	

Section A7.1.2.2 Biodegradation in freshwater (01)

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	analysis	involved solid phase extraction (SPE) and esterification in the case of Icaridin-acid. Analysis was done by gas chromatography-mass-spectrometry (GC/MS). Details of the sample preparation and analysis are described in Document IIIA, 7.1.2.1(02).
3.2	**Reference substance**	Not reported
3.2.1	Initial concentration of reference substance	Not applicable
3.3	**Testing procedure**	
3.3.1	Testing items	1. Fixed-bed bioreactor test (FBBR) to investigate the biodegradation of Icaridin and the formation and biodegradation of Icaridin-acid. The tests were conducted either with Rhine water or with effluent water of the WWTP Wiesbaden. 2. Measurement of Icaridin and Icaridin-acid concentrations in water samples from German rivers (only Icaridin-acid) and lakes. 3. Measurement of the Icaridin concentration in the wastewater influent and the Icaridin-acid concentration in the wastewater effluent of the WWTP Wiesbaden, Germany, over the years 2001, 2002 and 2003.
3.3.2	Analytical parameter	Test substance (Icaridin) and metabolite (Icaridin-acid) concentrations
3.3.3	Experimental set-up	1. Fixed-bed bioreactor test (FBBR): River water or effluent wastewater is running in circuit under aerobic conditions through a test vial containing porous glass beads. The glass beads serve as constituents for immobilisation of the microorganisms being present in the corresponding waters. The FBBR test was conducted either with Rhine water or with effluent wastewater of the WWTP Wiesbaden.
3.3.4	Test substance concentration	1. Fixed-bed bioreactor test (FBBR): 10 µg/L Icaridin and 100 µg/L Icaridin (results are not reported). Spiking was done one and three times in the test with Rhine river water and one and two times (25/04/2002 and 22/08/2002) in the test with WWTP effluent water.
3.3.5	Sampling location	2. Measurement of Icaridin and Icaridin-acid concentrations in water samples from German rivers and lakes: *cf.* Table A7_1_2_1-1 and Table A7_1_2_1-2 3. Measurement of Icaridin and Icaridin-acid concentrations in in-and effluents of a WWTP Wastewater treatment plant Wiesbaden, Germany
3.3.6	Sampling dates	1. Fixed-bed bioreactor test (FBBR): The sampling was done continuously over a period of 4 weeks up to 3 months 2. Measurement of Icaridin and Icaridin-acid concentrations in water samples from German rivers and lakes: *cf.* Table A7_1_2_1-1 and Table A7_1_2_1-2

Section A7.1.2.2 Biodegradation in freshwater (01)

Annex Point IIIA12.2 **ICARIDIN (KBR 3023)**

		3. Measurement of Icaridin and Icaridin-acid concentrations in in- and effluents of a WWTP *cf.* Figure A7_1_2_1-5
3.3.7	Sample quality	2. Measurement of Icaridin and Icaridin-acid concentrations in water samples from German rivers and lakes: Rivers: weekly mixed samples Lakes: randomly taken samples 3. Measurement of Icaridin and Icaridin-acid concentrations in in- and effluents of a WWTP Weekly mixed samples
3.3.8	Intermediates/ degradation products	Yes. Icaridin-acid
3.3.9	Statistics	None
		4 RESULTS
4.1	**Degradation of test substance**	
4.1.1	Graph	Figure A7_1_2_1-1 presents the results of the Fixed-bed bioreactor (FBBR) test conducted with Rhine water and one or three spikings of the water with 10 µg Icaridin/L. It resumes the primary degradation of the parent compound. Figure A7_1_2_1-3 presents the results of the Fixed-bed bioreactor (FBBR) test conducted with the effluent of the WWTP Wiesbaden and one or two spikings of the water with 10 µg Icaridin/L. It resumes the primary degradation of the parent compound. Figure A7_1_2_1-5 shows the Icaridin influent concentrations into the WWTP Wiesbaden from 2001 to 2003.
4.1.2	Concentration	Icaridin and Icaridin-acid concentrations in different German lakes sampled in 2003 are summarised in Table A7_1_2_1-2.
4.1.3	Other observations	No
4.1.4	Intermediates/ degradation products	Figure A7_1_2_1-2 demonstrates the formation and primary degradation of the metabolite Icaridin-acid in the bioreactor test conducted with Rhine water and one or three spikings of the water with 10 µg Icaridin/L. Figure A7_1_2_1-4 demonstrates the formation and primary degradation of the metabolite Icaridin-acid in the bioreactor test conducted with the effluent of the WWTP Wiesbaden and one or two spikings of the water with 10 µg Icaridin/L. **Table A7_1_2_1-1:summarises the Icaridin-acid concentrations in different German rivers sampled in 2003.** Icaridin-acid concentrations in different German lakes sampled in 2003 are summarised in Table A7_1_2_1-2.

Section A7.1.2.2 Biodegradation in freshwater (01)

Annex Point IIIA12.2 **ICARIDIN (KBR 3023)**

Figure A7_1_2_1-5 shows the Icaridin-acid effluent concentrations of the WWTP Wiesbaden from 2001 to 2003.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The biodegradation of Icaridin and the formation and biodegradation of the metabolite Icaridin-acid were investigated by using a Fixed-bed bioreactor (FBBR) and either surface Rhine water or the effluent of a WWTP.

Samples of three German rivers taken in spring and summer 2003 have been analysed for Icaridin-acid. In addition, randomly taken samples from German lakes were analysed for Icaridin and its metabolite.

Furthermore, Icaridin and Icaridin-acid concentrations in the in- and effluents of the WWTP Wiesbaden have been determined. The samples were taken in 2001, 2002 and 2003.

5.2 Results and discussion

Results of the FBBR tests

In the bioreactor test conducted with Rhine water it could be demonstrated, that biodegradation of Icaridin took place after the first spiking, however, it started after a lag phase. A significant increase in the rate and extent of conversion could be seen with the third spiking, demonstrating that an adaptation period is required for initiation of the microbial metabolisation. The rate of Icaridin-acid production following the third spiking was going hand-in-hand with the decline of the parent and thus significantly more rapid and reaching a higher concentration after the third spiking. Subsequent conversion of the metabolite can also be seen to be greater following the third spiking, where a lower final concentration was determined in this case.

Analogous to the surface water experiment, the conversion of Icaridin in the WWTP effluent was significantly enhanced after the second spiking compared to the first one.

The formation and conversion of the Icaridin-acid metabolite however showed a different pattern in the sewage treatment plant effluent by comparison with the surface water. Once again the formation following the second spiking was significantly more rapid as compared with the spiking before, with the maximum concentrations being reached after 5 days and 20 days, respectively. The rate of conversion of the metabolite thereafter however, was much more rapid for both spikings in the sewage effluent, as compared to the rates observed in the surface water experiment. Much higher peak concentrations and rates of formation of Icaridin-acid, and enhanced subsequent primary reduction were observed for the sewage effluent.

Icaridin acid concentrations in German rivers

The compound could not be detected above the detection limit in the rivers Rhine and Main. However, water samples taken from the river Nidda between March and July contained the metabolite at amounts between 0.07 µg/L and 0.36 µg/L.

Section A7.1.2.2 Biodegradation in freshwater (01)

Annex Point IIIA12.2 **ICARIDIN (KBR 3023)**

		Icaridin and Icaridin acid concentrations in German lakes Icaridin and Icaridin-acid could be identified in each 14 of 21 lake water samples. Their measured concentrations were in general < 0.1 µg/L (Icaridin) and ≤0.25 µg/L (Icardin-acid). Only in one lake (Neu-Malsch) remarkable high residues (up to 6.26 µg/L Icaridin and 0.41 µg/L Icaridin-acid) could be found. Icaridin and Icaridin acid concentrations in the in- and effluent of the WWTP Wiesbaden The Icaridin influent concentrations in 2001 were in general below 1.5 µg/L. Only one measurement conducted at the end of July/beginning of August of this year revealed a maximum amount of 2.6 µg/L. The concentrations of the metabolite were also higher during the summer months, reaching a maximum value of approximately 1.6 µg/L at the end of August/beginning of September. In 2002 influent concentrations of Icaridin increased compared to 2001, reaching a maximum value of approximately 2.5 µg/L in August. Even higher concentrations were observed during the extreme hot and dry summer 2003, where Icaridin concentrations in the influent reached values up to 4.5 µf/L. However, Icaridin-acid concentrations in the years 2002 and 2003 were lower compared to 2001, reaching maximum amounts of approximately 0.25 µg/L. In none of the years under consideration Icaridin or Icaridin-acid could be detected during the winter and early spring months (November to April).
5.3	**Conclusion**	The bioreactor tests reveal Icaridin to be quantitatively transferred into Icaridin acid. This process is forced by the adaptation of the microorganisms to the compound. Icaridin-acid in turn is further degraded after an adaptation of the microorganisms has taken place. The whole biodegradation process, especially the degradation of Icaridin-acid proceeds faster in a medium with a higher microoganism density (faster degradation (up to 90%) in effluent water compared to surface water). In lakes for bathing Icaridin and Icaridin-acid residues could be found occasionally and if detected at all, amounts were generally low. Only one lake (Neu-Malsch) exhibited high residues of the compounds, which might be the result of spillage. The occurrence of the metabolite Icardin-acid in bathing lakes is an indication for a degradation of the parent in natural surface water systems. The increase in the removal of Icaridin-acid following wastewater processing in the years 2002 and 2003 compared with 2001 can possibly be attributed to the adaptation of the microorganisms responsible for the degradation during wastewater treatment.
5.3.1	Reliability	2
5.3.2	Deficiencies	The publications show no significant deviations but they are brief or incomplete regarding sampling, test system description and the reporting of the results. They have to be seen in the context with the Documents A7.1.2.1(01), A7.1.2.1(02), and A7.1.2.1(04)

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	29/4-2010
Materials and Methods	The applicant's version is acceptable.
Results and discussion	Adopt applicant's version.
Conclusion	Include revised version: 5.3. Conclusion: the high concentrations found in lake Neu-Malsch is not attributed to a spillage in the article itself. This is speculative and should thus be removed from the study summary. The increase in removal of Icaridin-acid from 2001 to 2002 is in the article mentioned to might have been caused by a increase in hydraulic residence time in the STP from week 37 of 2001. Thus, there is no proof that the increased removal is due to adaptation of the microorganisms as mentioned in the study summary.
Reliability	3
Acceptability	not acceptable The data are in the form of a poster and an article in preparation for being published and the article is only half finished with a lot of questions not answered. The article is therefore not acceptable in the current form.
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

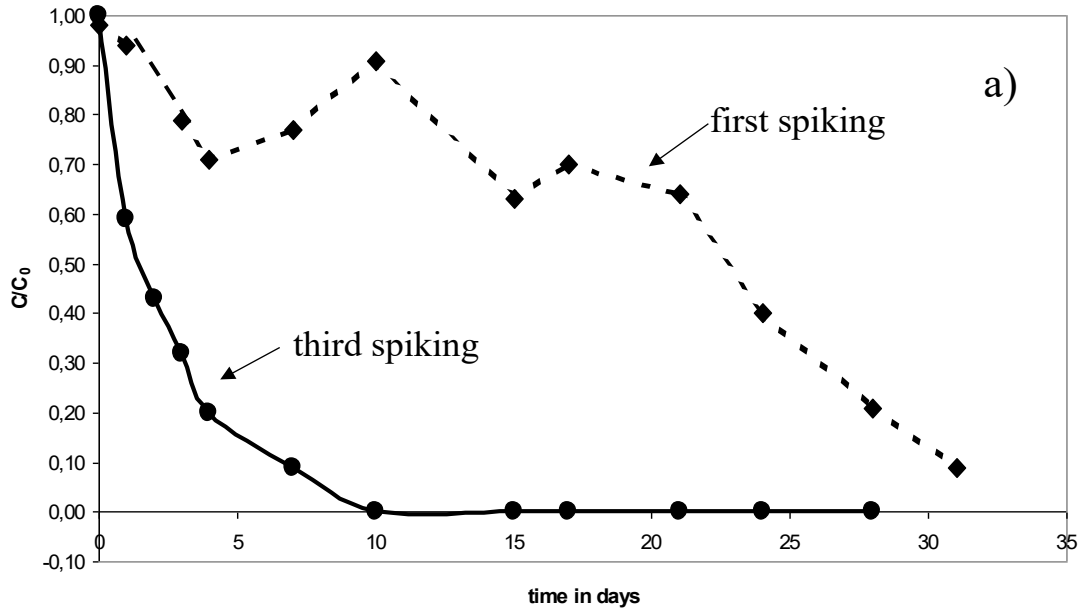


Figure A7_1_2_1-1: Fixed-bed bioreactor (FBBR) primary degradation of Icaridin in surface water (river Rhine) spiked one or three times with 10 µg/L Icaridin (♦= first spiking; ● = third spiking)

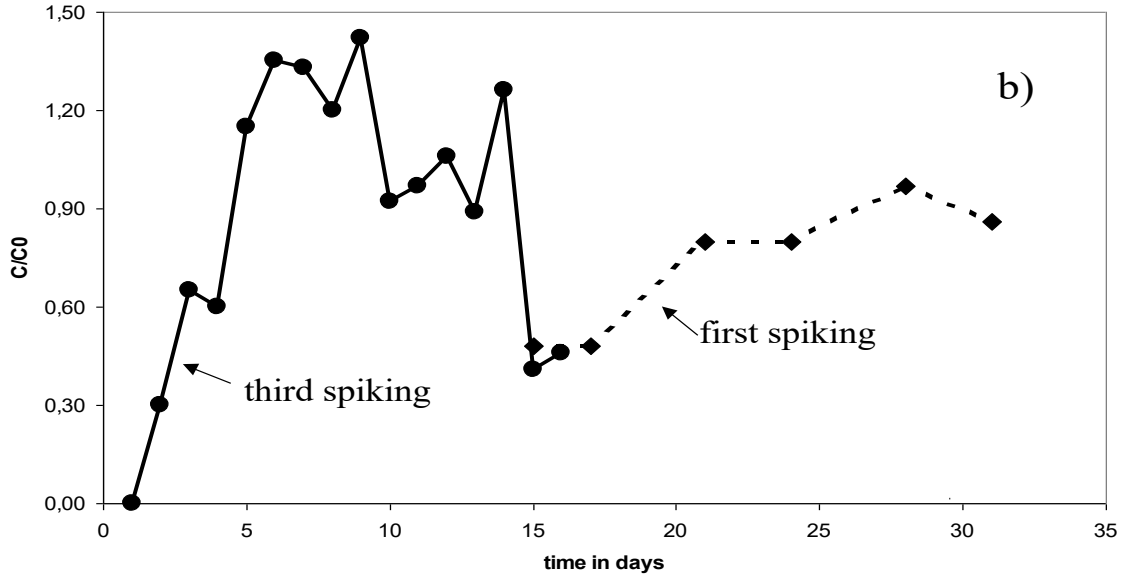


Figure A_7_1_2_1-2: Formation and primary degradation of Icaridin-acid in a FBBR test with surface water (river Rhine) spiked one or three times with 10 µg/L Icaridin (♦= first spiking; ● = third spiking)

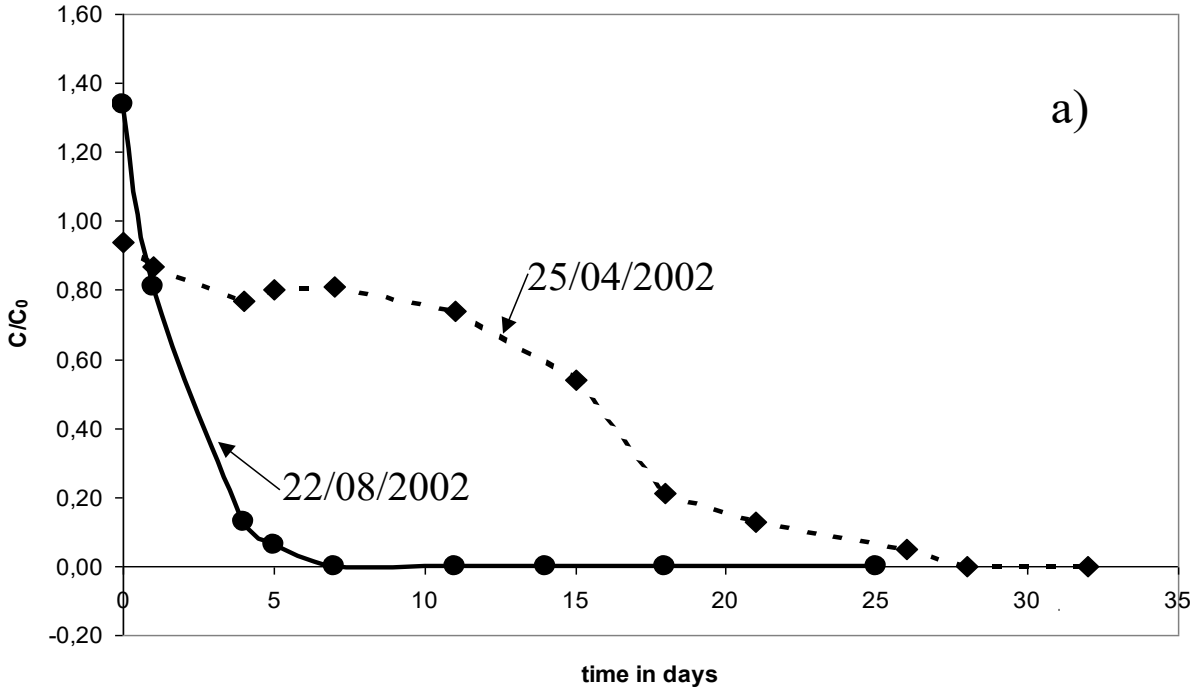


Figure A_7_1_2_1-3: Fixed-bed bioreactor (FBBR) primary degradation of Icaridin in the effluent of a sewage treatment plant (Wiesbaden) spiked one or two times with 10 µg/L Icaridin (♦= 25/04/2002, first spiking; ● = 22/08/2002, second spiking)

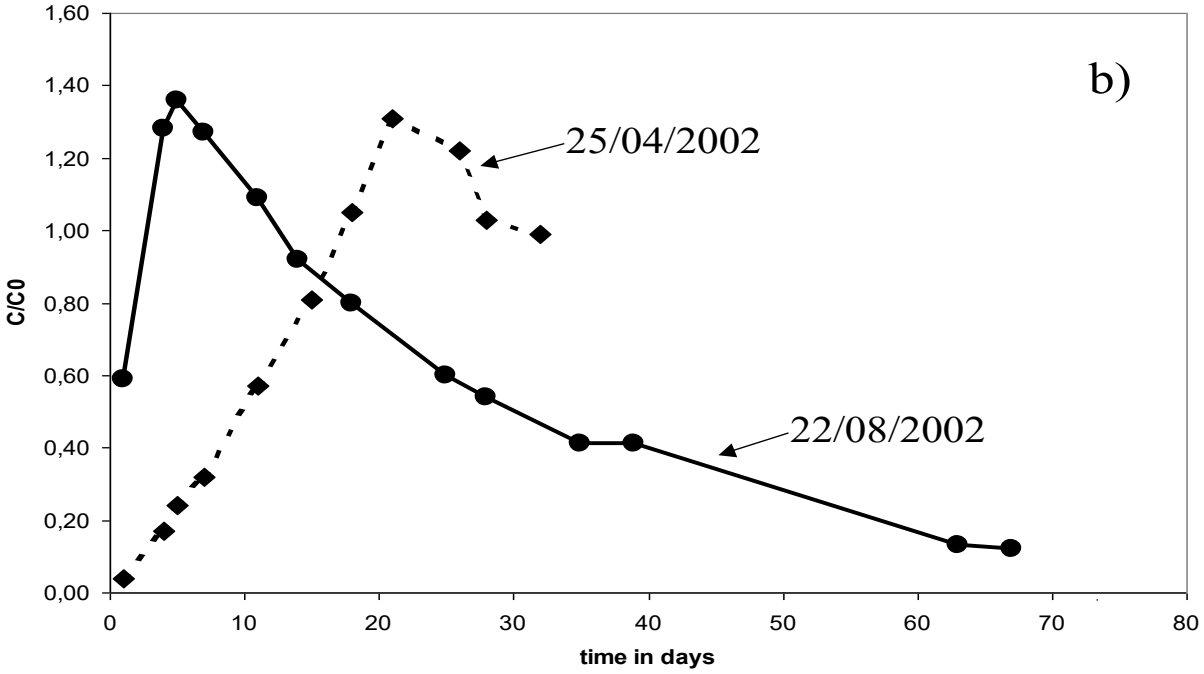


Figure A_7_1_2_1-4: Formation and primary degradation of Icaridin-acid in a FBBR test with the effluent of a sewage treatment plant (Wiesbaden) spiked one or two times with 10 µg/L Icaridin (♦= 25/04/2002, first spiking; ● = 22/08/2002, second spiking)

Table A7_1_2_1-1: Icaridin-acid concentrations in different rivers sampled in 2003 (weekly mixed samples)

River	Sampling period	Number of samples	Minimum concentration (µg/L)*	Maximum concentration (µg/L)*	Mean concentration (µg/L)
Rhine	05 - 09 2003	10	n.d.	n.d.	n.d.
Main	05 - 09 2003	17	n.d.*	n.d.	n.d.
Nidda	03 - 07 2003	17	0.07	0.36	0.18

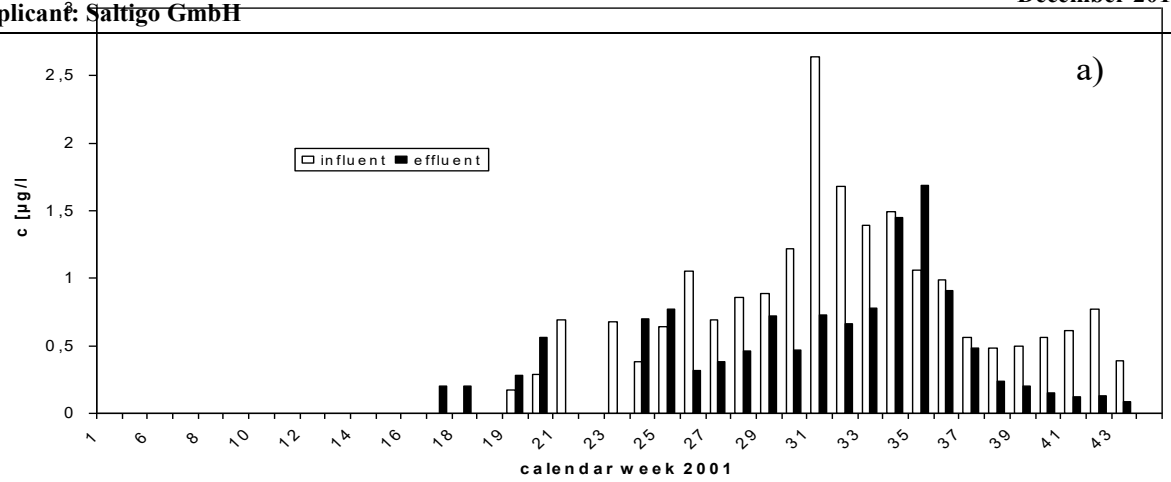
* LOQ = 0.03 µg/L, n.d. = not detectable

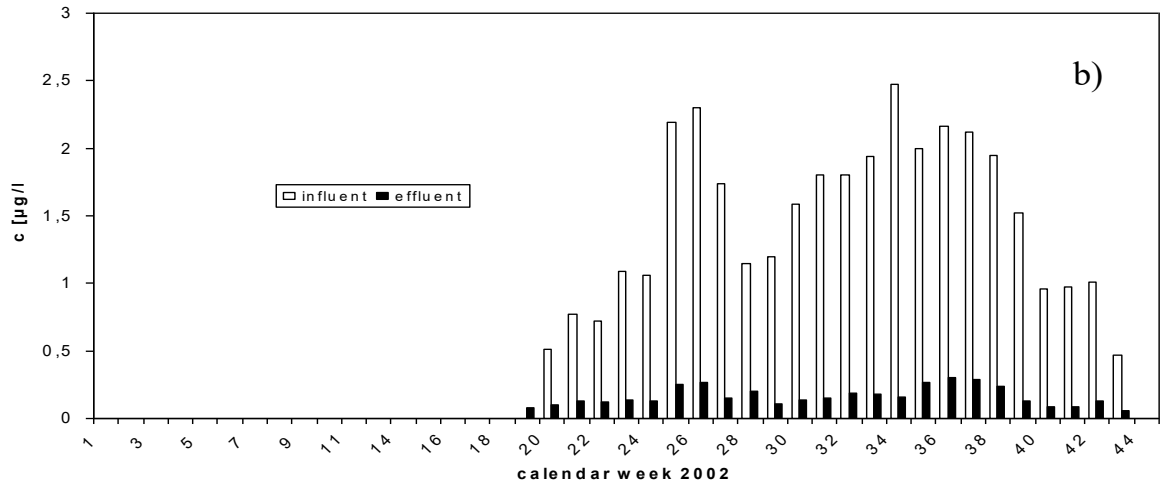
Table A7_1_2_1-2: Icaridin and Icaridin-acid concentrations in different lakes sampled in 2003 (randomly taken samples)

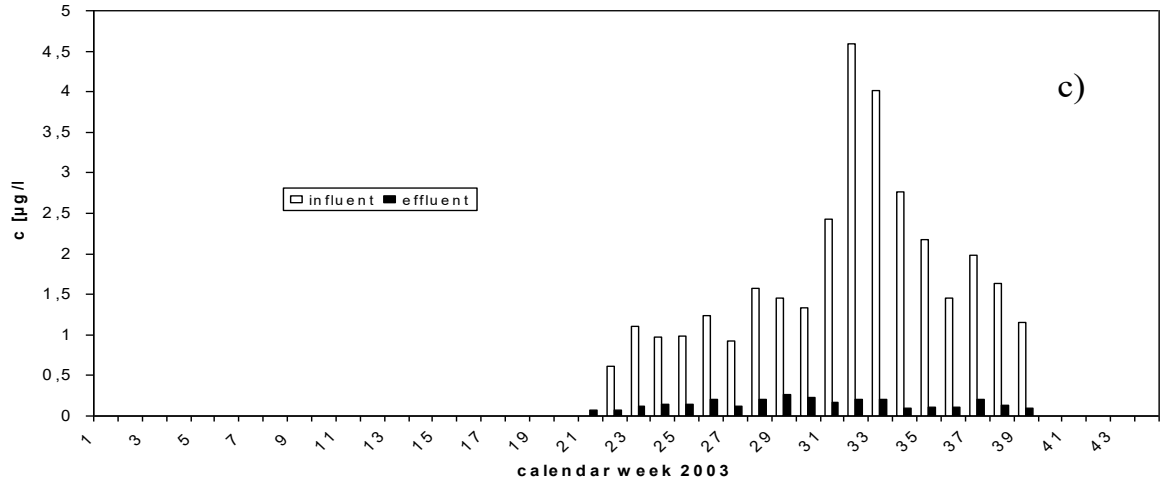
Lake	Sampling date	Icaridin concentration (µg/L)*	Icaridin-acid concentration (µg/L)*
Mariannenaue/Rhein	14-06-2003	n.d.	n.d.
HLUG-See 117	17-06-2003	n.d.	n.d.
Neu-Malsch	17-06-2003	0.76	0.31
Neu-Malsch	06-08-2003	6.26	0.41
Maroth (Westerwald)	22-06-2003	0.04	0.18
Strandbad Rodenbach	16-07-2003	0.07	0.07
Strandbad Rodenbach	01-08-2003	0.07	0.12
Schultheisweiher	16-07-2007	0.05	0.07
Schultheisweiher	01-08-2003	n.d.	0.09
Bärensee	16-07-2007	0.04	0.23
Bärensee	01-08-2003	0.07	0.25
Klein-Krotzenburg	11-06-2003	0.03	< LOQ
Klein-Krotzenburg	16-07-2007	0.05	0.03
Klein-Krotzenburg	01-08-2003	< LOQ	0.09
Waldschwimmbad Rüsselsheim	16-07-2007	0.08	0.07
Waldschwimmbad Rüsselsheim	01-08-2003	< LOQ	0.21
Birkensee	16-07-2007	0.04	< LOQ
Birkensee	01-08-2003	n.d.	0.08
Mainflingen	16-07-2007	0.04	< LOQ
Kärchersee Biblis	03-08-2003	n.d.	n.d.
Surfschule Biblis	03-08-2003	0.06	n.d.

* LOQ = 0.03 µg/L, n.d. = not detectable

Figure A7_1_2_1-5: Icaridin in the influent and Icaridin-acid in the effluent of the WWTP Wiesbaden plant in weekly mixed samples of the years 2001, 2002 and 2003


a)
influent
effluent
c [µg/l]
calendar week 2001


b)
influent
effluent
c [µg/l]
calendar week 2002


c)
influent
effluent
c [µg/l]
calendar week 2003

Section A7.1.2.2.2/01 Water/sediment degradation study

Annex Point IIIA XII2.1

			Official use only
		1 REFERENCE	
1.1	**Reference**	Fiebig, S. and Goller, St. (2014): SONC969 Saltidin, [carboxyl-^{14}C]-Aerobic Transformation in Aquatic Sediment Systems using ^{14}C-labelled Test Item. Dr. U. Noack Laboratorien, Sarstedt, Germany. Project No. 110817SH, Study No NAT15260 (unpublished), date: 2014-03-21	
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/list of approved active substances	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, OECD Guideline for the Testing of Chemicals 308, Aerobic and Anaerobic Transformation in Aquatic Sediment Systems, April 2002.	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	SONC969 Saltidin, [carboxyl^{14}C]-	
3.1.1	Lot/Batch number	Radio-labelled test substance: Batch number 198-190-0564-A-20120718-DRE Non-labelled test substance: Batch number CHCAEN0020	
3.1.2	Specification	Specific activity: 56.4 mCi/mmol	
3.1.3	Purity	Radiochemical purity was 99.7% Non-labelled test substance: Active ingredient content: 98.4%	
3.1.4	Further relevant properties	-	
3.1.5	Composition of Product	-	
3.1.6	TS inhibitory to micro-organisms	Not to be expected with reference to the activated sludge test with microorganisms.	
3.1.7	Specific chemical analysis	-	
3.2	**Reference**	No reference item is recommended for this test.	

Section A7.1.2.2.2/01 Water/sediment degradation study

Annex Point IIIA XII2.1

	substance	
3.2.1	Initial concentration of reference substance	-
3.3	**Test solution**	See table A7_1_2_2_2-3
3.4	**Testing procedure**	
3.4.1	Test system	Sediments and their associated waters (field fresh samples) originated from the rivers 'Rössing Bach' and 'Alte Leine'. Both sampling sites are classified as unpolluted. Samples were taken from the entire 5 to 10 cm upper layer of the sediment. The associated water was collected from the same site at the same time. A detailed description of the particle size distribution of the sediment is presented in table A7_1_2_2_2-1. Water as well as sediment parameter are summarised in in table A7_1_2_2_2-2. The sediment was separated from the water, manually cleared of large objects and then wet-sieved to a particle size of 2 mm. Sediments and water were mixed at the desired ratio in the incubation flasks and prepared for the acclimation phase. The water/sediment samples were preincubated in the incubation vessels under test conditions for 10 days ('Alte Leine') and 7 days ('Rössing Bach') to allow stabilisation of the systems.
3.4.2	Test conditions	See table A7_1_2_2_2-3
3.4.3	Method of preparation of test solution	See table A7_1_2_2_2-3
3.4.4	Initial TS concentration	See table A7_1_2_2_2-3
3.4.5	Number of replicates	
3.4.6	Duration of test	See table A7_1_2_2_2-3
3.4.7	Sampling	See table A7_1_2_2_2-3
3.4.8	Analytical methods	See table A7_1_2_2_2-4
3.4.9	Intermediates/ degradation products	See table A7_1_2_2_2-4
3.4.10	Controls	See table A7_1_2_2_2-3
3.4.11	Statistics	The kinetic evaluations were done based on the FOCUS guidance document on estimating persistence and degradation kinetics (SANCO/10058/2005, version 2.0, June 2006: Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration).
		4 RESULTS
4.1	**Degradation of**	

Section A7.1.2.2.2/01 Water/sediment degradation study

Annex Point IIIA XII2.1

	test substance	
4.1.1	Mass balance	The mass balance, distribution of radioactivity, $^{14}CO_2$ production and non-extractable residues formation is summarised in table A7_1_2_2_2-5 (test system 'Alte Leine') and in table A7_1_2_2_2-6 (test system 'Rössing Bach'). The corresponding figures are figure A7_1_2_2_2-1 (test system 'Alte Leine') and figure A7_1_2_2_2-2 (test system 'Rössing Bach'). The distribution of the applied radioactivity in the sediment is given in table A7_1_2_2_2-7 (test system 'Alte Leine') and in table A7_1_2_2_2-8 (test system 'Rössing Bach').
4.1.2	Transformation	The transformation of SONC969 Saltidin, [carboxyl-^{14}C]- and ^{14}C-Saltidin acid is summarised in tables A7_1_2_2_2-9 to A7_1_2_2_2-11 (test system 'Alte Leine') and in in tables A7_1_2_2_2-12 to A7_1_2_2_2-14 (test system 'Rössing Bach'). The proposed transformation pathway is presented in figure A7_1_2_2_2-9.
4.1.3	Kinetic analyses	The detailed results of the kinetic evaluations are given in table A7_1_2_2_2-15 and table A7_1_2_2_2-16 (test system 'Alte Leine') and in in table A7_1_2_2_2-17 and table A7_1_2_2_2-18 (test system 'Rössing Bach'). A graphical presentation of the kinetic analysis is given in figures A7_1_2_2_2-3 to A7_1_2_2_2-6 (test system 'Alte Leine') and figures A7_1_2_2_2-7 to A7_1_2_2_2-8 (test system 'Rössing Bach').
4.1.4	Other observations	-
4.1.5	Degradation of reference substance	n.a.
4.1.6	Intermediates/ degradation products	Please refer to Points 4.1.2 and 4.1.3.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The aerobic transformation and mineralisation rate of SONC969 Saltidin, [carboxyl^{14}C]- has been tested in 2 different water/sediment systems ('Alte Leine' and 'Rössing Bach') over a period of 100 and 102 days. Water/sediment samples have been treated with the test substance and incubated in closed biometer flasks in the dark at approximately 20°C. For the determination of the transformation rate the radio-labelled test substance SONC969 Saltidin [carboxyl-^{14}C] was used. The test substance was directly applied to the water phase of each replicate, yielding a test concentration of 3.33 kBq/mL, corresponding to 369 µg/L.
For the identification of the metabolites, the radio-labelled test substance as well as the non-radiolabelled test substance was used. Appropriate volumes of the stock solution containing the labelled test item and the stock solution containing the non-labelled test item were applied directly to the water phase of each replicate, yielding a test concentration of 6 mg/L.
Water and sediment sampling has been carried out directly after the application and at 9-10 additional sampling points. SONC969 Saltidin, [carboxyl-^{14}C]- in the water phase and sediment was determined by

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liquid scintillation counting (LSC) and HPLC coupled to a flow scintillation analyser (FSA). Prior to LSC analysis aliquots of sediment samples were combusted with an oxidizer. Prior to HPLC-FSA aliquots of wet sediment were extracted by a soxhlet extractor. The ethylene glycol traps for volatile compounds and the sodium hydroxide traps for carbon dioxide were analysed by LSC only. The radioactivity of degradation products was analysed via HPLC-FSA on a reversed phase column in gradient mode. The structure of the only detectable degradation product during the course of the study, ^{14}C-Saltidin acid, was elucidated via LC-MS/MS.
The DT_{50} and DT_{90}, the disappearance time within the concentration is reduced by 50% and 90%, respectively, was calculated with a single first order model (SFO).

5.2 Results and discussion

A mass balance of 90 - 110% AR (total applied radioactivity) was obtained up to day 100 for the water sediment system ‘Alte Leine’ and up to day 102 for the water sediment system ‘Rössing Bach’. Only at day 57, in the water sediment system ‘Alte Leine’ a mean mass balance of 85.2% AR was obtained due to an unusual low recovery of $^{14}CO_2$ in one replicate. Hence, the deviation from the 90 – 110 % range is regarded as a unique outlier.

The transformation rate of SONC969 Saltidin [carboxyl-^{14}C]- was high in both aquatic sediment systems. For both test systems approximately 14% AR diffused from the water phase into the sediment until day 15. Whereas the amount of AR in the sediment of the ‘Rössing Bach’ system remained in the range of 14.4 – 18.5% AR until the end of the study, in the ‘Alte Leine’ system from day 42 until test end a slow decrease was determined and at test end (day 100) 10.9% AR were determined in the sediment. The radioactivity was almost completely extractable from both sediments.

The $^{14}CO_2$ formation was below 10% AR in both test systems until day 57 (test system ‘Alte Leine’) and day 55 (test system ‘Rössing Bach’). Thereafter the CO_2 formation increased steadily until test end and reached 41.3% AR for ‘Alte Leine’ and 16.0% AR for ‘Rössing Bach’. Besides CO_2, no other volatile transformation products have been formed.

Simultaneously the amount of AR in the water phase decreased. At test end 46.9% AR (‘Alte Leine’) and 65.8% AR (‘Rössing Bach’) were determined in the water phases.

SONC969 Saltidin [carboxyl-^{14}C]- was transformed rapidly in the water phase of both test systems. As main transformation product ^{14}C-Saltidin acid was determined. Maximum concentration occurred on day 9 (‘Alte Leine’: 86.6% ARs (i.e. applied radioactivity of SONC969 Saltidin [carboxyl-^{14}C]- plus ^{14}C-Saltidin acid) and 12 (‘Rössing Bach’: 78.3% ARs). Thereafter, concentrations in the water phase declined, yielding 45.2% ARs (‘Alte Leine’) and 62.0% ARs (‘Rössing Bach’) at test end. No further metabolite was determined in the water phase. For the water phase, LSC measurements of the radioactivity and LC-FSA measurements have been compared in order to ensure that the LSC-

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radioactivity complies with the radioactivity found as SONC969 Saltidin [carboxyl-^{14}C]- and ^{14}C-Saltidin acid. With the exception of one replicate, the difference was < 10% AR for both test systems throughout the study. The remaining differences between both measurements can be associated with the typical fluctuations and analytical uncertainties of the LSC and LC-FSA measurements. The results reveal that no further metabolite at amounts > 10% AR were formed during the study in the water phase.

SONC969 Saltidin [carboxyl-^{14}C]- was found at a maximum concentration of 6.4% ARs on day 4 of exposure in the sediment extracts of the test system 'Alte Leine'. Thereafter, amounts declined and after day 15 values were below the LOQ or even LOD. In extracts of the 'Rössing Bach' system, SONC969 Saltidin [carboxyl-^{14}C]- was detectable until day 12, but only at one sampling (day 4) concentrations were slightly >LOQ (0.82% ARs). All further values were < LOQ or <LOD.

Saltidin acid could be observed in the sediments of both systems. Referring to 'Alte Leine', concentrations increased until day 42 (17.6% ARs), thereafter a slight decrease could be noticed. At termination of the test Saltidin acid in the sediment accounted for 9.7% ARs. In the sediment extract samples of 'Rössing Bach' Saltidin acid concentrations increased until day 41 (16.7% ARs). Until test end, a plateau between 16 and 18% ARs was observed. No further stable metabolites were determined in the sediment.

The transformation of SONC969 Saltidin [carboxyl-^{14}C]- and ^{14}C-Saltidin acid in aerobic water/sediment systems followed single first order (SFO) kinetics in the total system and the water phase of both test systems and in the sediment of 'Alte Leine'. The transformation of SONC969 Saltidin [carboxyl-^{14}C]- in the sediment 'Rössing Bach' was not determinable, because the transformation in the water phase was the dominating process. Therefore, a reliable DT calculation was not possible. For ^{14}C-Saltidin acid no decrease in the sediment of 'Rössing Bach' was determined within the testing period. The kinetic evaluation revealed DT_{50} values for SONC969 Saltidin [carboxyl-^{14}C]- between 1.8 and 3.3 days in the water and sediment phases, and the total system of both water/sediment systems.

For ^{14}C-Saltidin acid, DT_{50} values in the water phase account for 104 days ('Alte Leine') and 276 ('Rössing Bach'). Since sediment concentrations in the 'Rössing Bach' system did not indicate a noteworthy decrease over time, only DT_{50} values for Saltidin acid in the 'Alte Leine' system could be determined. They account for 78.1 days. Total system DT_{50} values for ^{14}C-Saltidin acid are considerably lower in the 'Alte Leine' system (DT_{50}: 128 days) compared to the 'Rössing Bach' system (DT_{50}: 420 days).

A proposal for the transformation pathway of SONC969 Saltidin [carboxyl-^{14}C]- in this study was elaborated by a combination of results obtained in a preliminary study (non-GLP) and the definitive study. The results of the preliminary study indicated that the ^{14}C-label in [Hydroxyethyl-1-^{14}C] Saltidin **1** (see figure A7_1_2_2_2-10) was lost

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		after an initial oxidation to Saltidin acid **2**. Simultaneously an increase of $^{14}CO_2$ in the corresponding traps was observed. Therefore a α- or β-oxidation was assumed to be responsible for this transformation step. The α-oxidation would lead to 1-[(butan-2-yloxy)carbonyl]piperidine-2-carboxylic acid **3** and the β-oxidation to butan-2-yl 2-oxopiperidine-1-carboxylate **4**. The results of the definitive study further demonstrated that also the ^{14}C-label in SONC969 Saltidin, [carboxyl-^{14}C]- was immediately lost during the transformation of Saltidin acid and accompanied by an increase of $^{14}CO_2$ in the corresponding traps. The initial oxidation from **1** to **2** was confirmed and further the carbamate moiety was cleaved probably under formation of CO_2, 2-butanol, piperidine-2-carboxylic acid **5** or piperidin-2-one **6**. Nevertheless no other stable metabolites than **2** could be detected during the course of the study.
5.3	**Conclusion**	The transformation rate of SONC969 Saltidin, [carboxyl-^{14}C]- was high in both aquatic sediment systems and proceeded via the major degradation product ^{14}C-Saltidin acid. DT_{50} values for SONC969 Saltidin, [carboxyl-^{14}C]- accounted for 3.3 days ('Alte Leine') and 1.9 days ('Rössing Bach') in the total system (water and sediment phase). The transformation of ^{14}C-Saltidin acid was progressing slower, but a steady decline following SFO kinetics was determined until test end. The DT_{50} values in the total system were 128 days ('Alte Leine') and 420 days ('Rössing Bach'). Simultaneously with the transformation of ^{14}C-Saltidin acid $^{14}CO_2$ was formed. No volatile, organic transformation products were formed during the test duration. No further stable transformation products (> 1.6 % of AR) were determined in the water and sediment phase at test end.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*21 August 2015*
Materials and Methods	*Adopt applicant's version*

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Results and discussion	*Adopt applicant's version; however; a minor correction of the DT50 value for SONC 969 Saltidin. We think that it should be between 1.9 and 3.3 days, instead of 1.8 and 3.3 days in the water and sediment phases under point 5.2.* *In section 5.2 it is stated that "Since sediment concentrations in the 'Rössing Bach' system did not indicate a noteworthy decrease over time, only DT_{50} values for Saltidin acid in the 'Alte Leine' system could be determined." We do agree that no decrease over time could be observed and therefore no DT_{50} values can be determined. However, after our oppinion then this information is important and shows that no dissipation is seen from the sediment. This indicate that Saltidin acid is persistent in sediment at this information is important and should be used in the risk assessment.*
Conclusion	*Adopt applicant's version, however it should be noted that based on the Rössing Bach system Saltidin acid should be considered as persistent in sediment under aerobic condition.*
Reliability	*Based on the assessment of materials and methods include appropriate reliability indicator*
Acceptability	2. Acceptable
Remarks	*According to OECD 308 the two sediments selected should differ with respect to organic carbon content and texture. One sediment should have a high organic carbon content (2.5-7.5%), the other sediment should have a low organic carbon content (0.5-2.5%). This is not the case in this study.*
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7_1_2_2_2-1: Particle size distribution of the sediments for the test systems 'Alte Leine' and 'Rössing Bach'

Texture	Particle size [mm]	Proportion [%]	
		'Alte Leine'	'Rössing Bach'
Sand	2.0 - 0.063	77.7	24.5
Silt	0.063 - 0.002	21.4	72.1
Clay	< 0.002	0.5	2.9

Table A7_1_2_2_2-2: Properties of the water/sediment systems used

Parameter	Stage of test procedure			
	Field sampling and handling	Acclimation phase (day -10/day -7/day -4)	Test start (day 0)	Test end [1)]
'Alte Leine' water				
Temperature [°C]	4.1	- / - / -	-	-
pH-value	8.0	8.02 / - / -	7.35	8.28
TOC [mg C/L]	3.39	- / - / -	8.30	8.52
O_2 concentration [mg O_2/L]	11.92	11.34 / - / 7.37	3.69	8.12
Microbial biomass [CFU/L]	$2.5*10^7$	- / - / $1.7*10^8$	$1.3*10^7$	-
Redox potential [mV]	-	184.7 / - / -	138	150
'Alte Leine' sediment				
pH-value	7.18	- / - / -	7.34	7.48
TOC [%]	2.8 / 2.7	- / - / -	2.6	2.5
Microbial biomass [CfU/g wet sediment]	$8.1 * 10^7$	- / - / -	$5.0*10^7$	$2.5*10^7$
Redox potential [mV]	-	42.3 / - / -	-192	-147
'Rössing Bach' water				
Temperature [°C]	4.6	- / - / -	-	-
pH-value	7.92	- / 7.91 / -	7.85	7.64
TOC [mg C/L]	2.36	- / - / -	17.30	9.56
O_2 concentration [mg O_2/L]	11.57	- / 11.03 / -	9.07	7.41
Microbial biomass [CFU/L]	$2.5*10^7$	- / - / -	$1.4*10^8$	-
Redox potential [mV]	-	- / 199.7 / -	228	202
'Rössing Bach' sediment				
pH-value	7.1	- / - / -	7.26	7.21
TOC [%]	3.0	- / - / -	3.2	3.0
Microbial biomass [CfU/g wet sediment]	$1.4*10^7$	- / - / -	$3.3*10^7$	$1.32*10^6$
Redox potential [mV]	-	- / 94.7 / -	-175	-160

[1)] 100 days for test system 'Alte Leine' and 102 days for test system 'Rössing Bach'

Table A7_1_2_2_2-3: Testing procedure and test solutions used in the aerobic water/sediment study

Parameter	Description
Temperature	Nominal: 20 ± 2 °C; Actual: 19.5 – 21.0 °C, short term deviations (< 12 h) to 19 °C and 22° C
Test duration	100 days (‘Alte Leine’), 102 days (‘Rössing Bach’)
Light/dark cycle	Darkness
Stock solution	Stock solution of labelled test item Nominal: 55.5 MBq/50 mL Actual: ‘Alte Leine’: 50.25 MBq/50 mL ‚Rössing Bach‘: 54 MBq/50 mL Stock solution of non-labelled test item 2.4 g/L in ultrapure water
Sediment/water ratio	1:3 Sediment: 120 g wet sediment per replicate corresponding to a sediment layer of 2.5 ± 0.5 cm : ‘Alte Leine’: 62.16 g dry weight ‘Rössing Bach’: 52.96 g dry weight Water: 300 mL corresponding to a water column of 7.5 ± 0.5 cm
Replicates	Duplicates per water/sediment system and per sampling time for the determination of the transformation rate. 4 replicates per water/sediment system for identification of metabolites. 4 control replicates per water/sediment system for measuring water/sediment parameter and biomass.
Test concentration	Transformation rate: Nominal: 3.33 kBq/mL (1 MBq/Replicate) corresponding to 369 µg/L Actual: ‘Alte Leine’: 3.33 kBq/mL (1 MBq/replicate) ‘Rössing Bach’: 3.70 kBq/mL (1.11 MBq/replicate) Identification of metabolites: Nominal: 6 mg/L, composed of 18.3 kBq/mL (2 mg/L) ^{14}C-labelled test item and 4 mg/L non-labelled test item Actual: ‘Alte Leine’: 6 mg/L, composed of 18.3 kBq/mL (2 mg/L) ^{14}C-labelled test item and 4 mg/L non-labelled test item ‘Rössing Bach’: 6.2 mg/L, composed of 20.4 kBq/mL (2.2 mg/L) ^{14}C-labelled test item and 4 mg/L non-labelled test item Solvent: ultrapure water
Application	Transformation rate: ‘Alte Leine’: Application of 0.995 mL stock solution directly to the water phase ‘Rössing Bach’: Application of 1.03 mL stock solution directly to the water phase Identification of metabolites: ‘Alte Leine’: Application of 5.474 mL stock solution (labelled test item) and 0.5 mL stock solution (non-labelled test item) directly to the water phase ‘Rössing Bach’: Application of 5.656 mL stock solution (labelled test item) and 0.5 mL stock solution (non-labelled test item) directly to the water phase Homogenous distribution of the test item in the water phase directly after application was done by using a paddle agitator.
Test vessels	Gas flow-through system: 500 mL glass flasks connected with an ethylene glycol trap for volatile organic transformation products and a series up to 4 sodium hydroxide traps for $^{14}CO_2$
Ethylene glycol trap	Crimped headspace bottle containing 50 mL ethylene glycol

Table A7_1_2_2_2-3 cont.: Testing procedure and test solutions used in the aerobic water/sediment study

Parameter	Description
$^{14}CO_2$ trap	Up to 4 crimped headspace bottles containing 50 mL 1mol/L aqueous sodium hydroxide.
Aeration	The test vessels were continuously supplied with air by gentle bubbling with compressed, moistened air at the surface of the water phase.
Sampling	Sampling for the determination of the transformation rate and the identification of metabolites proceeded at the same sampling dates. 'Alte Leine': 0, 4, 7, 9, 15, 28, 42, 57, 72, 86 and 100 days of exposure; 'Rössing Bach': 0, 1 (only water phase), 4, 6, 12, 28, 41, 55, 77 and 102 days of exposure. Two test item replicates were sacrificed at each sampling time. The water phase was carefully decanted to avoid disturbances of the sediment and the sediment and water were analysed separately. The sediment was homogenised by thorough stirring with a spatula. The traps were analysed for volatile transformation products.

Table A7_1_2_2_2-4: Analytical methods used in the aerobic water/sediment study

Parameter	Description
Determination of radioactivity by Liquid Scintillation Counter Analysis (LSC)	
Parameter	Radioactivity of the water phase, the sediment after combustion, the sodium hydroxide traps, the ethylene glycol traps, the sediment extracts, the extracted sediment (determination of non-extractable residues (NER))
Equipment	- LSC Counter : TRICARB 2100 TR, PACKARD (PERKIN ELMER) - Software : Ver. 1.05, PACKARD - Oxidizer : Model 307, PACKARD (PERKIN ELMER)
Reagents	LSC-Cocktail, UltimaGold XR, PERKIN ELMER Carbon dioxide absorber, Carbosorb E, PERKIN-ELMER LSC-Cocktail (for Carbosorb E), Permafluor E+, PERKIN-ELMER LSC-Cocktail for carbon dioxide traps, Hionic Fluor, PERKIN-ELMER
Counting Parameter	Counting Type: DPM (disintegrations per minute) Counting terminator: Until 2 x standard deviation of the counted disintegrations is < 0.5 %, but max. 20 min Lower energy level: 0 keV Upper energy level: 156 keV Quench indication paramter: tSIE (transformed spectral index of the external standard ^{133}Ba)
Quench Correction	A general quench curve of the analytical system was used to compensate for a decreased counting efficiency due to chemical or color quench in the different media. The extent of quench in the samples was described by the transformed spectral index of the external (tSIE) ^{133}Ba standard. The determined tSIE of a sample correlates with a counting efficiency.
Preparation of samples	
Water	0.5 mL of water (or 0.05 mL in case of samples for metabolite identification) were mixed with 10 mL of UltimaGold XR in a LSC-vial and measured with LSC.
Sediment	The radioactivity in sediment samples was determined via LSC after combustion with a sample oxidiser. 0.7 g wet sediment were directly weighed in 3 interlocked combusto cones followed by combustion for 5 min. using the sample oxidizer. The produced CO_2 was trapped in 10 mL of Carbosorb E, mixed with 10 mL Permafluor E+ and measured by LSC.
Sediment extracts	100 µL of the sediment extracts after extraction (see below) were mixed with 10 mL of UltimaGold XR and analysed via LSC.
Carbon dioxide traps	0.3 to 3 mL of the sodium hydroxide traps were mixed with 15 mL Hionic-Fluor in a LSC-vial and measured with LSC.
Traps for Volatiles	2 mL of the ethylene glycol trap were mixed with 8 mL of HPLC-water in a LSC-vial followed by addition of 10 mL UltimaGold XR.
Non Extractable Residues	0.2 g of the air dried extracted sediments were weighted in one combusto cone containing 3 combusto pads followed by moistening with 0.4 mL HPLC water. These samples were treated as described before for the unextracted sediment samples (see sediment radioactivity).
Method validation	Limit of Detection (LOD): ≤ 1 % AR for all media Limit of Quantification of the analytical method (LOQ): Water: 0.05% AR; Ethylene glycol traps: 0.04% AR; Sodium hydroxide traps: 0.03% AR; Sediments: 0.3% AR; Extracted sediments: 0.5% AR
Accuracy	The analytical methods for all media were validated on two fortification levels (1x and 10xLOQ). The mean recoveries at each fortification level were in the range of 95 and 105 %.

Precision	Relative standard deviations at each fortification level were lower than 5 %.

Table A7_1_2_2_2-4 cont.: Analytical methods used in the aerobic water/sediment study

Parameter	Description
Flow Scintillation analysis coupled with HPLC (HPLC-FSA)	
Parameter	Analysis of SONC969 Saltidin, [carboxyl-^{14}C]- in water and sediment extracts
Equipment	HPLC: 2695 Alliance separation module, WATERS Detector: 500TR FSA, PERKIN-ELMER Software: FlowOne, v3.65, PERKIN-ELMER Software LC: Mass Lynx^{TM} 4.1, WATERS
Reagents	ULTIMA-FLO^{TM} M (LSC-cocktail for Radio-HPLC), PERKIN-ELMER Disposable syringe filter: Chromafil RC-45/15 MS, MACHERY-NAGEL High DPM Spec-Chec-^{14}C, Part Number 6002135, 8.26*10^{5} dpm / mL, PERKIN-ELMER [hydroxyethyl-1-^{14}C] Saltidin, 3.55 MBq/ mg
Efficiency standard	Spec-Chec-^{14}C and [hydroxyethyl-1-^{14}C] Saltidin (corresponding to chemical identity of the test item but other carbon atom is labelled) with known activity. The dependence of the counting efficiency on the gradient conditions of the chromatographic system was determined by establishing efficiency tables. Therefore Spec-Chec-^{14}C and [hydroxyethyl-1-^{14}C] Saltidin with known activity were used.
Conditions of Analysis	Column: Discovery C18 5 µm, 250 x 4.6 mm, Batch 133820-01, SUPELCO Temperature: 25°C Mobile phase: A : 0.005 mol/L trifluoroacetic acid in HPLC water B : 0.005 mol/L trifluoroacetic acid in acetonitrile Gradient mode FSA Cell type, liquid, 500 µL Radio update 4 s Nuclide ^{14}C (LLD = 0 keV, ULD 156 keV) HPLC flow rate 1.0 mL / min LS flow rate 2.4 mL / min LS / HPLC ratio 2.4 : 1
Preparation of samples Water Sediment extracts	 0.9 mL of water were stabilized with 0.1 mL of ethanol prior to analysis. Samples for metabolite identification: 0.2 mL water was diluted with 0.8 mL HPLC water. Then 0.9 mL of this dilution were stabilized with 0.1 mL of ethanol prior to analysis. 25 g wet sediment were extracted in a soxhlet extractor with refluxing acetonitrile for 8 h. The extract was evaporated to dryness using a rotary evaporator. The residue was dissolved in 5 mL of a 1:1 mixture of ethanol and HPLC water and filtered over a disposable syringe filter (Chromafil RC-45/15 MS) prior to analysis.
Method validation	Limit of Detection (LOD): Signal-noise ratio of 3, corresponding to ≤ 0.12% AR for sediment and ≤ 1.6% for water. Limit of Quantification of the analytical method (LOQ): Water: 3.6 and 4.7% AR; Sediment extracts: 0.27 and 0.35% AR
Accuracy	Water: The analytical methods for all media were validated on two fortification levels (1x and 10xLOQ). The mean recoveries at each fortification level were in the range of 90 and 110 %. Sediment: The analytical method for sediment was not validated prior to study initiation but at every sampling time. Quality controls were prepared

	by spiking an equivalent amount of wet sediment from stock solutions of SONC969 Saltidin, [carboxyl-^{14}C]- in ethanol with the radioactivity, corresponding to the 3.6% AR. The mean recovery was between 90 and 110%.
Precision	Relative standard deviations at each fortification level were lower than 10%.

Table A7_1_2_2_2-4 cont.: Analytical methods used in the aerobic water/sediment study

Parameter	Description
Structure elucidation via combination of LC-MS and LC-MS/MS	
Parameter	The structure of the only detectable degradation product during the course of the study, ^{14}C-Saltidin acid, was elucidated via LC-MS/MS. The replicates for metabolite identification were used for these analyses. Firstly, the molecular weight of ^{14}C-Saltidin acid was derived in MS-scan mode. Secondly, structural units of the degradation product were derived by MS/MS. Finally the chemical structure of the degradation product was derived by combining all the information obtained from the measurements
Equipment	HPLC: 2695 Alliance separation module, WATERS Detector: Mass selective detector, Micromass Quattro Micro TM API (MS/MS-detector), WATERS Software: Mass Lynx^{TM} 4.1
Conditions of Analysis	The chromatographic method for these analyses corresponded to that of the FSA-analyses.
Preparation of samples Water Sediment extracts	 0.2 mL of water was diluted with 0.8 mL of HPLC water followed by a further dilution step (0.9 mL sample + 0.1 mL ethanol) prior to analysis. 25 g wet sediment were extracted in a soxhlet extractor with refluxing acetonitrile for 8 h. The extract was evaporated to dryness using a rotary evaporator. The residue was dissolved in 5 mL of a 1:1 mixture of ethanol and HPLC water and filtered over a disposable syringe filter (Chromafil RC-45/15 MS) prior to analysis.
Conditions of detection	General parameters of the mass spectrometer Ionisation mode: ES+ Capillary voltage: 2.00 kV Cone voltage: 20 V Source temperature: 120 °C Cone gas flow (N_2): 100 L/h Desolvation temperature: 350 °C Desolvation gas flow (N_2): 800 L/h Collision gas pressure (Ar): approx. 2.5 * 10^{-3} mbar (only for MS/MS) Data format: Continuum MS-scan Start mass: 200 Da End mass: 300 Da Scan time: 0.2 min MS/MS Daughters of: 244.3 Start mass: 50 End mass: 250 Scan time: 0.2 min Collision Energy: 20 eV

Table A7_1_2_2_2-5: Mass balance of the water/sediment test system 'Alte Leine'

Exposure Day	Repl.	Water (% of AR)	mv	Sediment (% of AR)	mv	$^{14}CO_2$ (% of AR)	mv	Mass Balance (% of AR)	mv
0	1 2	99.6 100.4	**100.0**	1.0 0.9	**1.0**	- -	**-**	100.6 101.3	**101.0**
4	1 2	89.8 90.4	**90.1**	9.3 8.9	**9.1**	0.178 0.221	**0.20**	99.3 99.5	**99.4**
7	1 2	87.7 81.7	**84.7**	9.8 11.4	**10.6**	0.98 2.85	**1.91**	98.5 95.9	**97.2**
9	1 2	81.6 81.1	**81.4**	12.5 12.1	**12.3**	2.81 3.06	**2.93**	96.9 96.3	**96.6**
15	1 2	78.4 76.6	**77.5**	13.7 14.0	**13.9**	3.31 8.08	**5.69**	95.4 98.7	**97.0**
28	1 2	70.4 73.3	**71.9**	13.6 14.4	**14.0**	11.9 6.59	**9.23**	95.9 94.3	**95.1**
42	1 2	74.5 75.4	**75.0**	16.3 16.5	**16.4**	6.72 5.50	**6.11**	97.5 97.4	**97.5**
57	1 2	65.9 68.9	**67.4**	15.6 14.5	**15.1**	3.70 8.25	**5.97**	85.2 91.6	**88.4**
72	1 2	62.1 59.7	**60.9**	14.4 13.3	**13.9**	17.7 22.9	**20.3**	94.2 95.9	**95.1**
86	1 2	62.4 49.5	**56.0**	14.5 11.7	**13.1**	21.8 32.1	**26.9**	98.7 93.3	**96.0**
100	1 2	46.8 47.0	**46.9**	11.4 10.4	**10.9**	41.3 (26.2)[#]	**41.3**	99.5 (83.6)[#]	**99.5**

AR = Applied Radioactivity

not included in calculations, $^{14}CO_2$ lost due technical fault between trap 2 and trap 3

- = not determined mv = mean values

Table A7_1_2_2_2-6: Mass balance of the water/sediment test system 'Rössing Bach'

Exposure Day	Repl.	Water (% of AR)	mv	Sediment (% of AR)	mv	$^{14}CO_2$ (% of AR)	mv	Mass Balance (% of AR)	mv
0	1	99.9	**99.6**	0.6	**0.6**	-	**-**	100.5	**100.2**
	2	99.3		0.5		-		99.8	
4	1	88.8	**88.5**	10.1	**9.9**	0.54	**0.43**	99.4	**98.7**
	2	88.1		9.6		0.33		98.0	
6	1	85.5	**85.8**	12.1	**11.8**	1.89	**1.69**	99.5	**99.2**
	2	86.0		11.4		1.50		98.9	
12	1	83.2	**80.7**	13.5	**14.4**	2.14	**3.26**	98.8	**98.4**
	2	78.2		15.3		4.39		97.9	
28	1	74.6	**74.6**	15.9	**16.8**	6.76	**7.17**	97.3	**98.5**
	2	74.6		17.6		7.57		99.8	
41	1	71.1	**72.6**	19.5	**18.0**	8.02	**7.58**	98.6	**98.1**
	2	74.0		16.5		7.14		97.6	
55	1	70.7	**72.0**	17.7	**17.0**	10.9	**10.0**	99.3	**98.9**
	2	73.2		16.2		9.12		98.5	
77	1	68.2	**68.1**	15.9	**17.4**	11.76	**13.1**	95.9	**98.5**
	2	68.0		18.8		14.4		101.2	
102	1	65.3	**65.8**	18.8	**18.5**	15.5	**16.0**	99.6	**100.3**
	2	66.3		18.2		16.4		100.9	

AR = Applied Radioactivity
n.d. = not determined mv = mean values

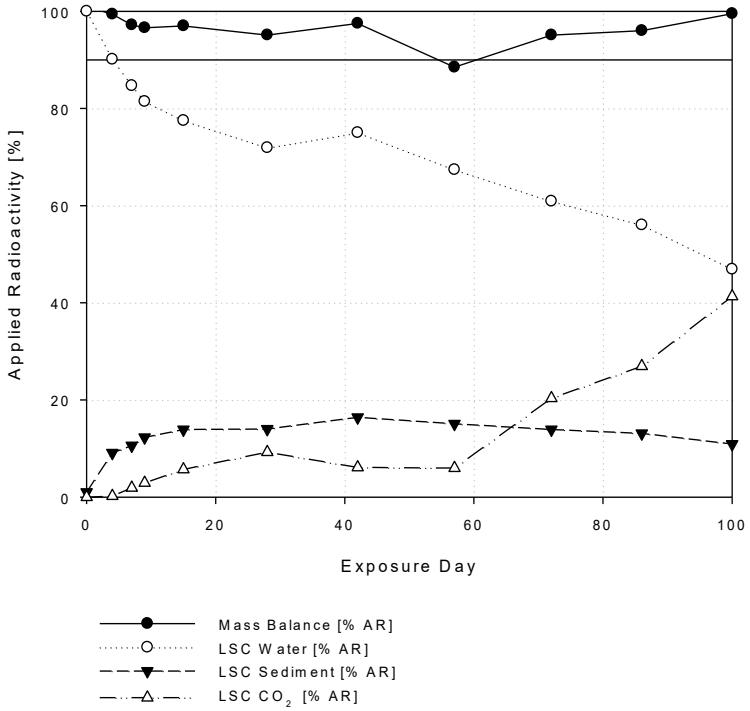

Figure A7_1_2_2_2-1: 'ALTE LEINE': Distribution of applied radioactivity

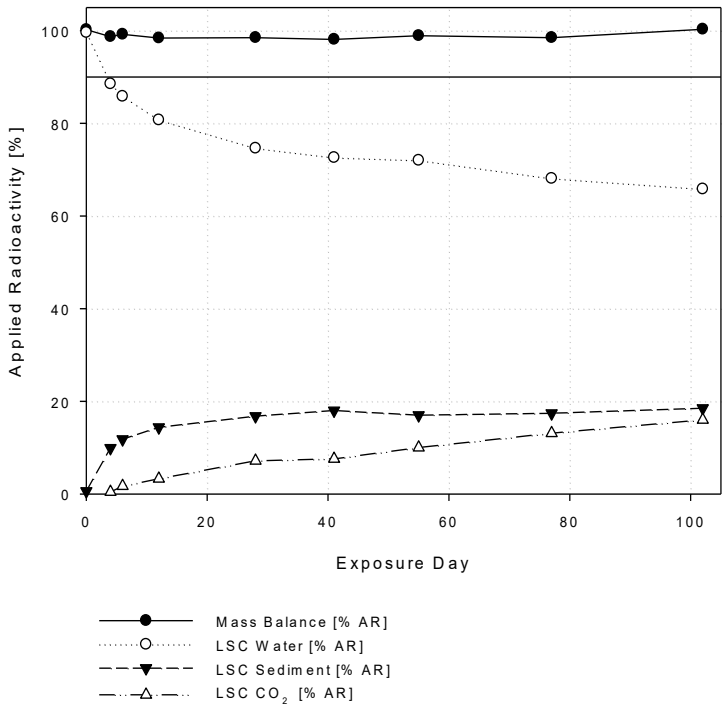

Figure A7_1_2_2_2-2: 'Rössing Bach': Distribution of applied radioactivity

Table A7_1_2_2_2-7: 'Alte Leine': Distribution of AR in the sediment

Exposure Day	Repl.	Sediment (% of AR)	mv	Sediment Extract (% of AR)	mv	Extracted Sediment (NER) (% of AR)	mv
4	1 2	9.3 8.9	**9.1**	8.9 9.6	**9.3**	0.1 0.2	**0.1**
7	1 2	9.8 11.4	**10.6**	9.5 10.5	**10.0**	0.3 0.5	**0.4**
9	1 2	12.5 12.1	**12.3**	11.1 11.5	**11.3**	0.7 0.5	**0.6**
15	1 2	13.7 14.0	**13.9**	12.9 12.7	**12.8**	0.5 0.7	**0.6**
28	1 2	13.6 14.4	**14.0**	13.4 14.0	**13.7**	0.7 0.7	**0.7**
42	1 2	16.3 16.5	**16.4**	15.7 16.1	**15.9**	0.5 0.4	**0.5**
57	1 2	15.6 14.5	**15.1**	13.8 14.0	**13.9**	0.8 0.6	**0.7**
72	1 2	14.4 13.3	**13.9**	12.6 13.0	**12.8**	1.0 1.0	**1.0**
86	1 2	14.5 11.7	**13.1**	13.5 10.6	**12.1**	0.9 1.1	**1.0**
100	1 2	11.4 10.4	**10.9**	10.7 9.2	**10.0**	1.2 1.3	**1.2**

AR = Applied Radioactivity
mv = mean values

Table A7_1_2_2_2-8: 'Rössing Bach': Distribution of AR in the sediment

Exposure Day	Repl.	Sediment (prior to extraction) (% of AR)	mv	Sediment Extract (% of AR)	mv	Non-Extractable Residues (NER) (% of AR)	mv
4	1	10.1	**9.9**	9.7	**10.3**	0.4	**0.4**
	2	9.6		10.8		0.3	
6	1	12.1	**11.8**	12.7	**12.0**	0.6	**0.6**
	2	11.4		11.4		0.6	
12	1	13.5	**14.4**	13.3	**14.1**	0.6	**0.8**
	2	15.3		14.9		0.9	
28	1	15.9	**16.8**	16.8	**16.0**	1.6	**1.4**
	2	17.6		15.2		1.1	
41	1	19.5	**18.0**	17.2	**16.4**	1.7	**1.4**
	2	16.5		15.7		1.0	
55	1	17.7	**17.0**	16.4	**16.1**	1.1	**1.0**
	2	16.2		15.7		0.8	
77	1	15.9	**17.4**	14.9	**16.5**	1.3	**1.2**
	2	18.8		18.1		1.1	
102	1	18.8	**18.5**	17.0	**17.0**	2.1	**1.6**
	2	18.2		17.0		1.2	

AR = Applied Radioactivity
mv = mean values

Table A7_1_2_2_2-9: 'Alte Leine': Transformation of SONC969 Saltidin, [carboxyl-^{14}C]- and ^{14}C-Saltidin acid in the total system

Exposure Day	Repl.	Total System (Water & Sediment) SONC969 Saltidin, [carboxyl-^{14}C]- (% of ARs)	Mean	^{14}C-Saltidin acid (% of ARs)	Mean
0	1	**95.8**	**97.6**	2.5	2.4
	2	**99.3**		2.4	
4	1	**63.3**	**60.6**	**31.2**	**33.7**
	2	**57.9**		**36.3**	
7	1	**18.0**	**9.8**	**84.1**	**90.7**
	2	**1.7**		**97.3**	
9	1	**1.5**	**2.0**	**100.6**	**98.2**
	2	**2.5**		**95.7**	
15	1	**0.47**	0.26	**96.8**	**97.5**
	2	*0.06*		**98.2**	
28	1	*0.06*	0.18	**85.7**	**87.8**
	2	0.30		**89.9**	
42	1	0.24	0.30	**92.7**	**93.2**
	2	0.36		**93.7**	
57	1	0.34	0.20	**83.3**	**84.6**
	2	*0.06*		**85.9**	
72	1	*0.06*	*0.06*	**75.2**	**73.5**
	2	*0.06*		**71.7**	
86	1	*0.06*	0.20	**78.6**	**68.4**
	2	0.34		**58.2**	
100	1	0.47	0.26	**56.2**	**54.9**
	2	*0.06*		**53.7**	

AR_S = Applied Radioactivity related to SONC969 Saltidin, [carboxyl-^{14}C]- and ^{14}C-Saltidin acid
n.a. = not applicable

Assignment of character style:
bold = values > LOQ, normal = values < LOQ, *italics* = values < LOD, calculated with ½ LOD

Table A7_1_2_2_2-10: 'Alte Leine': Transformation of SONC969 Saltidin, [carboxyl-^{14}C]- and ^{14}C-Saltidin acid in the water phase

Exposure Day	Repl.	Water Phase: SONC969 Saltidin, [carboxyl-^{14}C]- (% of ARs)	Mean	Water Phase: ^{14}C-Saltidin acid (% of ARs)	Mean
0	1	**95.8**	**97.6**	2.5	2.4
	2	**99.3**		2.4	
4	1	**58.2**	**54.2**	**30.5**	**32.0**
	2	**50.1**		**33.5**	
7	1	**14.3**	7.5	**77.2**	**81.5**
	2	*0.8*		**85.8**	
9	1	*0.8*	*0.8*	**88.1**	**86.6**
	2	*0.8*		**85.2**	
15	1	< LOD	< LOD	**83.3**	**83.2**
	2	< LOD		**83.2**	
28	1	< LOD	< LOD	**71.4**	**73.2**
	2	< LOD		**75.1**	
42	1	< LOD	< LOD	**75.2**	**75.6**
	2	< LOD		**75.9**	
57	1	< LOD	< LOD	**68.5**	**69.6**
	2	< LOD		**70.7**	
72	1	< LOD	< LOD	**61.0**	**59.5**
	2	< LOD		**58.1**	
86	1	< LOD	< LOD	**64.2**	**55.6**
	2	< LOD		**47.0**	
100	1	< LOD	< LOD	**45.8**	**45.2**
	2	< LOD		**44.6**	

AR_S = Applied Radioactivity related to SONC969 Saltidin, [carboxyl-^{14}C]- and ^{14}C-Saltidin acid
n.a. = not applicable

Assignment of character style:
bold = values > LOQ, normal = values < LOQ, *italics* = values < LOD, calculated with ½ LOD

Table A7_1_2_2_2-11: 'Alte Leine': Transformation of SONC969 Saltidin, [carboxyl-^{14}C]- and ^{14}C-Saltidin acid in the sediment

Exposure Day	Repl.	Sediment: SONC969 Saltidin, [carboxyl-^{14}C]- (% of ARs)	Mean	Sediment: ^{14}C-Saltidin acid (% of ARs)	Mean
0	1	n.a.	**n.a.**	n.a.	n.a.
	2	n.a.		n.a.	
4	1	**5.09**	**6.4**	**0.6**	**1.7**
	2	**7.77**		**2.7**	
7	1	**3.68**	**2.3**	**6.9**	**9.2**
	2	**0.90**		**11.5**	
9	1	**0.68**	**1.2**	**12.5**	**11.5**
	2	**1.74**		**10.6**	
15	1	**0.47**	0.3	**13.6**	**14.3**
	2	*0.06*		**15.0**	
28	1	*0.06*	0.2	**14.3**	**14.6**
	2	0.30		**14.8**	
42	1	0.24	0.3	**17.6**	**17.6**
	2	0.36		**17.7**	
57	1	0.34	0.2	**14.9**	**15.0**
	2	*0.06*		**15.2**	
72	1	*0.06*	*0.1*	**14.3**	**13.9**
	2	*0.06*		**13.6**	
86	1	*0.06*	0.2	**14.3**	**12.8**
	2	0.34		**11.2**	
100	1	**0.47**	0.3	**10.5**	**9.7**
	2	*0.06*		**9.0**	

AR_S = Applied Radioactivity related to SONC969 Saltidin, [carboxyl-^{14}C]- and ^{14}C-Saltidin acid
n.a. = not applicable

Assignment of character style:
bold = values > LOQ, normal = values < LOQ, *italics* = values < LOD, calculated with ½ LOD

Table A7_1_2_2_2-12: 'Rössing Bach': Transformation of SONC969 Saltidin, [carboxyl-^{14}C]- and ^{14}C-Saltidin acid in the total system

Exposure Day	Repl.	Total System (Water & Sediment) SONC969 Saltidin, [carboxyl-^{14}C]- (% of ARs)	Mean	^{14}C-Saltidin acid (% of ARs)	Mean
0	1 2	**97.5** **97.5**	**97.5**	2.5 2.6	2.5
1	1 2	n.a. n.a.	n.a.	n.a. n.a.	n.a.
4	1 2	**22.8** **27.6**	**25.2**	**70.9** **62.6**	**66.7**
6	1 2	**3.7** **8.4**	**6.1**	**92.0** **82.5**	**87.3**
12	1 2	2.9 0.69	1.8	**91.5** **92.4**	**91.9**
28	1 2	*0.92* 0.69	0.8	**86.4** **84.0**	**85.2**
41	1 2	0.12 0.24	0.2	**85.0** **85.8**	**85.4**
55	1 2	0.05 0.32	0.2	**83.9** **84.5**	**84.2**
77	1 2	*0.16* *0.05*	*0.1*	**79.2** **85.3**	**82.2**
102	1 2	0.28 *0.05*	0.2	**79.2** **79.4**	**79.3**

AR_S = Applied Radioactivity related to SONC969 Saltidin, [carboxyl-^{14}C]- and ^{14}C-Saltidin acid
n.a. = not applicable

Assignment of character style:
bold = values > LOQ, normal = values < LOQ, *italics* = values < LOD, calculated with ½ LOD

Table A7_1_2_2_2-13: 'Rössing Bach': Transformation of SONC969 Saltidin, [carboxyl-^{14}C]- and ^{14}C-Saltidin acid in the water phase

Exposure Day	Repl.	Water Phase: SONC969 Saltidin, [carboxyl-^{14}C]- (% of ARs)	Mean	Water Phase: ^{14}C-Saltidin acid (% of ARs)	Mean
0	1	**97.5**	**97.5**	2.5	2.5
	2	**97.5**		2.4	
1	1	**66.7**	**65.0**	**21.6**	**21.6**
	2	**63.3**		**21.6**	
4	1	**22.2**	**24.4**	**61.8**	**57.7**
	2	**26.5**		**53.6**	
6	1	3.6	**5.9**	**79.1**	**75.3**
	2	**8.2**		**71.5**	
12	1	2.6	1.6	**78.2**	**78.3**
	2	*0.6*		**78.4**	
28	1	*0.6*	*0.6*	**69.6**	**69.0**
	2	*0.6*		**68.5**	
41	1	< LOD	< LOD	**66.9**	**68.7**
	2	< LOD		**70.5**	
55	1	< LOD	< LOD	**67.1**	**67.9**
	2	< LOD		**68.7**	
77	1	< LOD	< LOD	**64.8**	**66.0**
	2	< LOD		**67.3**	
102	1	< LOD	< LOD	**62.1**	**62.0**
	2	< LOD		**62.0**	

ARs = Applied Radioactivity related to SONC969 Saltidin, [carboxyl-^{14}C]- and ^{14}C-Saltidin acid
n.a. = not applicable

Assignment of character style:
bold = values > LOQ, normal = values < LOQ, *italics* = values < LOD, calculated with ½ LOD

Table A7_1_2_2_2-14: 'Rössing Bach': Transformation of SONC969 Saltidin, [carboxyl-^{14}C]- and ^{14}C-Saltidin acid in the sediment

Exposure Day	Repl.	Sediment: SONC969 Saltidin, [carboxyl-^{14}C]- (% of ARs)	Mean	Sediment: ^{14}C-Saltidin acid (% of ARs)	Mean
0	1	n.a.	n.a.	n.a.	n.a.
	2	n.a.		n.a.	
1	1	n.a.	n.a.	n.a.	n.a.
	2	n.a.		n.a.	
4	1	**0.58**	0.82	**9.0**	**9.0**
	2	**1.05**		**9.0**	
6	1	0.13	*0.20*	**13.0**	**12.0**
	2	0.27		**11.0**	
12	1	**0.34**	0.19	**13.3**	**13.7**
	2	*0.05*		**14.1**	
28	1	0.27	*0.16*	**16.9**	**16.2**
	2	*0.05*		**15.5**	
41	1	0.12	0.18	**18.1**	**16.7**
	2	0.24		**15.3**	
55	1	*0.05*	0.18	**16.8**	**16.3**
	2	**0.32**		**15.8**	
77	1	0.16	*0.11*	**14.4**	**16.2**
	2	*0.05*		**18.0**	
102	1	0.28	0.17	**17.1**	**17.3**
	2	*0.05*		**17.4**	

ARs = Applied Radioactivity related to SONC969 Saltidin, [carboxyl-^{14}C]- and ^{14}C-Saltidin acid
n.a. = not applicable

Assignment of character style:
bold = values > LOQ, normal = values < LOQ, *italics* = values < LOD, calculated with ½ LOD

Table A7_1_2_2_2-15: Kinetic data for the test system 'Alte Leine' (total water and sediment as well as only water)

Endpoint / Statistic	System Compartment: Total (Water & Sediment)	System Compartment: Water
Model	**Single First Order (SFO)**	
C_0 (% of AR) *Initial value for fitting*	**104.8** ± 6.83 *100*	**98.9** ± 7.20 *100*
K_P (1/d) *Initial value for fitting*	**0.2135** ± 0.0232 *0.3*	**0.2232** ± 0.0283 *0.3*
ffM (as a fraction) *Initial value for fitting*	1 *1*	**0.942** ± 0.0987 *1*
K_m (1/d) *Initial value for fitting*	**0.00542** ± 0.00110 *0.008*	**0.00663** ± 0.00138 *0.008*
Data range (days)	0 - 100	0 - 100
χ^2 error SONC969 Saltidin, [carboxyl-^{14}C]-	32.3	23.2
t-Test (P=0.05)	Passed	Passed
χ^2 error ^{14}C-Saltindin acid	10.9	11.1
t-Test (P=0.05)	Passed	Passed
	DT_X values in days	
DT_{50} SONC969 Saltidin, [carboxyl-^{14}C]-	**3.3**	**3.1**
DT_{90} SONC969 Saltidin, [carboxyl-^{14}C]-	**10.8**	**10.3**
DT_{50} ^{14}C-Saltidin acid	**128**	**104**
DT_{90} ^{14}C-Saltidin acid	**425**	**347**

Table A7_1_2_2_2-16: Kinetic data for the test system 'Alte Leine' (sediment)

Endpoint / Statistic	System Compartment Sediment
SONC969 Saltidin, [carboxyl-^{14}C]-	
Model	**Single First Order (SFO)**
C_0 (% of AR) *Initial value for fitting*	**6.42** ± 0.494 *6.0*
K_M (1/d) *Initial value for fitting*	**0.3347** ± 0.0568 *0.03*
Data range (days)	4 - 100
χ^2 error	3.7
t-Test (P=0.05)	Passed
DT_X values in days	
DT_{50}	**2.1**
DT_{90}	**6.9**
^{14}C Saltidin acid	
Model	**Single First Order (SFO)**
C_0 (% of AR) *Initial value for fitting*	**17.7** ± 0.648 *20.0*
K_M (1/d) *Initial value for fitting*	**0.0088** ± 0.00124 *0.03*
Data range (days)	*42 - 100*
χ^2 error	3.0
t-Test (P=0.05)	Passed
DT_X values in days	
DT_{50}	**78.1**
DT_{90}	**260**

Table A7_1_2_2_2-17: Kinetic data for the test system 'Rössing Bach' (total water and sediment as well as only water)

Endpoint / Statistic	System Compartment	
	Total (Water & Sediment)	**Water**
Model	**Single First Order (SFO)**	
C_0 (% of AR) *Initial value for fitting*	**97.7** ± 2.27 *100*	**96.9** ± 2.19 *100*
K_P (1/d) *Initial value for fitting*	**0.3698** ± 0.0178 *0.4*	**0.3785** ± 0.0190 *0.2*
ffM (as a fraction) *Initial value for fitting*	**0.945** ± 0.0287 *0.9*	**0.807** ± 0.0276 *1*
K_m (1/d) *Initial value for fitting*	**0.00165** ± 0.00034 *0.004*	**0.00251** ± 0.00044 *0.006*
Data range (days)	0 - 102	0 - 102
χ^2 error SONC969 Saltidin, [carboxyl-^{14}C]-	8.9	5.4
t-Test (P=0.05)	Passed	Passed
χ^2 error ^{14}C-Saltindin acid	3.1	4.3
t-Test (P=0.05)	Passed	Passed
	DT_X values in days	
DT_{50} SONC969 Saltidin, [carboxyl-^{14}C]-	**1.9**	**1.8**
DT_{90} SONC969 Saltidin, [carboxyl-^{14}C]-	**6.2**	**6.1**
DT_{50} ^{14}C-Saltidin acid	**420**	**276**
DT_{90} ^{14}C-Saltidin acid	**1394**	**916**

Table A7_1_2_2_2-18: Kinetic data for the test system 'Rössing Bach' (sediment)

Endpoint / Statistic	System Compartment Sediment
SONC969 Saltidin, [carboxyl-^{14}C]-	
Model	**Single First Order (SFO)**
C_0 (% of AR) *Initial value for fitting*	**0.812** ± 0.146 *1.0*
K_M (1/d) *Initial value for fitting*	**0.6526** ± 0.3485 *0.6*
Data range (days)	4 - 102
χ^2 error	43.7
t-Test (P=0.05)	Passed

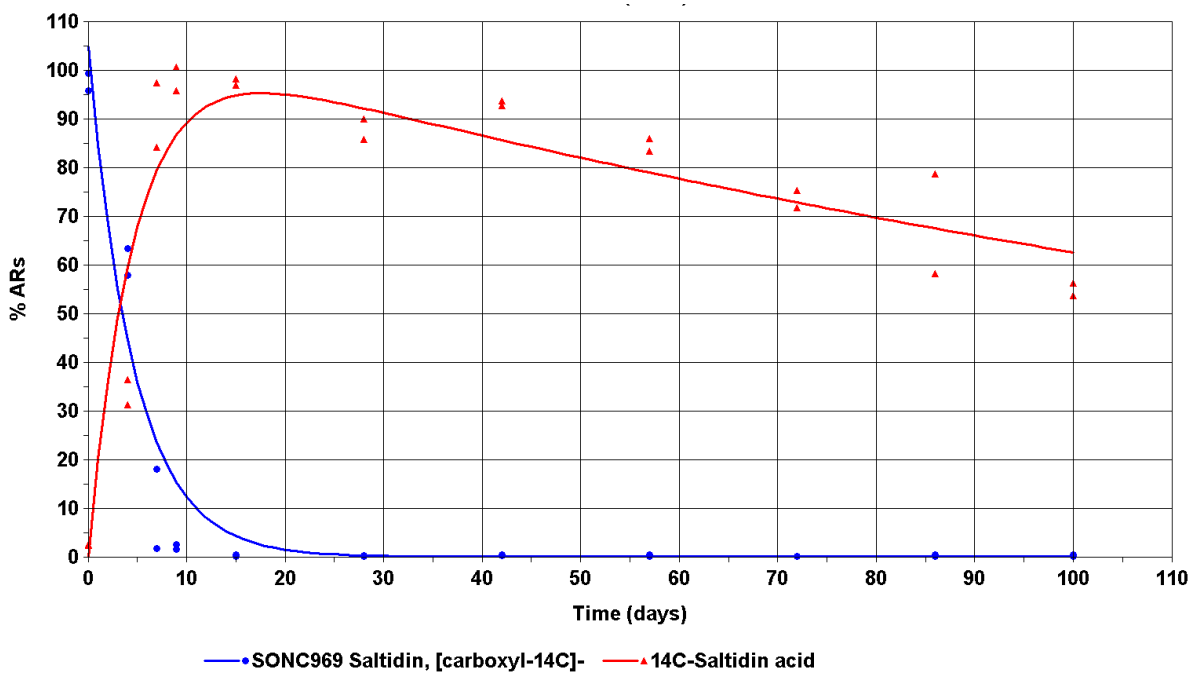

Figure A7_1_2_2_2-3: 'Alte Leine': Kinetic fit for SONC969 Saltidin, [carboxyl-^{14}C]- and ^{14}C-Saltidin acid in total system (water & sediment)

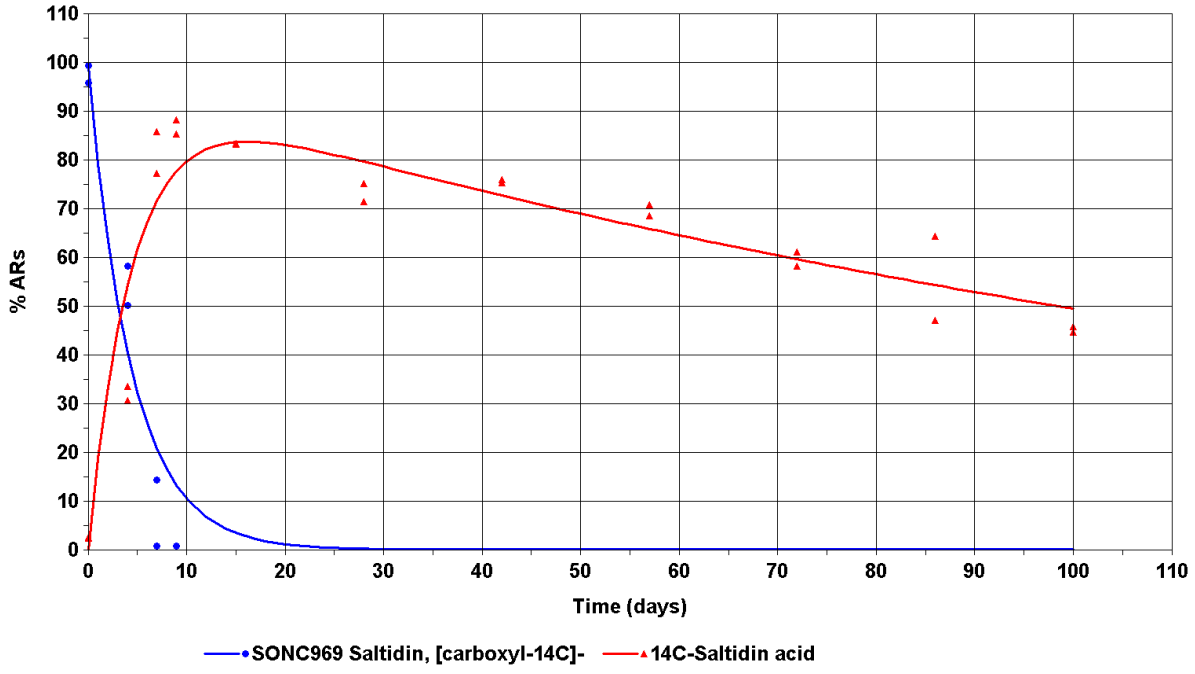

Figure A7_1_2_2_2-4: 'Alte Leine': Kinetic fit for SONC969 Saltidin, [carboxyl-^{14}C]- and ^{14}C-Saltidin acid in the water column

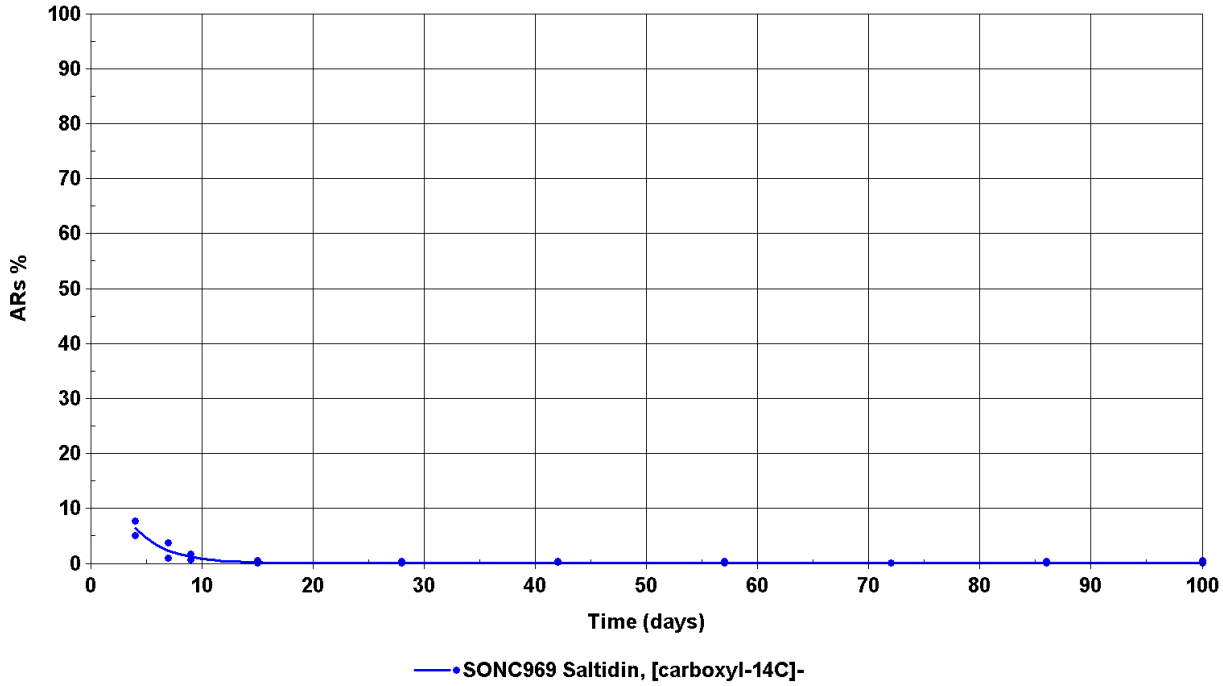

Figure A7_1_2_2_2-5: 'Alte Leine': Kinetic fit for SONC969 Saltidin, [carboxyl-^{14}C]- in sediment

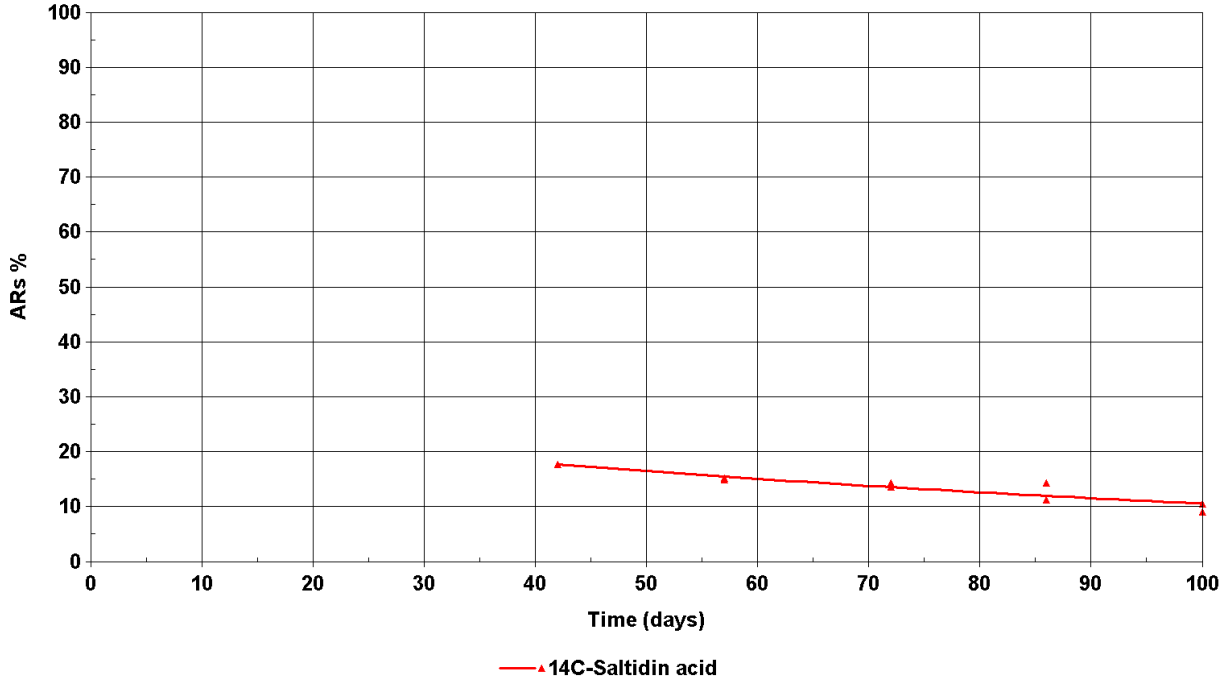

Figure A7_1_2_2_2-6: 'Alte Leine': Kinetic fit for ^{14}C-Saltidin acid in sediment

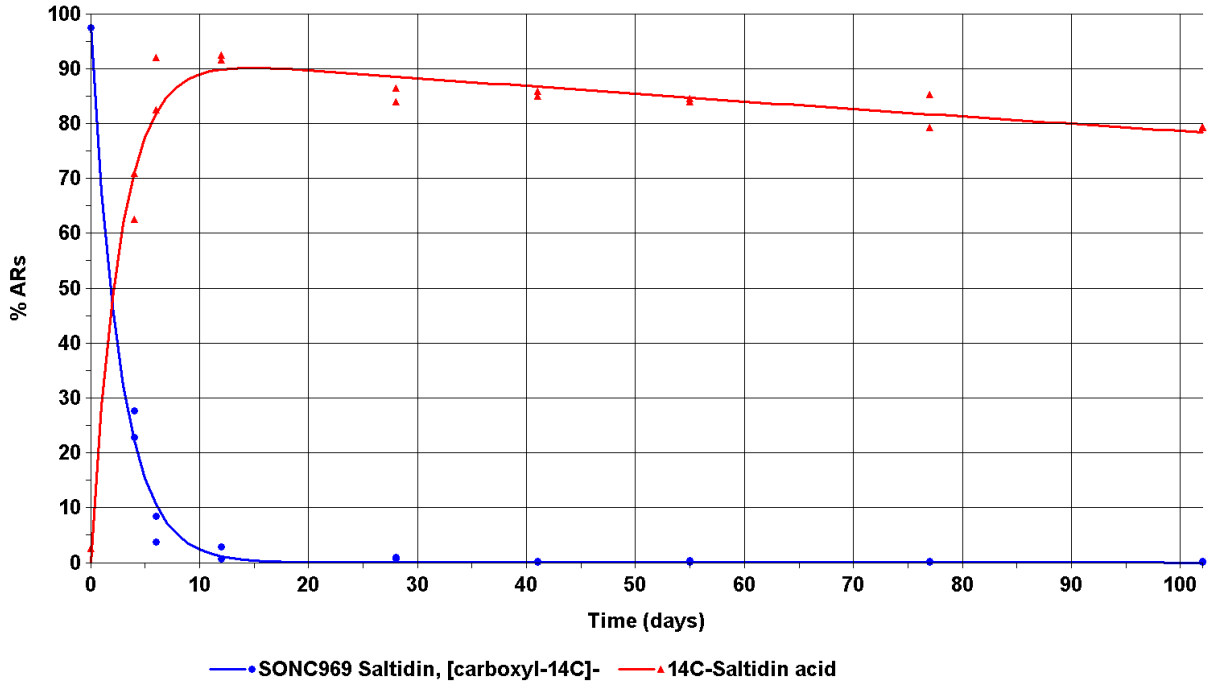

Figure A7_1_2_2_2-7: 'Rössing Bach': Kinetic fit for SONC969 Saltidin, [carboxyl-^{14}C]- and ^{14}C-Saltidin acid in total system (water & sediment)

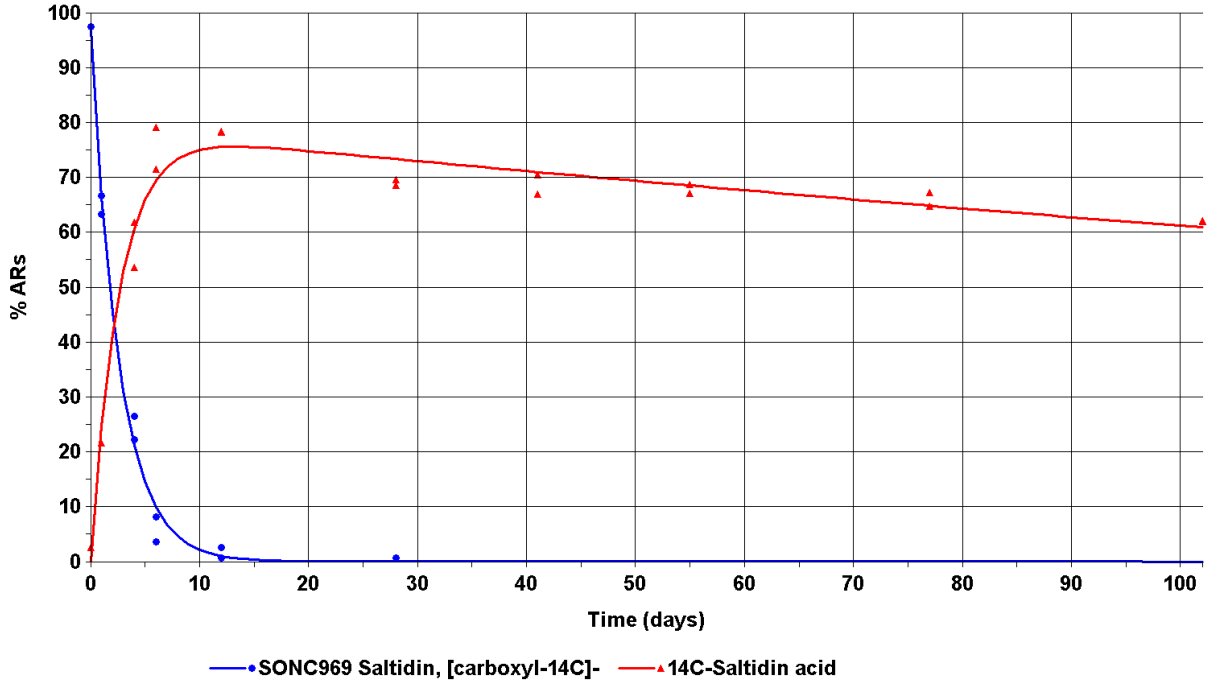

Figure A7_1_2_2_2-8: 'Rössing Bach': Kinetic fit for SONC969 Saltidin, [carboxyl-^{14}C]- and ^{14}C-Saltidin acid in the water column

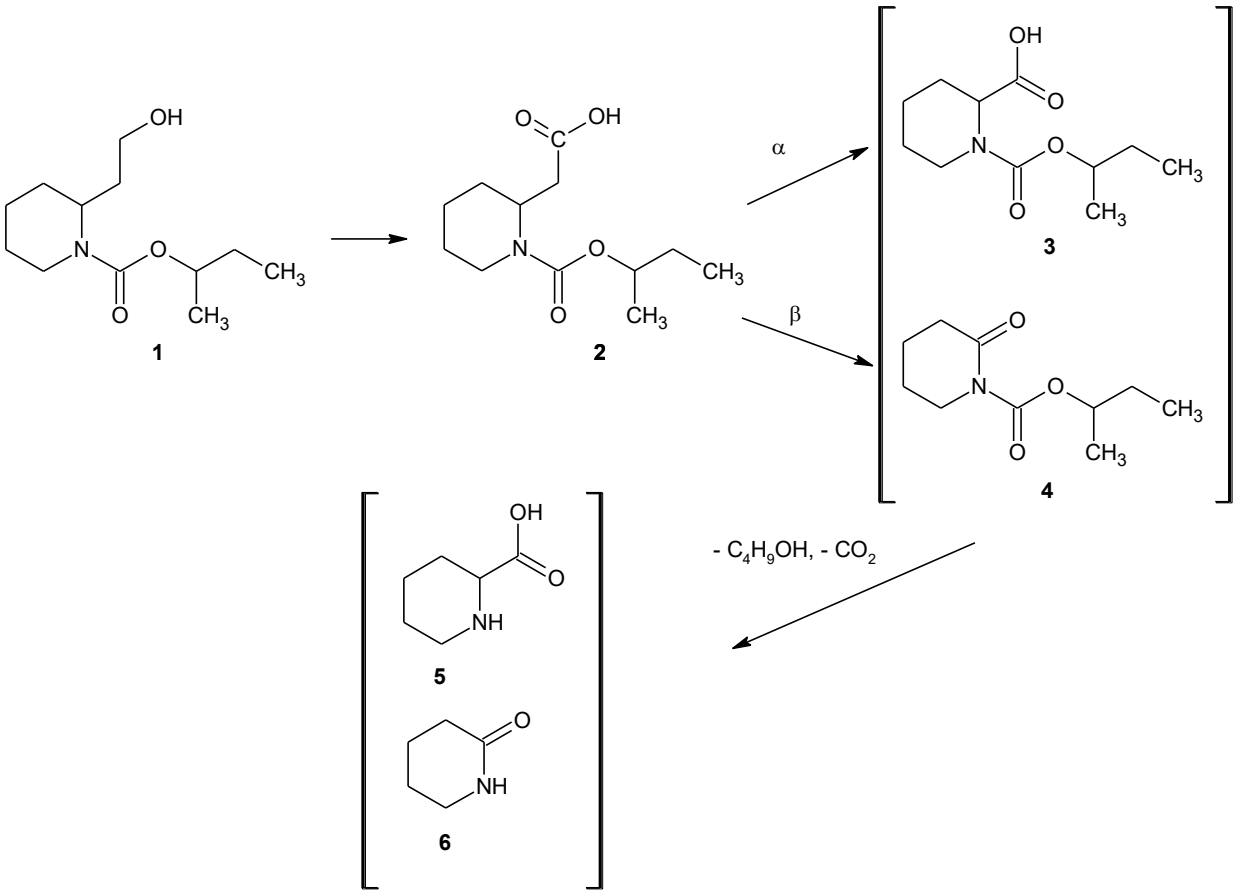

Figure A7_1_2_2_2-09: Proposed degradation pathway of Saltidin in aerobic aquatic systems

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		Official use only
	1 REFERENCE	
	1.1	
1.1 Reference	Fiebig, S. and Goller, St. (2014): SONC969 Saltidin, [carboxyl-^{14}C]-Anaerobic Transformation in Aquatic Sediment Systems using ^{14}C-labelled Test Item. Dr. U. Noack Laboratorien, Sarstedt, Germany. Project No. 110817SH, Study No NAN15260 (unpublished), date: 2014-03-14	
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/list of approved active substances	
	2 GUIDELINES AND QUALITY ASSURANCE	
2.1 Guideline study	Yes, OECD Guideline for the Testing of Chemicals 308, Aerobic and Anaerobic Transformation in Aquatic Sediment Systems, April 2002.	
2.2 GLP	Yes	
2.3 Deviations	No	
	3 MATERIALS AND METHODS	
3.1 Test material	SONC969 Saltidin, [carboxyl^{14}C]-	
3.1.1 Lot/Batch number	Radio-labelled test substance: Batch number 198-190-0564-A-20120718-DRE Non-labelled test substance: Batch number CHCAEN0020	
3.1.2 Specification	Specific activity: 56.4 mCi/mmol	
3.1.3 Purity	Radiochemical purity was 99.7% Non-labelled test substance: Active ingredient content: 98.4%	
3.1.4 Further relevant properties	-	
3.1.5 Composition of Product	-	
3.1.6 TS inhibitory to micro-organisms	Not to be expected with reference to the activated sludge test with microorganisms.	
3.1.7 Specific chemical analysis	-	

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3.2 Reference substance	No reference item is recommended for this test.
3.2.1 Initial concentration of reference substance	-
3.3 Test solution	See table A7_1_2_2_2-3 and table A7_1_2_2_2-4
3.4 Testing procedure	
3.4.1 Test system	Sediments and their associated waters (field fresh samples) originated from the river 'Alte Leine'. The sampling site is classified as unpolluted. Samples were taken from the anaerobic zone of the sediment. The associated water was collected from the same site at the same time. A detailed description of the particle size distribution of the sediment is presented in table A7_1_2_2_2-1. Water as well as sediment parameter are summarised in in table A7_1_2_2_2-2. The sediment and the water phase were handled and transported carefully under exclusion of oxygen as far as possible. The sediment was separated from the water, manually cleared of large objects and then wet-sieved to a particle size of 2 mm. The water was purged with nitrogen to reduce the oxygen concentration as far as possible. The specified amounts of sediments were filled into the incubation flasks. Afterwards the required volume of water was filled into the incubation flasks under exclusion of oxygen and constant nitrogen stream. The flasks were closed gastight so that no gas exchange with the atmosphere was possible. The water/sediment samples were preincubated in the incubation vessels under test conditions for 28 days until stable anaerobic conditions were reached.
3.4.2 Test conditions	See table A7_1_2_2_2-3
3.4.3 Method of preparation of test solution	See table A7_1_2_2_2-3
3.4.4 Initial TS concentration	See table A7_1_2_2_2-3 Investigation of the stock solution revealed a discrepancy between the total radioactivity of the stock solution determined by LSC and the radioactivity related to discrete peaks in the LC-FSA analysis. The major part (68.8 %) of the radioactivity in the LC-FSA analysis was related to SONC969 Saltidin, [carboxyl-^{14}C]- and 1.6 % to ^{14}C-Saltidin acid (see table A7_1_2_2_2-4). Furthermore a few other decomposition products at a level of < 2% for a single substance (mostly even < 1%) were additionally present and representing 11.2 % of the radioactivity. In total approximately 81.6 % of the radioactivity measured by LSC were related to discrete peaks. In contrast the remaining radioactivity of approximately 18.4% was not indicated by discrete peaks in the corresponding chromatogram during LC-FSA analysis of the stock solution. It was suspected that autoradiolysis had influenced the radiochemical purity. Nevertheless autoradiolysis of the test item did not impair the interpretation of the results of the study because there were no peaks interfering the monitoring of the test item and Saltidin acid. Therefore a reliable determination of DT_{50} and DT_{90} values was possible.

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	The total activity of the stock solution as well as the activity related to SONC969 Saltidin, [carboxyl-^{14}C]- and ^{14}C-Saltidin acid was determined and these values were the reference basis for all further calculations related to the transformation of the test item.
3.4.5 Number of replicates	See table A7_1_2_2_2-3
3.4.6 Duration of test	See table A7_1_2_2_2-3
3.4.7 Sampling	See table A7_1_2_2_2-3
3.4.8 Analytical methods	See table A7_1_2_2_2-5
3.4.9 Intermediates/ degradation products	See table A7_1_2_2_2-5
3.4.10 Controls	See table A7_1_2_2_2-3
3.4.11 Statistics	The kinetic evaluations were done based on the FOCUS guidance document on estimating persistence and degradation kinetics (SANCO/10058/2005, version 2.0, June 2006: Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration).
	4 RESULTS
4.1 Degradation of test substance	
4.1.1 Mass balance	The mass balance, distribution of radioactivity, $^{14}CO_2$ production and non-extractable residues formation is summarised in table A7_1_2_2_2-6. The corresponding illustration is figure A7_1_2_2_2-1. The distribution of the applied radioactivity in the sediment is given in table A7_1_2_2_2-7. The distribution of the applied radioactivity in the water phase is given in table A7_1_2_2_2-8. Due to a decline of the mass balance below 90% AR towards test termination, follow-up investigations have been made regarding $^{14}CO_2$ being dissolved in the water phase or being associated with the sediment phase. The results of these investigations are summarised in table A7_1_2_2_2-9.
4.1.2 Transformation	The anaerobic transformation of SONC969 Saltidin, [carboxyl-^{14}C]- and ^{14}C-Saltidin acid in the total system, the water phase and the sediment phase is summarised in tables A7_1_2_2_2-10 to A7_1_2_2_2-12.
4.1.3 Kinetic analyses	The detailed results of the kinetic evaluations are given in table A7_1_2_2_2-13 and table A7_1_2_2_2-14. A graphical presentation of the kinetic analysis is given in figures A7_1_2_2_2-2 to A7_1_2_2_2-4.
4.1.4 Other observations	-
4.1.5 Degradation of reference substance	n.a.
4.1.6 Intermediates/ degradation products	Please refer to Points 4.1.2 and 4.1.3.

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5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The anaerobic transformation and mineralisation rate of SONC969 Saltidin, [carboxyl^{14}C]- has been tested in the water/sediment system 'Alte Leine' over a period of 103 days. Water/sediment samples have been treated with the test substance and incubated in closed biometer flasks in the dark under anaerobic conditions at approximately 20°C.
For the determination of the transformation rate the radio-labelled test substance SONC969 Saltidin [carboxyl-^{14}C]- was used. For the identification of the metabolites, the radio-labelled test substance as well as the non-radiolabelled test substance was used. Appropriate volumes of the stock solution containing the labelled test item and the stock solution containing the non-labelled test item were applied directly to the water phase of each replicate. The application was carried out under anaerobic conditions and a constant nitrogen flow.
Water and sediment sampling has been carried out directly after the application and at 6 additional sampling points. SONC969 Saltidin, [carboxyl-^{14}C]- in the water phase and sediment was determined by liquid scintillation counting (LSC) and HPLC coupled to a flow scintillation analyser (FSA). Prior to LSC analysis aliquots of sediment samples were combusted with an oxidizer. Prior to HPLC-FSA aliquots of wet sediment were extracted by a soxhlet extractor. The ethylene glycol traps for volatile compounds and the sodium hydroxide traps for carbon dioxide were analysed by LSC, only. ^{14}C-Methane was combusted to $^{14}CO_2$ in a nitrogen/oxygen stream and trapped in two further aqueous sodium hydroxide traps. The radioactivity of degradation products was analysed via HPLC-FSA on a reversed phase column in gradient mode. The structure of the only detectable degradation product during the course of the study, ^{14}C-Saltidin acid, was elucidated via LC-MS/MS.
The DT_{50} and DT_{90}, the disappearance time within the concentration is reduced by 50% and 90%, respectively, was calculated with a single first order model (SFO).

5.2 Results and discussion

A mean mass balance of 90 - 110% (as % of total applied radioactivity (AR)) was obtained until day 42. At day 77 and day 103 the mass balance decreased to 85.1% and 83.0%, respectively.

The decrease of the mass balance indicated, that $^{14}CO_2$ was lost during sampling and not completely captured with the standard trapping method. The LC-FSA chromatograms indicated that further $^{14}CO_2$ might be dissolved in the water phase and is associated with the sediment. To confirm this assumption two further replicates were sampled and purged with N_2 vigorously below the water surface for a prolonged period of 11 days (traps 1 and 2, see table A7_1_2_2_2-9). After that, the NaOH traps were removed and 2 fresh NaOH traps (traps 3 and 4) were connected to the test vessels. The water phase was acidified with 5 mL phosphoric acid (85%) and the replicates were purged with N_2 for two further days.

The subsequent LSC measurements of all NaOH traps confirmed the assumption that $^{14}CO_2$ was captured incompletely. Approximately 7%

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$^{14}CO_2$ and 11% $^{14}CO_2$, respectively were determined in the NaOH traps after prolonged and vigorous purging. Further 4% $^{14}CO_2$ and 7% $^{14}CO_2$ were trapped in the NaOH traps after acidification.

On average approximately 15% $^{14}CO_2$ were captured with the extended trapping method whereas at test end (day 103) with the standard purging method only approx. 4% $^{14}CO_2$ was captured. Hence, the follow-up investigations clearly indicate that the decrease of the mass balance towards the test end can be attributed to $^{14}CO_2$ either being dissolved in the water phase or being associated with the sediment. However, the modified $^{14}CO_2$ trapping method was not applicable for the standard samplings as during the vigorously purging the sediment was disturbed and mixed with the water phase. Furthermore the sediment became spongy and gas bubbles were escaping from the sediment directly after acidification.

Until day 13 10.3% of the total applied radioactivity (AR) diffused from the water phase into the sediment. The amount of AR in the sediment remained in the range of 10.3 – 11.1% until day 42. From day 42 until test end a slight increase was determined and at test end (day 103) 13.2% of total AR were determined in the sediment. The radioactivity was almost completely extractable from the sediment until day 42 (≤ 1.5% NER). From day 42 until day 100 the amount of NER increased slowly up to 4.8% of AR.

Simultaneously with an increase of sediment concentrations, the amount of AR in the water phase decreased. At test end 65.9% of total AR was determined in the water phase.

As summarised under point 3.4.4, investigations on the stock solution came to the result, that a certain amount of radioactivity, which was applied to the water/sediment systems, was not associated with the test item or its major metabolite. To get a better impression about the behaviour of the radioactivity not associated with SONC969 Saltidin, [carboxyl-^{14}C]- and ^{14}C-Saltidin acid the distribution of the radioactivity in the water phase was investigated in more detail. Therefore, the total radioactivity measured by LSC and the activity related to SONC969 Saltidin, [carboxyl-^{14}C]- and ^{14}C-Saltidin acid were compared and the residual activity was calculated from this difference.

The comparison showed, that the difference residual activity remained almost constant until day 4 (approx. 30% AR, which is in line with the radioactivity not related to the parent compound or its metabolite in the stock solution). Between day 4 and day 28 a decrease to approx. 15% AR was determined. As at the same time the concentration of ^{14}C-Saltidin acid remained constant, it can be concluded that the decrease is due to mineralisation of the residual radioactivity. In the further course of the study, the difference decreased only slightly and remained in the range of 9.8% – 15.6% AR.

As supplemental mass balance investigations revealed a fraction of $^{14}CO_2$ being present dissolved in the test systems (mean approximately 15% AR) it can be concluded, that the remaining difference is < 10% AR and can be associated with the typical fluctuations and analytical

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uncertainties of the LSC and LC-FSA measurements.

Hence, the evaluation of the transformation of SONC969 Saltidin, [carboxyl-^{14}C]- is not influenced by the residue activity.

Only traces of methane were determined at test end, the methane production remained < 0.05% of AR until test end. At test end in one replicate 0.08% methane was determined. No radioactivity (all samples < LOQ) was determined in the ethylene glycol traps, indicating that no volatile transformation products were formed.

The transformation of SONC969 Saltidin, [carboxyl-^{14}C]- and ^{14}C-Saltidin acid was determined by LC-FSA measurements of the water phase and the sediment extract. The radioactivity of SONC969 Saltidin, [carboxyl-^{14}C]- and ^{14}C-Saltidin acid measured in the stock solution for application by LC-FSA at test start was set as 100% (= % ARs), and all further calculations of % transformation of SONC969 Saltidin, [carboxyl-^{14}C]- and ^{14}C-Saltidin acid were based on this value.

SONC969 Saltidin, [carboxyl-^{14}C]- was transformed in the water phase until day 13. As main transformation product ^{14}C-Saltidin acid was determined. The maximum concentration of ^{14}C-Saltidin acid occurred on day 28 (91.8% ARs), and between day 28 and day 103 a slow decrease of ^{14}C-Saltidin acid was observed. At test end, ^{14}C-Saltidin acid accounted for 77.8% ARs. No further stable ^{14}C-metabolites were determined in the water phase.

In the sediment extract samples, SONC969 Saltidin, [carboxyl-^{14}C]- was transformed until day 28. As main transformation product ^{14}C-Saltidin acid was determined. From day 13 until day 103, the concentration of ^{14}C-Saltidin acid remained in the range of 11.3 – 13.4% ARs. The maximum concentration of ^{14}C-Saltidin acid in the sediment (13.4% ARs) was determined on day 77. At test end, ^{14}C-Saltidin acid in the sediment was 12.8% of AR. No significant decrease of ^{14}C-Saltidin acid was determined in the sediment. Furthermore no other stable ^{14}C-metabolites were determined in the sediment.

The transformation of SONC969 Saltidin, [carboxyl-^{14}C]- and ^{14}C-Saltidin acid in anaerobic water/sediment systems followed single first order (SFO) kinetics in the total system and the water phase of the test system ‘Alte Leine’. The transformation of SONC969 Saltidin, [carboxyl-^{14}C]- in the sediment followed SFO kinetics as well, but only at one sampling day concentrations > LOQ were determined and no transfer rate from water to sediment could be calculated. For ^{14}C-Saltidin acid no decrease in the sediment was determined within the testing period, therefore the DT_{50} and DT_{90} values were not calculable.

The kinetic evaluation revealed DT_{50} values for the parent compound between 2.5 and 2.9 days in the water and sediment phases, and the total system.

For Saltidin acid, the DT_{50} value in the water phase accounted for 463 days. Total system DT_{50} values for Saltidin acid amounted to 778 days.

5.3 Conclusion

The transformation rate of SONC969 Saltidin, [carboxyl-^{14}C]- was fast in the total system and the water phase. Within 13 days SONC969

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		Saltidin, [carboxyl-^{14}C]-was transformed to ^{14}C-Saltidin acid, with DT_{50} values of 2.9 days (total system), 2.8 days (water phase) and 2.5 days (sediment phase). The transformation of ^{14}C-Saltidin acid was progressing much slower. The DT_{50} values were 778 days (total system) and 463 days (water phase). Noteworthy degradation of ^{14}C-Saltidin acid in the sediment could not be observed. No volatile, organic transformation products were formed during the test duration. No further stable transformation products (> 1.5% AR) were determined in the water and sediment phase during the course of the study and at test end.
5.3.1	Reliability	1
5.3.2	Deficiencies	Due to autoradiolysis, ^{14}C activity in the stock solution was not only associated with the parent compound or its major metabolite (approximately 70% AR), but also with other decomposition products (approximately 11% AR with <2% AR for a single substance) or diffuse radioactivity (approximately 18% AR), which did not contribute to discrete peaks. This autoradiolysis of the test item did not impair the interpretation of the results of the study because there were no peaks interfering the monitoring of the test item and Saltidin acid. At day 77 and day 103 the mass balance decreased to 85.1% and 83.0% AR, respectively. However, follow-up investigations clearly revealed, that the decrease of the mass balance could be attributed to $^{14}CO_2$ dissolved in the water phase and associated with the sediment. This amount of CO_2 could not be captured with the standard trapping method and modifications to trap the $^{14}CO_2$ completely were not possible without impacting the sampling of water and sediment for LSC and LC-FSA analysis negatively. In summary, the reduction of the mass balance towards the end of the study does not have a negative influence on the outcome of the 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	*24 August 2015*
Materials and Methods	*Adopt applicant's version or include revised version.*
Results and discussion	*Adopt applicant's version*
Conclusion	*Adopt applicant's version.*
Reliability	*Based on the assessment of materials and methods include appropriate reliability indicator* *2*
Acceptability	Acceptable; however, the RMS is concerned about the problem identified especially the problem due to autoradiolysis, ^{14}C activity in the stock solution. Therefore the radioactivity was not only associated with the parent compound or its major metabolite, but also with other decomposition products or diffuse radioactivity, which did not contribute to discrete peaks. The only reason that the RMS accept this study anyway is because the follow-up investigations the applicant has made which revealed, that this autoradiolysis of the test item did not impair the interpretation of the results of the study significantly. At some sampling points, the mass balances were slightly below 90% AR . This is also a problem; however the RMS accept the explanation given by the applicant. Due to autoradiolysis, ^{14}C activity in the stock solution was not only associated with the parent compound or its major metabolite (approximately 70% AR), but also with other decomposition products (approximately 11% AR with <2% AR for a single substance) or diffuse radioactivity (approximately 18% AR), which did not contribute to discrete peaks. A mean mass balance of 90- 110% (as% of total applied radioactivity) was only obtained until day 42 (here it was 90.9%). At day 77 and day 103 the mass balance decreased to 85.1% and 83.0%, respectively. Follow-up investigations revealed, that the decrease of the mass balance could be attributed to $^{14}CO_2$ dissolved in the water phase and associated with the sediment. This amount of CO_2 could not be captured with the standard trapping method. There was only used one sediment (including the associated water). The OECD 308 recommends two sediments (one sediment with high organic carbon content and one with low carbon content. In summary is there are several problems with this study; however as this study is not a key study for the environmental risk assessment the RMS accept this study as a supporting study.
Remarks	
	COMMENTS FROM ...

Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7_1_2_2_2-1: Particle size distribution of the sediment for the test system 'Alte Leine'

Texture	Particle size [mm]	Proportion [%]
Sand	2.0 - 0.063	79.7
Silt	0.063 - 0.002	18.1
Clay	< 0.002	1.4

Table A7_1_2_2_2-2: Properties of the water/sediment system used

Parameter	Stage of test procedure			
	Field sampling / handling	Acclimation phase (day -28 / day -22 / day -2)	Test start (day 0)	Test end (day 103)
'Alte Leine' water				
Temperature [°C]	14.4	21.2 / 20.3 / -	-	-
pH-value	7.54	7.46 / 7.37 / -	7.91	7.64
TOC [mg C/L]	17.2	27.4 / 8.27 / -	6.44	9.51
O_2 concentration [mg O_2/L]	7.69	0.18 / 2.16 / -	<0.1	<0.1
Microbial biomass [CFU/L]	$8.0*10^5$	- / - / -	$3.9*10^4$	-
Redox potential [mV]	-	105.8 / 200.6 / -36.3	-126.8	-127.8
'Alte Leine' sediment				
pH-value	7.34	- / - / -	7.51	7.37
TOC [%]	1.3	- / - / -	1.1	1.1
Microbial biomass [CfU/g wet sediment]	$1.64 * 10^6$	- / - / -	$1.5*10^6$	$1.2*10^6$
Redox potential [mV]	-	-288.9 / -298.3 / -	-358.5	-327.5

Table A7_1_2_2_2-3: Testing procedure and test solutions used in the anaerobic water/sediment study

Parameter	Description
Temperature	Nominal: 20 ± 2 °C; Actual: 19.0 – 21.0 °C, short term deviations (< 12 h) to 22° C
Test duration	103 days
Light/dark cycle	Darkness
Stock solution	Stock solution of labelled test item Nominal: 55.5 MBq/35 mL Actual: 46.9 MBq/35 mL Stock solution of non-labelled test item 2.4 g/L in ultrapure water
Sediment/water ratio	1:3 Sediment: 120 g wet sediment per replicate corresponding to a sediment layer of 2.5 ± 0.5 cm and 75.69 g dry weight Water: 300 mL corresponding to a water column of 7.5 ± 0.5 cm
Replicates	Duplicates per sampling time for the determination of the transformation rate. 2 replicates for identification of metabolites. 4 control replicates for measuring water/sediment parameter and biomass.
Test concentration	Transformation rate: Nominal: 3.33 kBq/mL (1 MBq/Replicate) corresponding to 369 µg/L Actual: Total activity (=AR): 4.47 kBq/mL (1.34 MBq/replicate) Activity related to SONC969 Saltidin, [carboxyl-^{14}C]- and ^{14}C-Saltidin acid (=ARs): 3.15 kBq/mL (0.944 MBq/replicate) Identification of metabolites: Nominal: 6 mg/L, composed of 18.3 kBq/mL (corresponding to 5.5 MBq/replicate and 2 mg/L) ^{14}C-labelled test item and 4 mg/L non-labelled test item Actual: 6 mg/L, composed of 17.3 kBq/mL (corresponding to 5.19 MBq/replicate and 2 mg/L) ^{14}C-labelled test item and 4 mg/L non-labelled test item Solvent: ultrapure water
Application	Application was carried out under anaerobic conditions and a constant nitrogen flow. Transformation rate: Application of 1.0 mL stock solution directly to the water phase Identification of metabolites: Application of 5.502 mL stock solution (labelled test item) and 0.5 mL stock solution (non-labelled test item) directly to the water phase Nitrogen was passed through the top layer of the water phase to achieve a homogeneous distribution of the test item in the water phase directly after application. Care was taken to disturb the sediment as little as possible.
Test vessels	Gastight 500 mL incubation flasks. The flasks were connected with the gas trapping system prior to sampling.
Ethylene glycol trap	Crimped headspace bottle containing 50 mL ethylene glycol

Table A7_1_2_2_2-3 cont.: Testing procedure and test solutions used in the anaerobic water/sediment study

Parameter	Description
$^{14}CO_2$ trap	Two crimped headspace bottles containing 50 mL 1mol/L aqueous sodium hydroxide.
^{14}C-Methane	^{14}C-Methane was combusted to $^{14}CO_2$ in a nitrogen/oxygen stream and trapped in two further aqueous sodium hydroxide traps. The combustion system for ^{14}C-methane consisted of a tube furnace for oxidation of methane to CO_2. Inside of the furnace, a glass tube filled with copper oxide as catalyst was placed. The combustion system was connected on one side to the outlet of the last $^{14}CO_2$ trap and on the other side to crimped bottles containing 50 mL 1 mol/L aqueous sodium hydroxide for trapping the $^{14}CO_2$ coming from the furnace.
Anaerobic conditions	The test vessels were purged with nitrogen during application of the test item and sealed gastight afterwards. 3-5 days prior to sampling the headspace gas was purged through the gas trapping system with nitrogen. Disturbance of the sediment was avoided as far as possible. The purging time depended on the amount of $^{14}CO_2$ and ^{14}C-methane produced.
Sampling	Sampling for the determination of the transformation rate: 0, 4, 13, 28, 42, 77, and 103 days of exposure. Two test item replicates were sacrificed at each sampling time. At least 3 days prior to sampling the replicates were connected to the gas trapping system and the headspace gas was purged with nitrogen through the gas trapping system. Produced ^{14}C-methane was purged with nitrogen and combusted in a nitrogen/oxygen stream. The newly formed $^{14}CO_2$ was trapped and analysed. Residual volatile ^{14}C and $^{14}CO_2$ in the traps was determined by LSC. Afterwards the water phase was carefully decanted to avoid disturbances of the sediment and the sediment and water were analysed separately. The sediment was homogenised by thorough stirring with a spatula. Two sub-samples of the water phase and 5 sub-samples of the sediment of each replicate were analysed.

Table A7_1_2_2_2-4: Activity of the stock solution for application

Analytical method	Parameter	Radioactivity (Bq/mL)	% of total activity
LSC	Total	5360	100
FSA	SONC969 Saltidin, [carboxyl-^{14}C]-	3686	68.8
	^{14}C-Saltidin acid	88	1.6
Remaining Activity		1586	29.6
Related to peaks		599	11.2
Not related to discrete peaks		987	18.4

Table A7_1_2_2_2-5: Analytical methods used in the anaerobic water/sediment study

Parameter	Description
Determination of radioactivity by Liquid Scintillation Counter Analysis (LSC)	
Parameter	Radioactivity of the water phase, the sediment after combustion, the sodium hydroxide traps, the ethylene glycol traps, the sediment extracts, the extracted sediment (determination of non-extractable residues (NER))
Equipment	- LSC Counter : TRICARB 2100 TR, CANBERRA-PACKARD - Software : Ver. 1.05, PACKARD - Oxidizer : Model 307, PACKARD (PERKINELMER)
Reagents	LSC-Cocktail, UltimaGold XR, PERKIN ELMER Carbon dioxide absorber, Carbosorb E, PERKIN-ELMER LSC-Cocktail (for Carbosorb E), Permafluor E+, PERKIN-ELMER LSC-Cocktail for carbon dioxide traps, Hionic Fluor, PERKIN-ELMER
Counting Parameter	Counting Type: DPM (disintegrations per minute) Counting terminator: Until 2 x standard deviation of the counted disintegrations is < 0.5 %, but max. 20 min Lower energy level: 0 keV Upper energy level: 156 keV Quench indication parameter: tSIE (transformed spectral index of the external standard ^{133}Ba)
Quench Correction	A general quench curve of the analytical system was used to compensate for a decreased counting efficiency due to chemical or color quench in the different media. The extent of quench in the samples was described by the transformed spectral index of the external (tSIE) ^{133}Ba standard. The determined tSIE of a sample correlates with a counting efficiency.
Preparation of samples	
Water	0.3 mL of water (or 0.05 mL in case of samples for metabolite identification) were mixed with 10 mL of UltimaGold XR in a LSC-vial and measured with LSC.
Sediment	The radioactivity in sediment samples was determined via LSC after combustion with a sample oxidiser. 0.7 g wet sediment were directly weighed in a combusto cone interlocked with 3 combusto pads followed by combustion for 5 min. using the sample oxidizer. The produced CO_2 was trapped in 10 mL of Carbosorb E, mixed with 10 mL Permafluor E+ and measured by LSC.
Sediment extracts	100 µL of the sediment extracts after extraction (see below) were mixed with 10 mL of UltimaGold XR and analysed via LSC.
Carbon dioxide traps	1.0 to 3 mL of the sodium hydroxide traps were mixed with 15 mL Hionic-Fluor in a LSC-vial and measured with LSC.
Ethylene glycol traps	2 mL of the ethylene glycol trap were mixed with 8 mL of HPLC-water in a LSC-vial followed by addition of 10 mL UltimaGold XR.
Non Extractable Residues	0.2 g of the air dried extracted sediments were weighted in one combusto cone containing 3 combusto pads followed by moistening with 0.4 mL HPLC water. These samples were treated as described before for the unextracted sediment samples (see sediment radioactivity).
Method validation	Limit of Detection (LOD): ≤ 1 % AR for all media Limit of Quantification of the analytical method (LOQ_M): Water: 0.04% AR; Ethylene glycol traps: 0.03% AR; Sodium hydroxide traps: 0.02% AR; Sediments: 0.3% AR
Accuracy	The analytical methods for all media were validated on two fortification levels (1x and 10xLOQ_M). The mean recoveries at each fortification level were in the range of 95 and 105 % (please refer to Doc. IIIA, 7.1.2.2.2(01)).

Precision	Relative standard deviations at each fortification level were lower than 5% (please refer to Doc. IIIA, 7.1.2.2.2(01)).

Table A7_1_2_2_2-5 cont.: Analytical methods used in the anaerobic water/sediment study

Parameter	Description
Flow Scintillation analysis coupled with HPLC (HPLC-FSA)	
Parameter	Analysis of SONC969 Saltidin, [carboxyl-^{14}C]- in water and sediment extracts
Equipment	HPLC: 2695 Alliance separation module, WATERS Detector: 500TR FSA, PERKIN-ELMER Software: FlowOne, v3.65, PERKIN-ELMER Software LC: Mass Lynx^{TM} 4.1, WATERS
Reagents	ULTIMA-FLO^{TM} M (LSC-cocktail for Radio-HPLC), PERKIN-ELMER Disposable syringe filter: Chromafil RC-45/15 MS, MACHERY-NAGEL High DPM Spec-Chec-^{14}C, Part Number 6002135, 8.26*10^{5} dpm / mL, PERKIN-ELMER
Efficiency standard	Spec-Chec-^{14}C was used with known activity.
Conditions of Analysis	Column: Discovery C18 5 µm, 250 x 4.6 mm, Batch 133820-01, SUPELCO Temperature: 25°C Mobile phase: A : 0.005 mol/L trifluoroacetic acid in HPLC water B : 0.005 mol/L trifluoroacetic acid in acetonitrile Gradient mode FSA Cell type, liquid, 500 µL Radio update 4 s Nuclide ^{14}C (LLD = 0 keV, ULD 156 keV) HPLC flow rate 1.0 mL / min LS flow rate 2.4 mL / min LS / HPLC ratio 2.4 : 1
Preparation of samples	
Water	0.9 mL of water was stabilized with 0.1 mL of ethanol prior to analysis. Samples for metabolite identification: 0.2 mL water was diluted with 0.8 mL HPLC water. Then 0.9 mL of this dilution was stabilized with 0.1 mL of ethanol prior to analysis.
Sediment	25 g wet sediment were extracted in a soxhlet extractor with refluxing acetonitrile for 8 h. The extract was evaporated to dryness using a rotary evaporator. The residue was dissolved in 5 mL of a 1:1 mixture of ethanol and HPLC water and filtered over a disposable syringe filter (Chromafil RC-45/15 MS) prior to analysis.
Method validation	Limit of Detection (LOD): Signal-noise ratio of 3,l corresponding to 0.1% AR for sediment and 1.5% for water Limit of Quantification of the analytical method (LOQ_M): Water: 4.5% AR; Sediment: 0.3% AR
Accuracy	Water: The analytical methods for all media were validated on two fortification levels (1x and 10xLOQ_M). The mean recoveries at each fortification level were in the range of 90 and 110 % (please refer to Doc. IIIA, 7.1.2.2.2(01)). Sediment: Quality controls were treated as the sediment samples from the definitive test at every sampling time. Quality controls were prepared by spiking an equivalent amount of wet sediment with SONC969 Saltidin, [carboxyl-^{14}C]-, corresponding to 2.7% AR. The mean recovery was between 90 and 110%.
Precision	Relative standard deviations at each fortification level were lower than 10%.

Table A7_1_2_2_2-5 cont.: Analytical methods used in the anaerobic water/sediment study

Parameter	Description
Structure elucidation via combination of LC-MS and LC-MS/MS	
Parameter	The structure of the only detectable degradation product during the course of the study, ^{14}C-Saltidin acid, was elucidated via LC-MS/MS during the corresponding aerobic water/sediment study with the test item (please refer to Doc. IIIA, 7.1.2.2.2(01)).
Equipment	Please refer to Doc. IIIA, 7.1.2.2.2(01)
Conditions of Analysis	Please refer to Doc. IIIA, 7.1.2.2.2(01)
Preparation of samples	Please refer to Doc. IIIA, 7.1.2.2.2(01)
Conditions of detection	Please refer to Doc. IIIA, 7.1.2.2.2(01)

Table A7_1_2_2_2-6: Mass balance of the anaerobic water/sediment test (system 'Alte Leine')

Exposure Day	Repl.	Water % of AR	mv	Sediment % of AR	mv	$^{14}CO_2$ % of AR	mv	Mass Balance % of AR	mv
0	1	100.9	**100.6**	0.5	**0.4**	-	**-**	101.4	**101.0**
	2	100.3		0.3		-		100.6	
4	1	89.8	**89.5**	8.5	**9.3**	-	**-**	98.3	**98.8**
	2	89.2		10.0		-		99.2	
13	1	83.4	**83.9**	9.9	**10.3**	1.55	**1.61**	94.9	**95.8**
	2	84.4		10.7		1.68		96.8	
28	1	80.5	**80.0**	10.6	**10.6**	2.72	**1.97**	93.8	**92.5**
	2	79.5		10.5		1.23		91.2	
42	1	77.0	**77.0**	11.3	**11.1**	3.19	**2.87**	91.5	**90.9**
	2	76.9		10.8		2.56		90.3	
77	1	69.1	**69.6**	12.6	**12.2**	3.63	**3.32**	85.4	**85.1**
	2	70.0		11.8		3.02		84.8	
103	1	66.4	**65.9**	13.5	**13.2**	4.16	**3.90**	84.1	**83.0**
	2	65.4		12.9		3.64		82.0	

AR = Applied Radioactivity
- = not determined mv = mean values

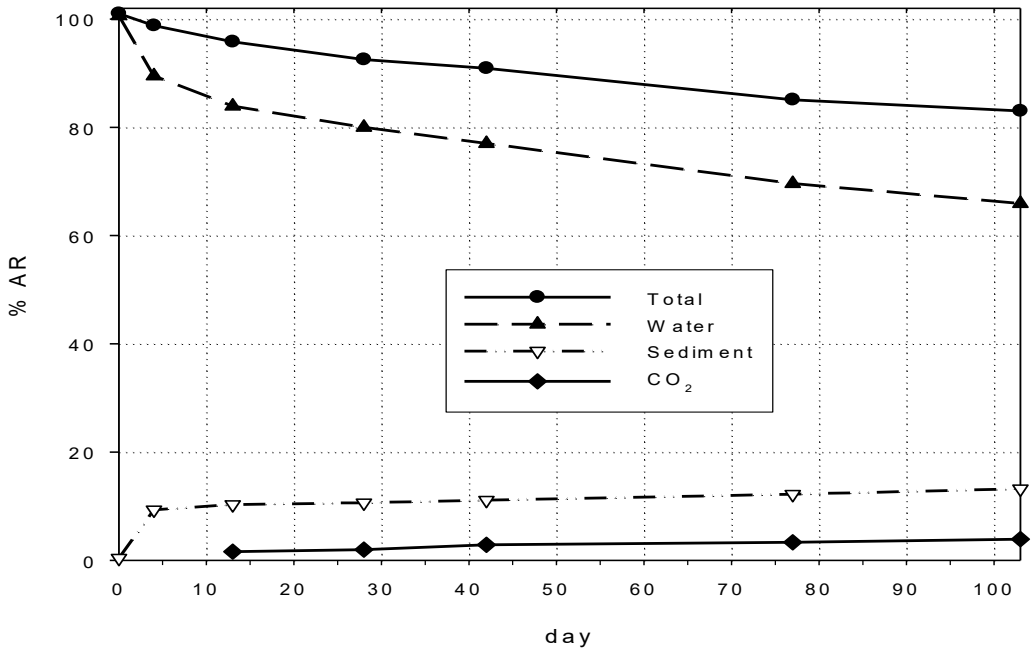

Figure A7_1_2_2_2-1: 'ALTE LEINE': Distribution of applied radioactivity

Table A7_1_2_2_2-7: 'Alte Leine': Distribution of AR in the sediment

Exposure Day	Repl.	Sediment % of AR	mv	Sediment Extract % of AR	mv	Extracted Sediment (NER) % of AR	mv
4	1 2	8.5 10.0	**9.3**	7.9 11.3	**9.6**	0.2 0.1	**0.2**
13	1 2	9.9 10.7	**10.3**	9.5 10.6	**10.0**	0.4 0.5	**0.4**
28	1 2	10.6 10.5	**10.6**	9.7 9.7	**9.7**	0.8 1.1	**1.0**
42	1 2	11.3 10.8	**11.1**	9.4 9.3	**9.4**	1.6 1.5	**1.5**
77	1 2	12.6 11.8	**12.2**	8.5 7.9	**8.2**	3.7 3.2	**3.4**
103	1 2	13.5 12.9	**13.2**	10.2 7.9	**9.1**	4.3 5.2	**4.8**

AR = Applied Radioactivity, mv = mean values

Table A7_1_2_2_2-8: 'Alte Leine': Distribution of AR in the water phase

Exposure Day	Repl.	Total Activity % of AR	mv	Activity of Test Item and Metabolite % of AR	mv	Residue Activity % of AR	mv
0	1 2	100.9 100.3	**100.6**	71.9 70.1	**71.0**	29.0 30.2	**29.6**
4	1 2	89.8 89.2	**89.5**	58.7 62.8	**60.7**	31.1 26.4	**28.8**
13	1 2	83.4 84.4	**83.9**	62.0 64.0	**63.0**	21.4 20.4	**20.9**
28	1 2	80.5 79.5	**80.0**	64.2 65.2	**64.7**	16.3 14.3	**15.3**
42	1 2	77.0 76.9	**77.0**	61.6 61.1	**61.4**	15.4 15.8	**15.6**
77	1 2	69.1 70.0	**69.6**	59.9 59.7	**59.8**	9.2 10.3	**9.8**
103	1 2	66.4 65.4	**65.9**	55.9 53.6	**54.8**	10.5 11.8	**11.1**

AR = Applied Radioactivity; mv = mean values

Table A7_1_2_2_2-9: Course of $^{14}CO_2$ formation

Trap	%CO_2 Formation	
	Replicate 1	Replicate 2
1	6.8	10.1
2	0.6	1.3
3	**3.4**	**6.1**
4	**0.6**	**0.9**
Sum	11.4	18.4

Bold = $^{14}CO_2$ sampled after acidification

Table A7_1_2_2_2-10: 'Alte Leine': Anaerobic transformation of SONC969 Saltidin, [carboxyl-^{14}C]- and ^{14}C-Saltidin acid in the total system

Exposure Day		Total System (Water & Sediment)			
		SONC969 Saltidin, [carboxyl-^{14}C]- % of ARs		^{14}C- Saltidin acid % of ARs	
	Repl.		mv		mv
0	1 2	**98.6** **97.8**	**98.2**	3.6 1.8	2.7
4	1 2	**36.8** **39.5**	**38.2**	**55.6** **62.4**	**59.0**
13	1 2	**1.0** 1.9	1.4	**100.9** **102.1**	**101.5**
28	1 2	*0.1* *0.1*	*0.1*	**102.7** **104.5**	**103.6**
42	1 2	< LOD < LOD	< LOD	**99.2** **97.6**	**98.4**
77	1 2	< LOD < LOD	< LOD	**98.1** **98.6**	**98.4**
103	1 2	< LOD 0.2	*0.1*	**93.6** **87.6**	**90.6**

AR_S = Applied Radioactivity related to SONC969 Saltidin, [carboxyl-^{14}C]- and ^{14}C-Saltidin acid
n.a. = not applicable; mv = mean values

Assignment of character style:

bold = values > LOQ, normal = values < LOQ, *italics* = values < LOD, calculated with ½ LOD

Table A7_1_2_2_2-11: 'Alte Leine': Anaerobic transformation of SONC969 Saltidin, [carboxyl-^{14}C]- and ^{14}C-Saltidin acid in the water phase

Exposure Day	Repl.	Water Phase: SONC969 Saltidin, [carboxyl-^{14}C]- % of ARs	mv	Water Phase: ^{14}C- Saltidin acid % of ARs	mv
0	1	**98.6**	**98.2**	3.6	2.7
	2	**97.8**		1.8	
4	1	**32.4**	**32.5**	**51.0**	**53.7**
	2	**32.7**		**56.5**	
13	1	*0.8*	1.0	**87.3**	**88.4**
	2	*1.2*[1)]		**89.6**	
28	1	< LOD	< LOD	**91.1**	**91.8**
	2	< LOD		**92.6**	
42	1	< LOD	< LOD	**87.5**	**87.1**
	2	< LOD		**86.8**	
77	1	< LOD	< LOD	**85.1**	**84.9**
	2	< LOD		**84.8**	
103	1	< LOD	< LOD	**79.4**	**77.8**
	2	< LOD		**76.1**	

ARs = Applied Radioactivity related to SONC969 Saltidin, [carboxyl-^{14}C]- and ^{14}C-Saltidin acid
n.a. = not applicable; mv = mean value

Assignment of character style:

bold = values > LOQ, normal = values < LOQ, *italics* = values < LOD, calculated with ½ LOD

1) one subsample slightly > LOD

Table A7_1_2_2_2-12: 'Alte Leine': Anaerobic transformation of SONC969 Saltidin, [carboxyl-^{14}C]- and ^{14}C-Saltidin acid in the sediment

Exposure Day	Repl.	Sediment: SONC969 Saltidin, [carboxyl-^{14}C]- % of ARs	mv	Sediment: ^{14}C- Saltidin acid % of ARs	mv
0	1 2	n.a. n.a.	n.a.	n.a. n.a.	n.a.
4	1 2	**4.4** **6.8**	**5.6**	**4.7** **5.9**	**5.3**
13	1 2	0.23 0.63	0.4	**13.6** **12.5**	**13.0**
28	1 2	*0.05* *0.05*	*0.05*	**11.6** **11.9**	**11.7**
42	1 2	< LOD < LOD	< LOD	**11.7** **10.8**	**11.3**
77	1 2	< LOD < LOD	< LOD	**13.0** **13.8**	**13.4**
103	1 2	< LOD 0.24	< LOD	**14.2** **11.5**	**12.8**

AR_S = Applied Radioactivity related to SONC969 Saltidin, [carboxyl-^{14}C]- and ^{14}C-Saltidin acid
n.a. = not applicable; mv = mean value

Assignment of character style:

bold = values > LOQ, normal = values < LOQ, *italics* = values < LOD, calculated with ½ LOD

Table A7_1_2_2_2-13: Kinetic data for the test system 'Alte Leine' (total water and sediment as well as only water)

Endpoint / Statistic	System Compartment	
	Total (Water & Sediment)	**Water**
Model	**Single First Order (SFO)**	
C_0 (% of AR) *Initial value for fitting*	**102.3** ± 1.33 *100*	**96.9** ± 2.12 *100*
K_P (1/d) *Initial value for fitting*	**0.2392** ± 0.0097 *0.2*	**0.2491** ± 0.0121 *0.2*
ffM (as a fraction) *Initial value for fitting*	1 *1*	**0.955** ± 0.0298 *1*
K_m (1/d) *Initial value for fitting*	**0.00089** ± 0.00026 *0.001*	**0.00150** ± 0.00035 *0.001*
Data range (days)	0 – 103	0 – 103
χ^2 error SONC969 Saltidin, [carboxyl-^{14}C]-	8.0	5.2
t-Test (P=0.05)	Passed	Passed
χ^2 error ^{14}C-Saltindin acid	3.0	2.8
t-Test (P=0.05)	Passed	Passed
	DT_X values in days	
DT_{50} SONC969 Saltidin, [carboxyl-^{14}C]-	**2.9**	**2.8**
DT_{90} SONC969 Saltidin, [carboxyl-^{14}C]-	**9.7**	**9.2**
DT_{50} ^{14}C-Saltidin acid	**778**	**463**
DT_{90} ^{14}C-Saltidin acid	**2584**	**1537**

Table A7_1_2_2_2-14: Kinetic data for the test system 'Alte Leine' (sediment)

Endpoint / Statistic	System Compartment Sediment
SONC969 Saltidin, [carboxyl-14C]-	
Model	**Single First Order (SFO)**
C_0 (% of AR) *Initial value for fitting*	**5.60** ± 0.3887 *7*
K_P (1/d) *Initial value for fitting*	**0.281** ± 0.1003 *0.3*
Data range (days)	4 - 103
χ^2 error	4.0
t-Test (P=0.05)	Passed
DT_X values in days	
DT_{50}	**2.5**
DT_{90}	**8.2**

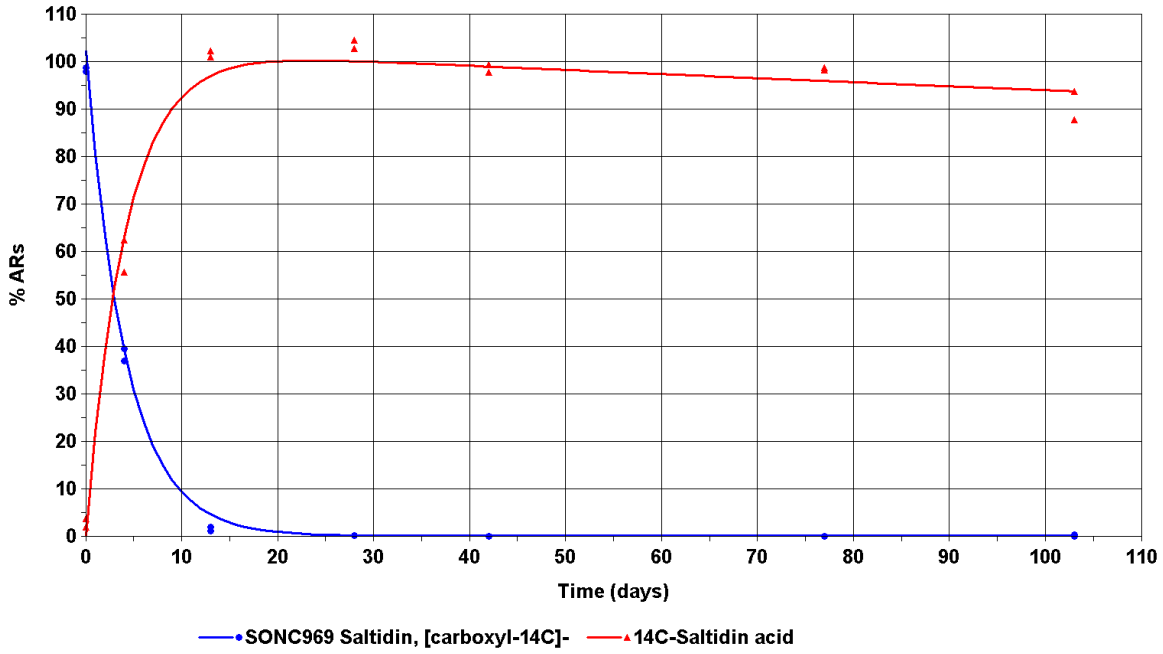

Figure A7_1_2_2_2-2: 'Alte Leine': Kinetic fit for SONC969 Saltidin, [carboxyl-^{14}C]- and ^{14}C-Saltidin acid in total system (water & sediment)

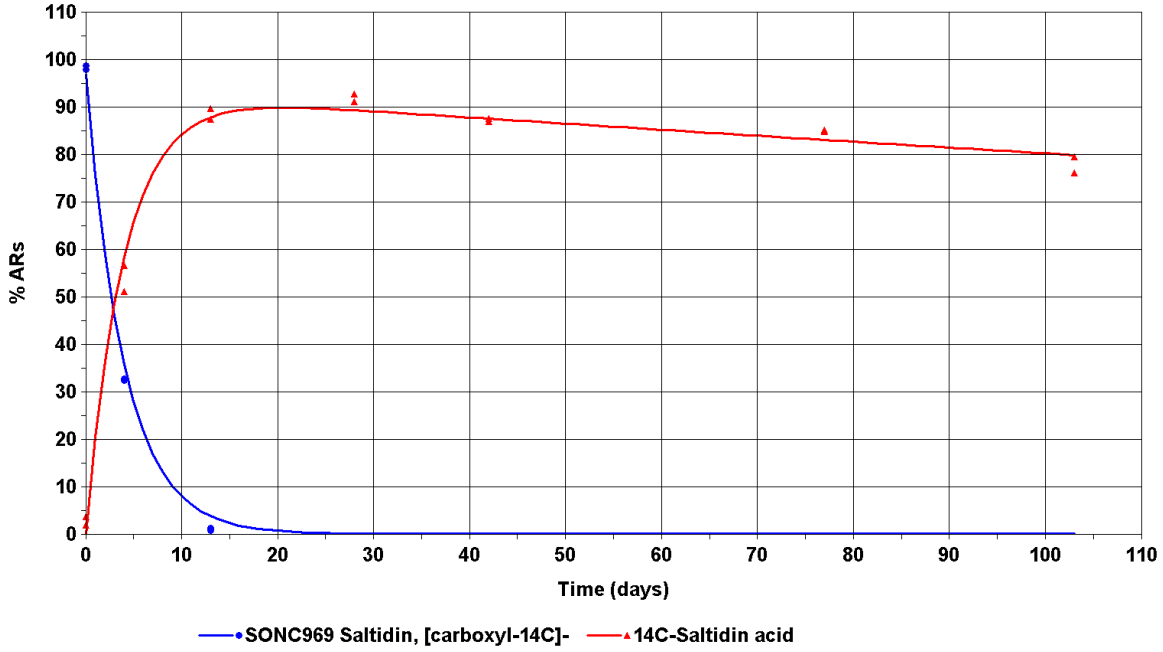

Figure A7_1_2_2_2-3: 'Alte Leine': Kinetic fit for SONC969 Saltidin, [carboxyl-^{14}C]- and ^{14}C-Saltidin acid in the water column

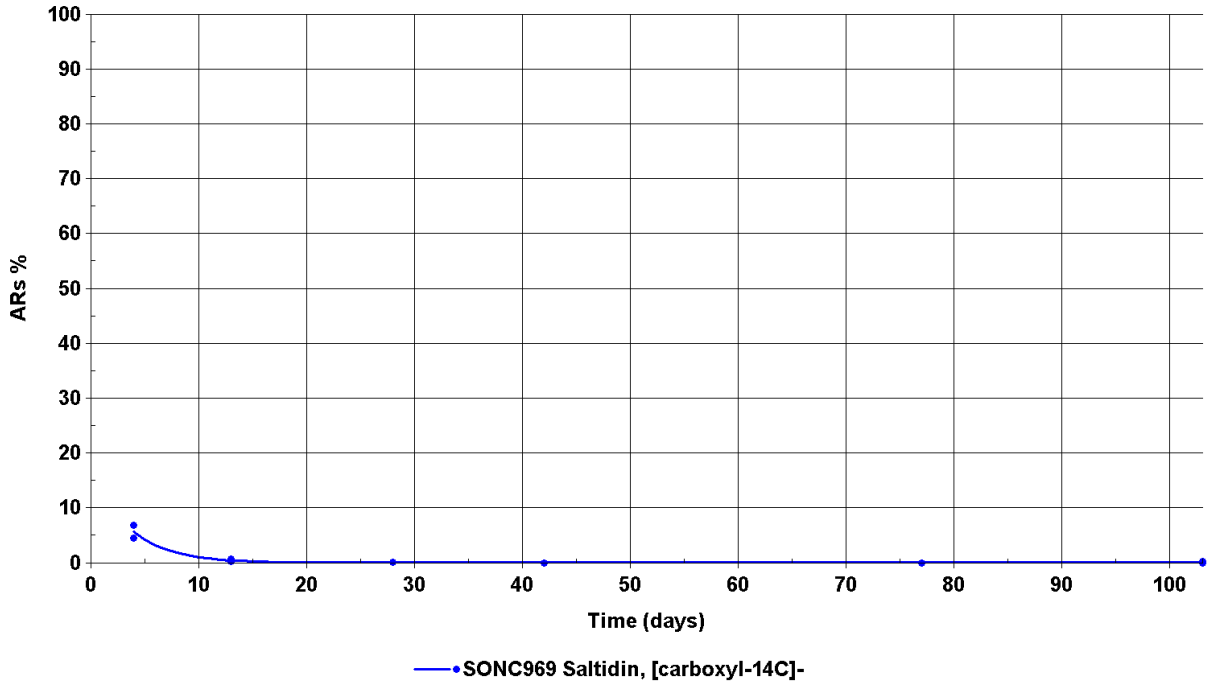

Figure A7_1_2_2_2-4: 'Alte Leine': Kinetic fit for SONC969 Saltidin, [carboxyl-^{14}C]- in sediment

Section A7.1.3 Adsorption / Desorption screening test

Annex Point IIA.7.7

			Official use only
		1 REFERENCE	
1.1	**Reference**	Jungheim (2001): Bayrepel – Adsorption//Desorption. Bayer AG, ZF-Zentrale Analytik, Leverkusen, Germany, Report No. N-01/0026/00 LEV, Date: 2001-04-02.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Lanxess Deutschland 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**	Yes OECD Guideline 121 (Proposal for a New Guideline 121, January 2001)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Icaridin (Bayrepel)	
3.1.1	Lot/Batch number	898711001, Source: Bayer AG, PF-CAE, Monheim	
3.1.2	Specification	As given in section 2 of the dossier	
3.1.3	Purity	Not given in report	
3.1.4	Further relevant properties	Water solubility of Icaridin: about 8.2 g/l (Krohn, 1996)	
3.1.5	Method of analysis	HPLC, fitted with a pulse-free pump and a suitable detection device according to OECD Guideline 121. Column type: Length 250 mm, inner diameter 4 mm, Stationary phase: LiChronopher 100 CN, particle diameter 5 µm; Mobile phase: 500 ml acetonitrile : 85 ml buffer solution pH 6 : 415 ml water; Flow rate: 0.7 ml, column temperature: 40 °C, injection volume: 10 µl; Detection: UV, 220 nm pH determination: pH meter with a calibrated single-rod glass electrode	
3.2	**Degradation products**	Not relevant	
3.2.1	Method of analysis for degradation products	-	

Section A7.1.3 Adsorption / Desorption screening test

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3.3	**Reference substance**	Yes, six reference compounds were used to determine an average capacity factor k': 2-nitrobenzamide, 3-nitrobenzamide, N,N-dimethylbenzamide, methylbenzoate, naphthalene, 1,2,3-trichlorobenzene. Sodium nitrate was used to determine the HPLC dead time (t_0).
3.3.1	Method of analysis for reference substance	HPLC, See point 3.1.5 for detailed description of analytical method
3.4	**Testing procedure**	
3.4.1	Test system	HPLC with UV detection. Chromatographic conditions: See point 3.1.5 As a result of partitioning between mobile and stationary phases the test substance is retarded. The dual composition of the stationary phase having polar and non-polar sites allows for interaction of a molecule in the similar way as in the case for organic matter in soil. This enables the relationship between the retention time on the column and the adsorption coefficient on organic matter to be established.
3.4.2	Test solution and Test conditions	According to guideline, maximum concentration of the test substance should not exceed 50% of the solubility in the solvent. Therefore the measurements were carried out at concentrations of approx. 2 %. In compliance with the guideline and considering environmental relevance, pH 6 was chosen as pH value for the buffer solution. The pH of a 2 % solution of Icaridin (in 0.1 % aqueous NaCl) is 8.9. Therefore the pH value for the non-ionic form of the test substance is outside the field of application for the pH value of the column, which is pH 4.0-7.5. For this reason a test with the non-ionic form of the test substance could not be performed. The reference Koc values in the OECD guideline presumply refer to 25 °C. However, the column temperature was 40 °C; this temperature is without influence on the result of the determination. The calibration was done referring to Koc values of reference substances determined at 25 °C. HPLC parameters: See point 3.1.5.
3.5	**Calculations**	**Kd:** Distribution coefficient is defined as the ratio of equilibration concentrations C of a dissolved test substance in a two phase system consisting of a sorbent (soil or sewage sludge) and an aqueous phase. It can be dimensionless or have the dimension ml/g. **Koc:** Distribution coefficient (Kd) or Freundlich adsorption coeffcient (Kf) normalised to the organic carbon content (foc) of a sorbent. Depending on the dimensions of Kd and Kf, Koc can be dimensionless or have the dimensions ml/g or µg/g organic matter, respectively. Using the HPLC estimation method Koc is deduced from the capacity factor (k') using a calibration plot of log k' versus log Koc of the selected reference compounds. Koc is an indicator for the extension of adsorption between a substance and the sorbent and allows comparisons to be made between different chemicals. **k':** Capacity factor = $(t_R - t_0)/t_0$; t_R = HPLC retention time of test and

Section A7.1.3 Adsorption / Desorption screening test

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reference substances (min); t_0 = HPLC dead time (min).
log Koc = Slope x log k' + Intercept; Slope and intercept derived from the linear regression of the reference standards using Koc.

4 RESULTS

4.1	**Measurements**	HPLC retention time data for the reference compounds and Icaridin (Bayrepel) are given in Table A7_1_3-1. The dead time t_0 was determined to be 2.429 min using sodium nitrate.
4.2	**Calculations**	Calculated adsorption parameter for the reference compounds and Icaridin (Bayrepel) are given in Table A7_1_3-1. Regression plot (log k' versus log Koc) available in original report (page 7).
4.3	**Degradation product(s)**	Not relevant

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The adsorption koefficient Koc of Icaridin (Bayrepel) on soil was estimated using High Performance Liquid Chromatography (HPLC). The test was performed according to OECD Guideline 121 (Proposal for New Guideline, 2001). Six reference standards of known Koc were analysed on a HPLC system to determine an average capacity factor k'. Sodium nitrate was used to determine the HPLC system dead time (t_0). A regression line was plotted with the determined k' values and the known Koc values (log k' versus log Koc).
5.2	**Results and discussion**	Icaridin (Bayrepel) was analysed on the same HPLC system during the same sample sequence as the reference substances and an average k' value of 0.912 was determined. The Koc value for Icaridin (Bayrepel) was estimated by interpolation from the reference substance regression line. The linear regression of measured k' values against literature Koc values yielded a line with a slope of 0.264, an intercept of -0.548 and a correlation coefficient R^2 of 0.908. The estimated Koc value for Icaridin is 85.1, whereas log Koc amounts to 1.93.
5.3	**Conclusion**	Based on classifications of Briggs (Proc. 7th British Insecticide and Fungicide Conference, Nottingham, UK, 83-86, 1973) and Verdam et al. (RIVM Report No. 728473001, NL, 1988) for the estimation of the mobility of plant protectants in soil based on Kd and/or Koc-values, Icaridin (Bayrepel) is to be classified as a substance with intermediate mobility.
5.3.1	Reliability	2
5.3.2	Deficiencies	Purity of test substance not reported. The reference Koc values in the OECD guideline presumply refer to 25 °C. However, the column temperature was 40 °C; this temperature is without influence on the result of the determination.

Section A7.1.3 Adsorption / Desorption screening test

Annex Point IIA.7.7

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 2007
Materials and Methods	The adsorption coefficient Koc of Icaridin (Bayrepel) on soil was estimated using High Performance Liquid Chromatography (HPLC). The test was performed according to OECD Guideline 121 (Proposal for New Guideline, 2001). Six reference standards of known Koc were analysed on a HPLC system to determine an average capacity factor k'. Sodium nitrate was used to determine the HPLC system dead time (t_0). A regression line was plotted with the determined k' values and the known Koc values (log k' versus log Koc).
Results and discussion	Icaridin (Bayrepel) was analysed on the same HPLC system during the same sample sequence as the reference substances and an average k' value of 0.912 was determined. The Koc value for Icaridin (Bayrepel) was estimated by interpolation from the reference substance regression line. The linear regression of measured k' values against literature Koc values yielded a line with a slope of 0.264, an intercept of -0.548 and a correlation coefficient R^2 of 0.908. The estimated Koc value for Icaridin is 85.1, whereas log Koc amounts to 1.93.
Conclusion	The Koc of Icaridin (Bayrepel) was in an OECD 121 test determined as Koc = 85.1 (log Koc = 1.93).
Reliability	Based on the assessment of the study a reliability indicator of 2 is considered appropriate for the study
Acceptability	The indications are that the study has been performed according to the guideline and that the major validity criteria of the study can be considered as fulfilled. The study is thus considered acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7_1_3-1: HPLC retention time data and Koc calculations

Substance	Mean Retention Time [min]	MeanDead Time [min]	Capacity factor (k')	log k'	log Koc
Sodium nitrate	-	2.429	-	-	-
2-Nitrobenzamide	3.971	-	0.635	-0.197	1.45
3-Nitrobenzamide	4.092	-	0.685	-0.164	1.95
N,N-dimethylbenzamide	4.220	-	0.737	-0.133	1.52
Methylbenzoate	5.141	-	1.116	0.048	1.80
Naphthalene	6.511	-	1.680	0.225	2.75
1,2,3-Trichlorobenzene	6.852	-	1.821	0.260	3.16
Icaridin (Bayrepel)	**4.646**	-	**0.912**	**-0.040**	**1.93**

Section 7.1.4.1 Annex Point IIIA 12.2	Field study on accumulation in the sediment	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [...]**	**Technically not feasible [ ] Scientifically unjustified [ ]** **Other justification [X].**	
Detailed justification:	A specific field study on accumulation in sediment was not performed, because this is not a data requirement. In addition sediment is not the compartment at risk for Icaridin according to the risk assessment.	
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	April 2007	
Evaluation of applicant's justification	Applicant's justification is OK, based on the adsorption/desorption properties	
Conclusion	Applicant's justification is acceptable	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section 7.2 Annex Point IIIA 12.2.2	Fate and behaviour in soil	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [...] **Limited exposure [X]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	Further studies on the degradation, adsorption and mobility of Icaridin in soils were not performed, because the soil is not a compartment at risk for the compound. According to the risk assessment Icaridin is mainly released to the environment via STPs. Soils could theoretically be exposed to Icaridin residues by an application of sewage sludge or by dry and wet deposition from the atmosphere. Since Icaridin is predominantly present in the water phase of a STP, the exposure route via sewage sludge treatment is of no concern. In addition, the short atmospheric half-life of Icaridin prevents the compound to be deposed to soils. Further investigations on the fate of Icaridin in the soil compartment are 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	April 2007	
Evaluation of applicant's justification	Applicant's justification is OK	
Conclusion	Applicant's justification is acceptable	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A7.2.1/01 and A7.2.2.1/01 — Aerobic degradation in soil

Annex Point: IIIA VII.4, XII 1.1

		Official use only
	1 REFERENCE	
1.1 Reference	Fiebig, S. and Goller, St. (2014): SONC969 Saltidin, [carboxyl-^{14}C]-Aerobic Transformation in Soil. Dr. U. Noack Laboratorien, Sarstedt, Germany. Project No. 120814SB, Study No NAB15260 (unpublished), date: 2014-03-24	
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/list of approved active substances	
	2 GUIDELINES AND QUALITY ASSURANCE	
2.1 Guideline study	Yes, OECD Guideline for the Testing of Chemicals 307, Aerobic Transformation in Soil, April 2002.	
2.2 GLP	Yes	
2.3 Deviations	The soil was stored at 6 ± 2°C instead of 4 ± 2°C due to organizational reasons. This deviation was considered to have no impact on quality and integrity of the study.	
	3 MATERIALS AND METHODS	
3.1 Test material	SONC969 Saltidin, [carboxyl^{14}C]-	
3.1.1 Lot/Batch number	6 Batch number 198-190-0564-A-20120718-DRE	
3.1.2 Specification	Specific activity: 56.4 mCi/mmol	
3.1.3 Purity	7 Radiochemical purity was 99.7%	
3.1.4 Further relevant properties	-	
3.1.5 Method of analysis	See table A7_2_1-5.	
3.2 Reference substance	No reference item is recommended for this test.	
3.2.1 Method of analysis for reference substance	-	
3.3 Soil types	Four soils were used for the study: two loamy sands, a silty sand, and a clayey loam. For soil properties see table A7_2_1-1.	8
3.4 Testing procedure	9	
3.4.1 Test system	4 different standard soils (LUFA 2.2, 5M, 2.3 and 2.4, field fresh sampled) were used, representing a range of relevant soils. The soils vary in their organic carbon content, pH, clay content and microbial biomass. The soil was manually cleared of large objects	

	and then sieved to a particle size of 2 mm. The maximal water holding capacity and the pH-value were determined. The soil moisture content was adjusted to 40 - 60 % of its maximum water holding capacity with demineralised water after receipt. Dry out of the soil was prevented by moistening with demineralised water as necessary until test start. The soil was checked for a detectable microbial biomass (result in terms of percentage of total organic carbon). The soil was stored for 2 days (soil 2.2 and soil 5M) and 38 days (soil 2.3 and soil 2.4) at 6 ± 2 °C and afterwards preincubated at room temperature (ca. 20°C) for 26 days (soil 2.2), 34 days (soil 5M), 4 days (soil 2.3) and 11 days (soil 2.4), respectively, before application to allow germination and removal of seeds and to guarantee a temperature adaptation of the micro-organisms.
3.4.2 Test conditions	See table A7_2_1-2
3.4.3 Method of preparation of test solution	See table A7_2_1-2
3.4.4 Initial TS concentration	See table A7_2_1-2, table A7_2_1-3, table A7_2_1-4 Investigation of the stock solution revealed a discrepancy between the total radioactivity of the stock solution determined by LSC and the radioactivity related to discrete peaks in the LC-FSA analysis. The major part (59.3%) of the radioactivity in the LC-FSA analysis was related to SONC969 Saltidin, [carboxyl-^{14}C]- and 2.8% to ^{14}C-Saltidin. Furthermore a few other decomposition products at a level of < 2% for a single substance (mostly even < 1%) were additionally present. In total approximately 73% of the radioactivity measured by LSC were related to discrete peaks. In contrast the remaining radioactivity of approximately 26.5% was not indicated by discrete peaks in the corresponding chromatogram during LC-FSA analysis of the stock solution. It was suspected that autoradiolysis had influenced the radiochemical purity. Nevertheless autoradiolysis of the test item did not impair the interpretation of the results of the study because there were no peaks interfering the monitoring of the test item and Saltidin acid. Therefore a reliable determination of DT_{50} and DT_{90} values was possible. To verify the amount of applied radioactivity and to have a confident starting point for the calculation of the transformation, the stock solution was analysed prior to the application to each soil. The total activity of the stock solution as well as the activity related to SONC969 Saltidin, [carboxyl-^{14}C]- and ^{14}C-Saltidin acid was determined prior the application of each soil and these values were the reference basis for all further calculations for the respective soil. The results for the analysis of the stock solution prior to each application are given in table A7_2_1-4.
3.4.5 Number of replicates	See table A7_2_1-2
3.4.6 Duration of test	See table A7_2_1-2

3.4.7	Sampling	See table A7_2_1-2
3.4.8	Analytical methods	See table A7_2_1-5
3.4.9	Intermediates/ degradation products	See table A7_2_1-5
3.4.10	Controls	See table A7_2_1-2
3.4.11	Statistics	The kinetic evaluations were done based on the FOCUS guidance document on estimating persistence and degradation kinetics (SANCO/10058/2005, version 2.0, June 2006: Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration).

4 RESULTS

4.1 Aerobic soil metabolism

4.1.1	Mass Balance	The mass balance, distribution of radioactivity, $^{14}CO_2$ production and non-extractable residues formation in the four soils is summarised in table A7_2_1-6 to table A7_2_1-9. The corresponding illustration is given in figure A7_2_1-1 to figure A7_2_1-4. The distribution of the applied radioactivity in the soil extracts is given in table A7_2_1-10 to table A7_2_1-13.
4.1.2	Transformation	The aerobic transformation of SONC969 Saltidin, [carboxyl-^{14}C]- and ^{14}C-Saltidin acid in the four soils is summarised in tables A7_2_1-14 to A7_2_1-17. The proposed metabolic pathway for Saltidin degradation in soils under aerobic conditions is given in figure A7_2_1-9.
4.1.3	Kinetic analysis	The detailed results of the kinetic evaluations are given in table A7_2_1-18 and table A7_2_1-19. A graphical presentation of the kinetic analysis is given in figures A7_2_1-5 to A7_2_1-8.
4.1.4	Other observations	-
4.1.5	Degradation of reference substance	n.a.
4.1.6	Intermediates/degradation products	Please refer to Points 4.1.2 and 4.1.3.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The aerobic transformation rate of SONC969 Saltidin, [carboxyl-^{14}C]- has been tested in 4 different soils over a period of 21 - 31 days. Soil samples have been treated with SONC969 Saltidin [carboxyl-^{14}C]- (nominal test concentration: 3.66 kBq/g soil dry weight, corresponding to 405 µg/kg soil dry weight) and incubated in biometer flasks in the dark at approximately 20°C. Sampling was done immediately after the application and at 7-9 further sampling points.

For the calculation of the mass balance the radioactivity in the soil extracts, the extracted soil and the evolved $^{14}CO_2$ was determined by LSC. The amount of test item and transformation products in the soil

extracts was determined by HPLC-FSA on a reversed phase column in gradient mode. The structure of the only relevant degradation product during the course of the study, ^{14}C-Saltidin acid, was elucidated via LC-MS/MS.

The DT_{50} and DT_{90}, the disappearance time within the concentration is reduced by 50 % and 90 %, respectively, was calculated with a single first order model (SFO).

5.2 Results and discussion

10

11

5.2.1 Mass balance

12 A mass balance of 90 – 100% AR (= applied radioactivity) was obtained for soils 2.2, 2.3 and 5M for almost all samplings of the study.

13 As at the 2 h and 4 h samplings $^{14}CO_2$ determination was not feasible due to practical reasons, the mass balance decreased slightly below 90% within the samplings on day 0 for soil 5M, soil 2.3 and soil 2.4.

14 Moreover for all 4 soils the course of mass balance showed a similar pattern. The mass balance was high at test start, decreased slightly during the study and once transformation was completed the mass balance increased, and was in the range of the initial values. It is assumed, that this effect is due to the $^{14}CO_2$ determination from separate replicates. During the main transformation phase the replicates for $^{14}CO_2$ determination and the replicates for determination of transformation presumably showed a slightly different course of transformation. Anyway, as indicated above, the mass balance for soils 2.2, 2.3 and 5M is in most cases within the limit of 90 – 100% and if not (at 4 occasions), at least a mass balance of 88.3% is achieved.

15 As for soils 2.2, 2.3 and 5M, for soil 2.4 the $^{14}CO_2$ production started immediately after the application and as a result $^{14}CO_2$ was lost during the preparation of the replicates for $^{14}CO_2$ determination. Therefore the mass balance remained in the range of 83.6 – 88.0% between day 2 and day 7, but increased to 90.0% and 91.8% at test end (day 10 and day 31). Therefore, mass balances of slightly below 90% can be attributed to the immediate start of $^{14}CO_2$ production and the very fast mineralisation.

5.2.2 Extractable/non-extractable residues (NER)

16 The extractability changed during the study but showed a similar course for all 4 soils. The non-extractable residues (NER) increased during the transformation of SONC969 Saltidin, [carboxyl-^{14}C]- to ^{14}C-Saltidin acid and reached a maximum once the transformation was completed. In course of the further transformation and mineralization of ^{14}C-Saltidin acid the amount of NER decreased continuously until the end of the respective exposure period and was <10% for all 4 soils at the end of the respective exposure periods. The amount of NER was lowest for soil 2.3 and remained <10% throughout the study duration for this soil. For the other 3 soils the amount of NER was comparable and reached a maximum of 22.0 – 34.6% AR. It can be assumed that most of the NER still present at the end of the study is

related to radioactivity incorporated in the bacteria biomass during the transformation and mineralization.

17 Investigations on the stock solution came to the result, that a certain amount of radioactivity applied to the soils was not associated with the test item or its major metabolite. To get a better impression about the behaviour of the radioactivity not associated with SONC969 Saltidin, [carboxyl-^{14}C]- and ^{14}C-Saltidin acid the distribution of the radioactivity in the soil extracts was investigated in more detail. Therefore, the total radioactivity measured by LSC and the activity related to SONC969 Saltidin, [carboxyl-^{14}C]- and ^{14}C-Saltidin acid were compared and the residual activity was calculated from this difference.

18 The comparison showed that the difference decreased from approx. 30% AR to approx. 10% within the first 24 h after the application for all 4 soils (see tables A7_2_1-10 to A7_2_1-13). During the further course of the study, the difference fluctuated and remained in the range of 8% - 1% or it was even negative. This remaining difference can be associated with the typical fluctuations and analytical uncertainties of the LSC and LC-FSA measurements. This means that the soil extracts from day one onwards contained mainly the radioactivity related to SONC969 Saltidin, [carboxyl-^{14}C]- and ^{14}C-Saltidin acid and further transformation products appearing within the course of the study. These results confirm that no additional degradation products (besides M2-M9) with > 10% AR were formed at any sampling point.

19 It can be concluded, that the evaluation of the transformation and the mineralisation is not influenced by the residue activity.

5.2.3 CO_2 formation

20 The $^{14}CO_2$ formation started almost immediately after the application in all 4 soils. The mineralization progressed steadily and was > 90% AR for all 4 soils at test end, except for soil 2.4, where it accounted for 85.4% AR. However, although for soil 2.4 the amount of $^{14}CO_2$ determined in the NaOH traps was < 90 %, the study results indicated that $^{14}CO_2$ losses occurred during the preparation of the replicates for $^{14}CO_2$ determination. Hence, $^{14}CO_2$ formation in this soil can be assumed to be in the same order of magnitude as in the other soils.

21 No radioactivity (all samples < LOQ) was determined in the ethylene glycol traps, indicating that no volatile transformation products were formed.

5.2.4 Transformation

22 The radioactivity of SONC969 Saltidin, [carboxyl-^{14}C]- and ^{14}C-Saltidin acid measured in the stock solution by LC-FSA prior to application was set as 100 % (= % ARs), and all further calculations of % transformation of SONC969 Saltidin, [carboxyl-^{14}C]- and ^{14}C-Saltidin acid were done based on this value.

23 SONC969 Saltidin, [carboxyl-^{14}C]- was transformed rapidly in all four soils, yielding ^{14}C-Saltidin acid as main transformation product. ^{14}C-Saltidin acid had also a transient nature and was further transformed efficiently. Up to 8 different further degradation products (M2 – M9) were detected in the soils.

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Annex Point: IIIA VII.4, XII 1.1

24 These transformation products were not stable and the quantities were <10% for a single substance, except for metabolite M3 in soil 2.3. However, an exceedance of 10% ARs (14.7%) could only be observed 0.33 h after the application, whereas 2 h after the application the amounts fell already below the limit of detection. In general, all transformation products were further degraded rapidly, and no longer detectable after 4-6 days.

25 Based on the findings summarised in chapter 5.2.2 and detailed in-depth evaluation of all chromatograms and peak pattern it was stated, that all metabolites determined during the course of the study are transformation products from the transformation of ^{14}C-Saltidin acid and not related to the remaining radioactivity detected in the stock solution. Furthermore the relative retention time (RRT) of the metabolites differed from the RRT's of most of the discrete peaks determined in the stock solutions. An exception were M5 and M6 which were detected at low amounts (1% – 2% of AR) in the stock solution, but the activity related to these transformation products had disappeared already at the first sampling directly after application. Therefore it can be concluded, that the appearance of M5 and M6 is related to the transformation of ^{14}C-Saltidin acid.

5.2.5 Kinetic analysis

The kinetic evaluation revealed DT_{50} values (20°C) for SONC969 Saltidin, [carboxyl-^{14}C]- between 0.02 and 0.055 days. For ^{14}C-Saltidin acid, DT_{50} values (20°C) were in the range of 0.6 to 2.7 days.

5.2.6 Transformation pathway

The results of a preliminary study indicated that the ^{14}C-label in [hydroxyethyl-1-^{14}C] Saltidin **1** was lost after an initial oxidation to Saltidin acid **2** (see figure A7_2_1-9). Simultaneously an increase of $^{14}CO_2$ in the corresponding traps was observed. Therefore a α- or β-oxidation was assumed to be responsible for this transformation step. The α-oxidation would lead to 1-[(butan-2-yloxy)carbonyl]piperidine-2-carboxylic acid **3** and the β-oxidation to butan-2-yl 2-oxopiperidine-1-carboxylate **4**.

The results of the definitive study further demonstrated that also the ^{14}C-label in SONC969 Saltidin, [carboxyl-^{14}C]- was immediately lost during the transformation of Saltidin acid and accompanied by an increase of $^{14}CO_2$ in the corresponding traps. The initial oxidation from **1** to **2** was confirmed and further the carbamate moiety was cleaved probably under formation of CO_2, 2-butanol, piperidine-2-carboxylic acid **5** or piperidin-2-one **6.** In all 4 soils, besides Saltidin acid, further metabolites could be detected during the course of the study. But these metabolites were not stable (present only at one sampling time and for < 2 h) or the quantities were < 10%. It was assumed that these metabolites corresponded to intermediates occurring in the initial oxidation of the alcohol moiety (for example an aldehyde) or during α-and ß-oxidation of Saltidin acid (for example α- and ß-oxygenated derivatives or α,β-unsaturated derivatives of Saltidin acid).

5.3 Conclusion

The transformation rate of SONC969 Saltidin, [carboxyl-^{14}C]- was fast in all 4 soils. Within 2 hours (soil 2.3 and soil 2.4) and 4 hours (soil 5M, soil 2.2), respectively SONC969 Saltidin, [carboxyl-^{14}C]- was transformed to ^{14}C-Saltidin acid, with DT_{50} values of 0.52 hours (soil

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		2.2), 1.31 hours (soil 5M), 0.57 hours (soil 2.3) and 0.47 hours (soil 2.4). The transformation of ^{14}C-Saltidin acid was progressing slightly slower. However, a steady and fast decline following SFO kinetics was determined in all 4 soils and ^{14}C-Saltidin acid was transformed completely (> 90%) within 6-7 days in soil 2.2, soil 5M and soil 2.3 and within 3 days in soil 2.4. The DT_{50} values were 54.2 hours (soil 2.2), 64.5 hours (soil 5M), 53.2 hours (soil 2.3) and 14.3 hours (soil 2.4), respectively. Simultaneously with the transformation of ^{14}C-Saltidin acid additional metabolites were detected and $^{14}CO_2$ was formed. These transformation products were not stable and the quantities were <10% for a single substance, except for metabolite M3 in soil 2.3. However, an exceedance of 10% ARs (14.7% ARs) could only be observed 0.33 h after the application, whereas 2 h after the application the amounts fell already below the limit of detection. In general, all transformation products were transformed rapidly and no longer detectable after 4-6 days. No volatile, organic transformation products were formed during the test duration.
5.3.1	Reliability	1
5.3.2	Deficiencies	Due to autoradiolysis, ^{14}C activity in the stock solution was not only associated with the parent compound or its major metabolite, but also with other decomposition products or diffuse radioactivity, which did not contribute to discrete peaks. However, follow-up investigations revealed, that this autoradiolysis of the test item did not impair the interpretation of the results of the study. At some sampling points, the mass balances were slightly below 90% AR (83.6% at minimum). This can be attributed to the immediate start of $^{14}CO_2$ production when the test item was applied and the very fast mineralisation especially when the test started. This reduced mass does not have an impact on the interpretation of the results.

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. August 2015
Materials and Methods	Applicants version is acceptable.
Results and discussion	Adopt applicant's version
Conclusion	Adopt applicant's version
Reliability	Based on the assessment of materials and methods include appropriate reliability indicator 2
Acceptability	Acceptable; however, the RMS is concerned about the problem identified especially the problem due to autoradiolysis, ^{14}C activity in the stock solution. Therefore the radioactivity was not only associated with the parent compound or its major metabolite, but also with other decomposition products or diffuse radioactivity, which did not contribute to discrete peaks. The only reason that the RMS accept this study anyway is because the follow-up investigations the applicant has made which revealed, that this autoradiolysis of the test item did not impair the interpretation of the results of the study significantly. At some sampling points, the mass balances were slightly below 90% AR (83.6% at minimum). This is also a problem; however again the RMS accept the explanation made by the applicant.
Remarks	26
	COMMENTS FROM ...
Date	Give date of comments submitted
Materials and Methods	Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion. Discuss if deviating from view of rapporteur member state
Results and discussion	Discuss if deviating from view of rapporteur member state
Conclusion	Discuss if deviating from view of rapporteur member state
Reliability	Discuss if deviating from view of rapporteur member state
Acceptability	Discuss if deviating from view of rapporteur member state
Remarks	27

Table A7_2_1-1: Physicochemical properties of the test soils

Parameter	LUFA-soil 2.2 Batch-No. F2.2 4113	LUFA-soil 5M Batch-No. F5.M 4213	LUFA-soil 2.3 Batch-No. F2.3 4113	LUFA-soil 2.4 Batch-No. F2.4 4113
Sampling depth*	ca. 20 cm	ca. 20 cm	ca. 20 cm	ca. 20 cm
pH-value*	5.5 ± 0.2	7.3 ± 0.1	6.0 ± 0.9	7.2 ± 0.2
Dry weight (DW) before application [g/100 g soil]	84.4	87.3	86.7	83.1
Maximum water holding capacity* [g/100 g DW]	42.5 ± 3.2	39.2± 2.8	36.2 ± 2.0	43.8 ± 1.3
Particle size distribution acc. to DIN*				
Sand:				
0.63 - 2.0 mm [%]	0.7 ± 0.3	1.3 ± 0.2	2.1 ± 0.6	1.7 ± 0.2
0.2 - 0.63 mm [%]	41.8 ± 2.4	14.5 ± 1.8	30.1 ± 0.1	6.9 ± 2.2
0.063 - 0.2 mm [%]	32.5 ± 2.2	37.9 ± 1.5	25.4 ± 2.0	19.0 ± 0.3
Silt:				
0.02 - 0.063 mm [%]	8.2 ± 1.3	21.8 ± 1.1	20.2 ± 2.0	23.0 ± 1.0
0.006 - 0.02 mm [%]	5.1 ± 1.1	9.3 ± 1.0	11.4 ± 1.0	14.8 ± 1.1
0.002 - 0.006 mm [%]	3.6 ± 0.9	4.4 ± 0.9	5.1 ± 0.1	8.1 ± 1.0
Clay:				
< 0.002 mm [%]	8.1 ± 1.4	10.8 ± 1.2	5.9 ± 2.5	26.5 ± 1.9
Organic carbon content [%][1]	1.4	0.91	0.65	2.1
Microbial biomass [%] of total organic carbon[2)]	1.53	3.78	2.85	2.21
Cation exchange capacity [mVal/100 g]*	10.2 ± 0.5	17.1 ± 3.3	6.9 ± 1.0	32.2 ± 4.4
Weight per Volume (g/1000) mL)	1247 ± 45	1314 ± 68	1335 ± 8	1289 ± 32
Soil texture*	loamy sand (lS)[#]	Loamy sand (lS)[#]	silty sand (uS)[#]	clayey loam (tL)[#]
Sampling date	2013-10-11	2013-10-14	2013-10-10	2013-10-09

*) data provided by LUFA Speyer

#) acc. to German DIN classification

[1)] data determined by Institut Koldingen GmbH (non GLP)

[2)] determined prior to test start

Table A7_2_1-2:Testing procedure and test solutions used in the aerobic soil study

<table>
<tr><th>Parameter</th><th>Description</th></tr>
<tr><td>Temperature</td><td>Nominal: 20 ± 2 °C; Actual: 18.2 – 22.2°C (mean value: 19.87 – 20.55°C)</td></tr>
<tr><td>Test duration</td><td>23 days (soil 2.2), 21 days (soil 5M), 16 days (soil 2.3), and 31 days (soil 2.4)</td></tr>
<tr><td>Light/dark cycle</td><td>Darkness</td></tr>
<tr><td>Soil moisture content</td><td>At the beginning of the test the soils were adjusted to 46-50 % of the maximum water holding capacity. Soil moisture content was not checked for replicates for determination of mineralisation because this was technically not feasible. The replicates for transformation were weighted to check for losses by evaporation.</td></tr>
<tr><td>Stock solution</td><td>65.5 MBq/100mL ultrapure water</td></tr>
<tr><td>Test concentration</td><td>Nominal test concentration: 3.66 kBq/g soil dry weight (183 kBq/replicate), corresponding to 405 µg/kg soil dry weight.
Actual test concentration:
<table>
<tr><th></th><th colspan="3">Activity related to SONC969 Saltidin, [carboxyl-^{14}C]- and ^{14}C-Saltidin acid (=AR$_S$)</th><th>Total Activity (=AR)</th></tr>
<tr><th>Soil</th><th>kBq/g soil DW</th><th>kBq/Replicate</th><th>µg/kg soil DW</th><th>kBq/Replicate</th></tr>
<tr><td>2.2</td><td>3.83</td><td>192</td><td>425</td><td>296</td></tr>
<tr><td>5M</td><td>3.97</td><td>198</td><td>439</td><td>303</td></tr>
<tr><td>2.3</td><td>3.87</td><td>193</td><td>428</td><td>303</td></tr>
<tr><td>2.4</td><td>4.13</td><td>207</td><td>459</td><td>303</td></tr>
</table>
AR = applied radioactivity;
bold = Basis for further calculation of mass balance and transformation</td></tr>
<tr><td>Replicates</td><td>Two replicates per sampling interval for each soil treated with test item and one control was tested. Individual flasks were prepared for each sampling time.</td></tr>
<tr><td>Application</td><td>The aqueous solution of the test item was applied on the surface of each soil. Soils were mixed carefully (for ≥ 2 min.) to insure homogeneous distribution. Subsequently samples for extraction were taken and the soils were distributed to the test replicates. To minimize losses due to fast transformation of SONC969 Saltidin, [carboxyl-^{14}C]- and mineralization (formation of $^{14}CO_2$) during set up of the individual replicates, all working steps were carried out as quick as feasible.
Test item concentrations, application conditions, exact application volumes and amounts of soil are listed in table A7_2_1-3.</td></tr>
<tr><td>Test vessels</td><td>250 mL (control 500 mL), biometer type flasks with gas outlet and connected with appropriate traps for volatile transformation products and $^{14}CO_2$ to determine the mineralization.
250 mL, biometer type flasks with funnel filled with soda lime to trap $^{14}CO_2$. These test replicates were used for soil extraction and determination of the transformation.
500 mL, biometer type flasks for the controls</td></tr>
</table>

Table A7_2_1-2 cont.: Testing procedure and test solutions used in the aerobic soil study

<table>
<tr><th>Parameter</th><th>Description</th></tr>
<tr><td>Volatile traps for determination of mineralisation</td><td>Crimped headspace bottles containing 50 mL ethylene glycol were used for trapping volatile organic transformation products. Crimped headspace bottle containing 50 mL 1 mol/L aqueous sodium hydroxide traps for trapping CO_2.</td></tr>
<tr><td>Aeration</td><td>Biometer type flasks with funnel: exchange of air was maintained by diffusion from the headspace.
Biometer type flasks with gas outlet and traps: gas exchange by continuously aeration</td></tr>
<tr><td>Sampling</td><td>Sampling for determination of the transformation rate:

<table>
<tr><th>Soil</th><th>Number of Samplings</th><th>Sampling Times</th></tr>
<tr><td>2.2</td><td>8</td><td>0h, 4h,
1, 2, 3, 4, 7 and 23 days</td></tr>
<tr><td>5M</td><td>10</td><td>0h, 2h, 4h,
1, 2, 3, 4, 6, 14 and 21 days</td></tr>
<tr><td>2.3</td><td>10</td><td>0h, 2h, 4h,
1, 2, 3, 4, 7, 10 and 16 days</td></tr>
<tr><td>2.4</td><td>10</td><td>0h, 2h, 4h,
1, 2, 3, 4, 7, 10 and 31 days</td></tr>
</table>

2 test item replicates were sacrificed at each sampling time. At test start and the sampling at 2 h and 4 h 4 subsamples of the total applied soil amount were analysed.</td></tr>
<tr><td>Biomass activity</td><td>To check the biomass activity glucose induced respiration rates of the controls were determined at least at test start and test end. Depending on the course of the biodegradation further determinations were done within the main transformation phase of each soil.</td></tr>
</table>

Table A7_2_1-3:Application conditions of the soil

Lufa Soil		**2.2**	**5M**	**2.3**	**2.4**
Maximal water holding capacity (MWHC)	[g/100 g soil DW]	42.5	39.2	36.3	43.8
Dry Weight (DW) before application	[g/100 g soil]	84.4	87.3	86.7	83.1
Nominal test item concentration in soil	[kBq/g soil DW]	3.66			
Nominal concentration of stock solution	[MBq/100 mL]	65.5			
Total applied soil amount					
Control / Test item (corresponding to 2.0 kg DW)	[kg]	2.370	2.291	2.307	2.407
Applied volume of water to adjust the DW	mL	36.68	66.68	7.68	12.68
Volume of test item stock solution	[mL]	18.32	18.32	18.32	18.32
% of MWHC after application	%	50	48	46	50
Dry Weight (DW) after application	%	82.5	84.2	85.7	82.0
Moist soil amount per replicate (control)	[g]	242.43	237.5	233.4	243.9
Moist soil amount per replicate (test item)	[g]	60.6	59.4	58.3	61.0

DW = dry weight

Table A7_2_1-4: Activity of the stock solution for application

Soil			Radioactivity Bq/mL	% of total activity
2.2	LSC	Total	6457	100
	FSA	SONC969 Saltidin, [carboxyl-^{14}C]-	4032	62.4
		^{14}C-Saltidin acid	150	2.3
	Remaining Activity		2275	35.2
	Related to peaks		882	13.7
	Not related to discrete peaks		1393	21.6
5M	LSC	Total	6608	100
	FSA	SONC969 Saltidin, [carboxyl-^{14}C]-	4078	61.7
		^{14}C-Saltidin acid	253	3.8
	Remaining Activity		2278	34.5
	Related to peaks		1196	18.1
	Not related to discrete peaks		1082	16.4
2.3	LSC	Total	6612	100
	FSA	SONC969 Saltidin, [carboxyl-^{14}C]-	3963	59.9
		^{14}C-Saltidin acid	259	3.9
	Remaining Activity		2390	36.1
	Related to peaks		1071	16.2
	Not related to discrete peaks		1319	20.0
2.4	LSC	Total	6613	100
	FSA	SONC969 Saltidin, [carboxyl-^{14}C]-	4226	63.9
		^{14}C-Saltidin acid	287	4.3
	Remaining Activity		2100	31.8
	Related to peaks		888	13.4
	Not related to discrete peaks		1212	18.3

Table A7_2_1-5:Analytical methods used in the aerobic soil study

Parameter	Description
Determination of radioactivity by Liquid Scintillation Counter Analysis (LSC)	
Parameter	Radioactivity of the soil extracts, the extracted soil after combustion, the sodium hydroxide traps, the ethylene glycol traps
Equipment	- LSC Counter : TRICARB 2100 TR, CANBERRA-PACKARD - Software : Ver. 1.05, PACKARD - Oxidizer : Model 307, PACKARD (PERKINELMER)
Reagents	LSC-Cocktail, UltimaGold XR, PERKIN ELMER Carbon dioxide absorber, Carbosorb E, PERKIN-ELMER LSC-Cocktail (for Carbosorb E), Permafluor E+, PERKIN-ELMER LSC-Cocktail for carbon dioxide traps, Hionic Fluor, PERKIN-ELMER
Counting Parameter	Counting Type: DPM (disintegrations per minute) Counting terminator: Until 2 x standard deviation of the counted disintegrations is < 0.5 %, but max. 20 min Lower energy level: 0 keV Upper energy level: 156 keV Quench indication paramter: tSIE (transformed spectral index of the external standard ^{133}Ba)
Quench Correction	A general quench curve of the analytical system was used to compensate for a decreased counting efficiency due to chemical or color quench in the different media. The extent of quench in the samples was described by the transformed spectral index of the external (tSIE) ^{133}Ba standard. The determined tSIE of a sample correlates with a counting efficiency.
Preparation of samples	
Soil dry weight	The soil dry weight was determined by drying a soil sample for at least 3 h at 105°C.
Carbon dioxide traps	0.3 to 3 mL of the sodium hydroxide traps were mixed with 15 mL Hionic-Fluor in a LSC-vial and measured with LSC.
Soil extract	0.25 – 1.0 mL of the soil extract were mixed with 10 mL UltimaGold XR in an LSC vial and measured with LSC
Ethylene glycol traps	2 mL of the ethylene glycol trap were mixed with 8 mL of HPLC-water in a LSC-vial followed by addition of 10 mL UltimaGold XR.
Non Extractable Residues	0.2 g of the dried extracted soils were weighted in one combusto cone containing 3 combusto pads followed by moistening with 0.4 – 0.5 mL HPLC water. These samples were combusted for 5 min. using the sample oxidizer. The produced CO_2 was trapped in 10 mL of Carbosorb E, mixed with 10 mL Permafluor E+ and measured by LSC.
Method validation	Limit of Detection (LOD): ≤ 1 % AR for all media Limit of Quantification of the analytical method (LOQ_M): Ethylene glycol traps: 0.1% AR; Sodium hydroxide traps: 0.1% AR; soil extracts: 0.14; extracted soil: 1.4% AR
Accuracy	The analytical methods for the carbon dioxide traps and ethylene glycol traps were validated with satisfactory results on two levels à 5 replicates in a separate study % (please refer to Doc. IIIA, 7.1.2.2.2(01)). The validation parameters are also relevant for this study. Extracted soil samples were combusted with a sample oxidizer followed by analysis with LSC analogous to the treatment of extracted sediment samples in the corresponding aerobic water sediment study with the test item. The mean recoveries at each fortification level were in the range of 95 and 105 %.
Precision	Relative standard deviations at each fortification level were lower than 5% (please refer to Doc. IIIA, 7.1.2.2.2(01)).

Table A7_2_1-5 cont.: Analytical methods used in the aerobic soil study

Parameter	Description
Flow Scintillation analysis coupled with HPLC (HPLC-FSA)	
Parameter	Analysis of SONC969 Saltidin, [carboxyl-^{14}C]- and degradation products in soil extracts
Equipment	HPLC: 2695 Alliance separation module, WATERS Detector: 500TR FSA, PERKIN-ELMER Software: FlowOne, v3.65, PERKIN-ELMER Software LC: Mass Lynx^{TM} 4.1, WATERS
Reagents	ULTIMA-FLO^{TM} M (LSC-cocktail for Radio-HPLC), PERKIN-ELMER High DPM Spec-Check-^{14}C, Part Number 6002135, 8.26*10^5 dpm/mL. PERKIN EKMER
Efficiency standard	Spec-Check^{14}C was used as standard with known activity.
Conditions of Analysis	Column: Discovery C18 5 µm, 250 x 4.6 mm, Batch 133820-01, SUPELCO Temperature: 25°C Mobile phase: A : 0.005 mol/L trifluoroacetic acid in HPLC water B : 0.005 mol/L trifluoroacetic acid in acetonitrile Gradient mode FSA Cell type, liquid, 500 µL Radio update 4 s Nuclide ^{14}C (LLD = 0 keV, ULD 156 keV) HPLC flow rate 1.0 mL / min LS flow rate 2.4 mL / min LS / HPLC ratio 2.4 : 1
Extraction methods	Due to the different soil characteristics, the extraction methods had to be adapted within the study.
Method 1	10 g DW of the soil sample were weighed into a screw top tube (50 mL) followed by addition of 30 mL of acetonitrile. This suspension was agitated by an overhead shaker at 90 rpm for 1 h followed by centrifugation at 3000 rpm for 3 min. The supernatant was decanted into a round bottom flask. 5 mL of acetonitrile were added and the soil suspension was extracted again with an overhead shaker at 90 rpm for 5 min followed by centrifugation at 3000 rpm for 3 min. This extraction cycle was repeated once. The combined extracts were evaporated to dryness under reduced pressure. Finally, the residue was dissolved in 5 mL of a 1:1 mixture of ethanol and HPLC water prior to analysis. An appropriate volume of the extract was dissolved in 10 mL Ultima Gold XR followed by analysis with LSC. For FSA, 100 µL of the extract were analysed.
Method 2	The extraction was performed as described before. But a mixture of acetonitrile and HPLC water 90 : 10 (v/v) at pH 2 was used as extraction solvent.
Method 2.1	If necessary extracted soil samples were extracted for again. The extraction was carried out overnight with 30 mL of a mixture of acetonitrile and HPLC water 90 : 10 (v/v) at pH 2. After that the suspension was centrifuged at 3000 rpm for 3 min and the supernatant was decanted into a round bottom flask. The extract was evaporated to dryness under reduced pressure and the residue was dissolved in 5 mL of a 1:1 mixture of ethanol and HPLC water prior to analysis.

Table A7_2_1-5 cont.: Analytical methods used in the aerobic soil study

Parameter	Description
Flow Scintillation analysis coupled with HPLC (HPLC-FSA)	
Method validation	No method validation was performed with SONC969 Saltidin, [carboxyl-^{14}C]- in the different soils because preliminary investigations (non-GLP) demonstrated that the biodegradation of the test item was faster than the time period necessary for soil sample preparation with different extraction methods. Therefore, soil samples were extracted based on the described methods during the definitive study. In addition extracted soil samples were combusted followed by LSC analysis to determine the amount of NERs. The mass balances obtained during the study confirmed the validity of the extraction methods. Limit of Detection (LOD): Signal-noise ratio of 3 Limit of Quantification of the analytical method (LOQ): Signal-noise ratio of 9.
Structure elucidation via combination of LC-MS and LC-MS/MS	
Parameter	One relevant degradation product, ^{14}C-Saltidin acid, was elucidated via a combination of LC-MS and LC-MS/MS during the corresponding aerobic water sediment study with the test item (please refer to Doc. IIIA, 7.1.2.2.2(01)). During this study further transformation products were detected in the different soil extracts, but these transformation products were short-lived and the quantities for a single substance were < 10% (with one exception) . All further transformation products were transformed rapidly and not detectable after 4 days anymore. Therefore no further work was carried out to identify these transformation products.
Equipment	Please refer to Doc. IIIA, 7.1.2.2.2(01)
Conditions of Analysis	Please refer to Doc. IIIA, 7.1.2.2.2(01)
Preparation of samples	Please refer to Doc. IIIA, 7.1.2.2.2(01)
Conditions of detection	Please refer to Doc. IIIA, 7.1.2.2.2(01)

Table A7_2_1-6: Mass Balance of the aerobic soil degradation in soil 2.2

Exposure Day	Repl.	Soil % of AR	mv	Non-Extractable Residues (NER) % of AR	mv	$^{14}CO_2$ % of AR	mv	Mass Balance % of AR	mv
0 (0.33 h)	4#	87.3*	**87.3**	10.1*	**10.1**	-	–	97.3	**97.3**
0 (4 h)	4#	70.4*	**70.4**	25.2*	**25.2**	-	–	95.8 95.3	**95.6**
1	1 2	48.3 50.8	**49.6**	23.7 24.3	**24.0**	20.4 21.3	**20.8**	92.4 96.5	**94.4**
2	1 2	36.2 37.6	**36.9**	23.1 21.4	**22.3**	33.5 35.5	**34.5**	92.7 94.6	**93.7**
3	1 2	27.3 27.4	**27.3**	21.8 23.4	**22.5**	45.5 44.2	**44.8**	94.5 95.0	**94.8**
4	1 2	19.8 21.0	**20.4**	18.5 16.5	**17.5**	- -	–	- -	–
7	1 2	8.2 7.9	**8.0**	11.7 13.9	**12.8**	82.8 59.1	**70.9**	102.7 81.0	**91.8**
23	1 2	2.4 2.1	**2.3**	6.8 6.7	**6.7**	98.5 93.1	**95.8**	107.6 101.9	**104.7**

AR = Applied Radioactivity; - = not determined; mv = mean values

4 subsamples of the total applied soil batch

* mean values of 4 subsamples of the total applied soil batch

Table A7_2_1-7: Mass Balance of the aerobic soil degradation in soil 5M

Exposure Day	Repl.	Soil % of AR	mv	Non-Extractable Residues (NER) % of AR	mv	$^{14}CO_2$ % of AR	mv	Mass Balance % of AR	mv
0 (0.33 h)	4#	84.2*	**84.2**	15.1*	**15.1**	-	–	99.3	**99.3**
0 (2 h)	4#	65.7*	**65.7**	28.0*	**28.0**	-	–	93.8	**93.8**
0 (4 h)	4#	55.2*	**55.2**	34.6*	**34.6**	-	–	89.8	**89.8**
1	1 2	61.1 59.7	**60.4**	10.5 10.1	**10.3**	24.7 (2.2)§	**24.7**	96.2 (71.9)§	**96.2**
2	1 2	43.1 45.3	**44.2**	9.3 8.6	**9.0**	38.0 39.4	**38.7**	90.4 93.3	**91.8**
3	1 2	31.3 33.3	**32.3**	6.3 8.0	**7.1**	53.0 44.7	**48.9**	90.5 86.0	**88.3**
4	1 2	20.3 24.9	**22.6**	8.6 7.5	**8.1**	73.5 70.7	**72.1**	102.4 103.0	**102.7**
6	1 2	5.9 5.5	**5.7**	5.8 5.4	**5.6**	92.6 98.6	**95.6**	104.4 109.5	**106.9**
14	1 2	1.2 1.1	**1.2**	3.5 4.2	**3.9**	94.5 84.7	**89.6**	99.3 90.0	**94.6**
21	1 2	0.9 1.0	**1.0**	2.7 2.5	**2.6**	102.0 87.7	**94.9**	105.7 91.2	**98.4**

AR = Applied Radioactivity; - = not determined; mv = mean values

4 subsamples of the total applied soil batch

* mean values of 4 subsamples of the total applied soil batch

§ Replicate for CO_2 determination leaked, value not taken into account for mass balance calculation

Table A7_2_1-8: Mass Balance of the aerobic soil degradation in soil 2.3

Exposure Day	Repl.	Soil % of AR	mv	Non-Extractable Residues (NER) % of AR	mv	$^{14}CO_2$ % of AR	mv	Mass Balance % of AR	mv
0 (0.33 h)	4#	93.3*	**93.3**	1.0*	**1.0**	-	**-**	94.3	**94.3**
0 (2 h)	4#	89.5*	**89.5**	1.9*	**1.9**	-	**-**	91.4	**91.4**
0 (4 h)	4#	86.4*	**86.4**	2.6*	**2.6**	-	**-**	89.0	**89.0**
1	1 2	69.7 67.3	**68.5**	7.6 10.1	**8.8**	16.0 13.7	**14.8**	93.2 91.1	**92.1**
2	1 2	58.1 57.2	**57.7**	7.4 8.2	**7.8**	24.9 26.2	**25.6**	90.4 91.6	**91.0**
3	1 2	44.8 45.2	**45.1**	5.4 6.2	**5.8**	39.6 39.2	**39.4**	89.7 90.9	**90.3**
4	1 2	36.7 35.5	**36.1**	5.3 5.0	**5.2**	46.6 50.3	**48.5**	88.6 90.9	**89.8**
7	1 2	9.2 8.0	**8.6**	4.8 5.7	**5.2**	74.3 78.2	**76.3**	88.3 91.9	**90.1**
10	1 2	4.0 4.1	**4.1**	4.8 5.2	**5.0**	92.4 92.5	**92.5**	101.2 101.8	**101.5**
16	1 2	2.4 3.6	**3.0**	2.8 3.2	**3.0**	77.6 91.7	**84.6**	82.8 98.4	**90.6**

AR = Applied Radioactivity; - = not determined; mv = mean values

4 subsamples of the total applied soil batch

* mean values of 4 subsamples of the total applied soil batch

Table A7_2_1-9: Mass Balance of the aerobic soil degradation in soil 2.4

Exposure Day	Repl.	Soil % of AR	mv	Non-Extractable Residues (NER) % of AR	mv	$^{14}CO_2$ % of AR	mv	Mass Balance % of AR	mv
0 (0.33 h)	4#	83.4*	**83.4**	5.1*	**5.1**	-	**-**	88.5	**88.5**
0 (2 h)	4#	74.7*	**74.7**	13.8*	**13.8**	-	**-**	88.5	**88.5**
0 (4 h)	4#	66.6*	**66.6**	16.1*	**16.1**	-	**-**	82.6	**82.6**
1	1 2	27.3 26.1	**26.7**	22.8 21.2	**22.0**	49.4 51.9	**50.6**	99.4 99.2	**99.3**
2	1 2	8.8 8.8	**8.8**	17.2 19.4	**18.3**	56.8 56.2	**56.5**	82.8 84.4	**83.6**
3	1 2	4.8 5.3	**5.0**	14.1 15.8	**14.9**	74.3 61.7	**68.0**	93.1 82.8	**88.0**
4	1 2	2.6 3.1	**2.8**	12.8 13.6	**13.2**	70.4 69.3	**69.9**	85.8 86.0	**85.9**
7	1 2	1.8 1.9	**1.9**	10.6 10.9	**10.7**	(4.1)§ 75.1	**75.1**	(16.6)§ 87.9	**87.9**
10	1 2	1.6 1.7	**1.6**	8.5 9.1	**8.8**	80.3 79.0	**79.6**	90.4 89.7	**90.0**
31	1 2	0.8 0.8	**0.8**	5.6 6.1	**5.7**	84.7 85.7	**85.2**	91.1 92.6	**91.8**

AR = Applied Radioactivity; - = not determined; mv = mean values

4 subsamples of the total applied soil batch

***** mean values of 4 subsamples of the total applied soil batch

§ Replicate for CO_2 determination leaked, value not taken into account for mass balance calculation

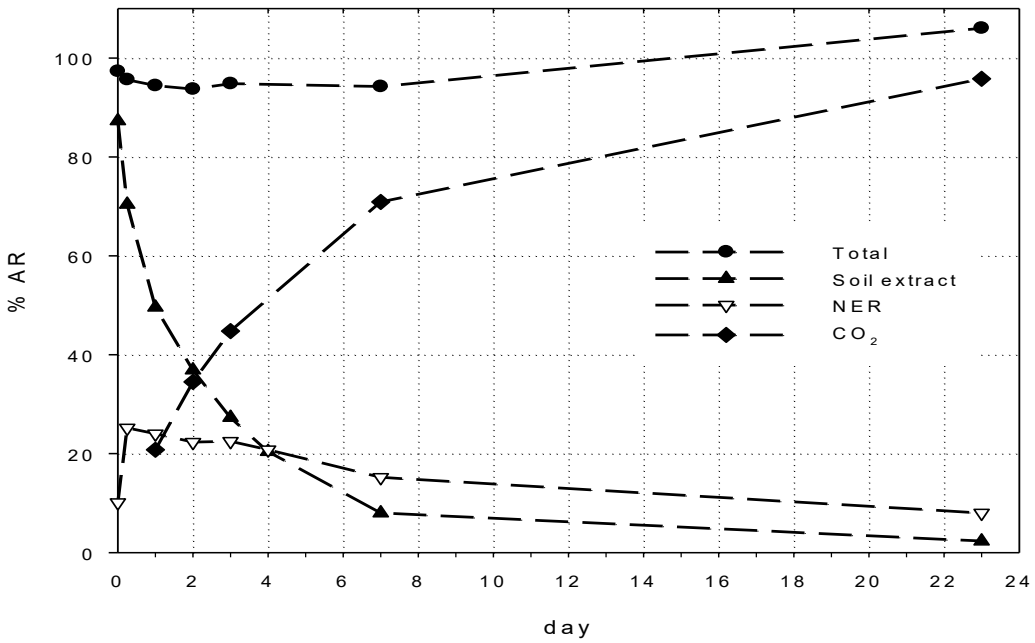

Figure A7_2_1-1: Soil 2.2: Distribution of applied radioactivity and mineralisation

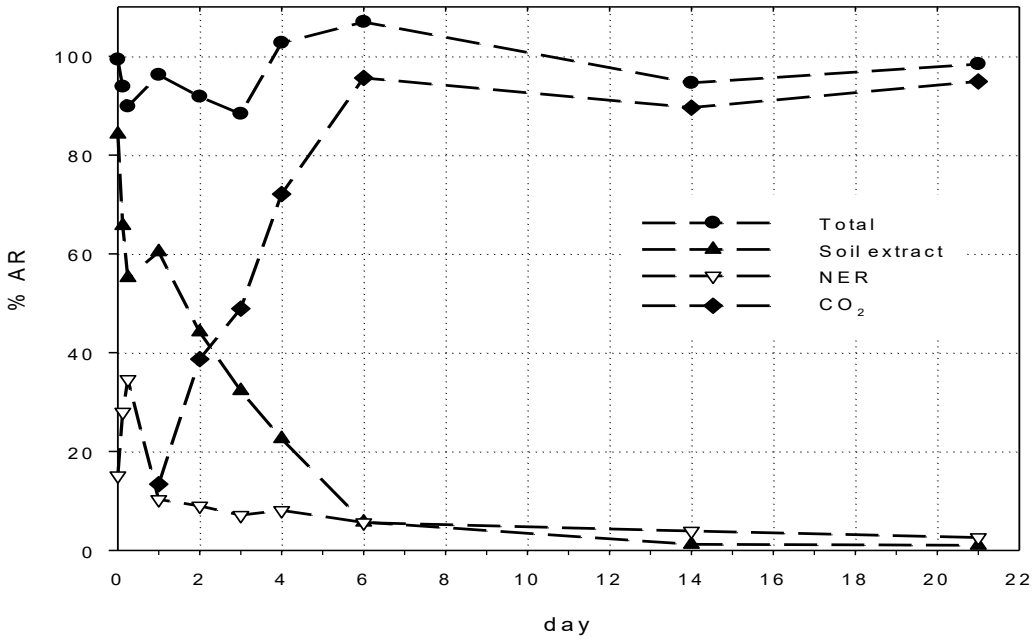

Figure A7_2_1-2: Soil 5M: Distribution of applied radioactivity and mineralization

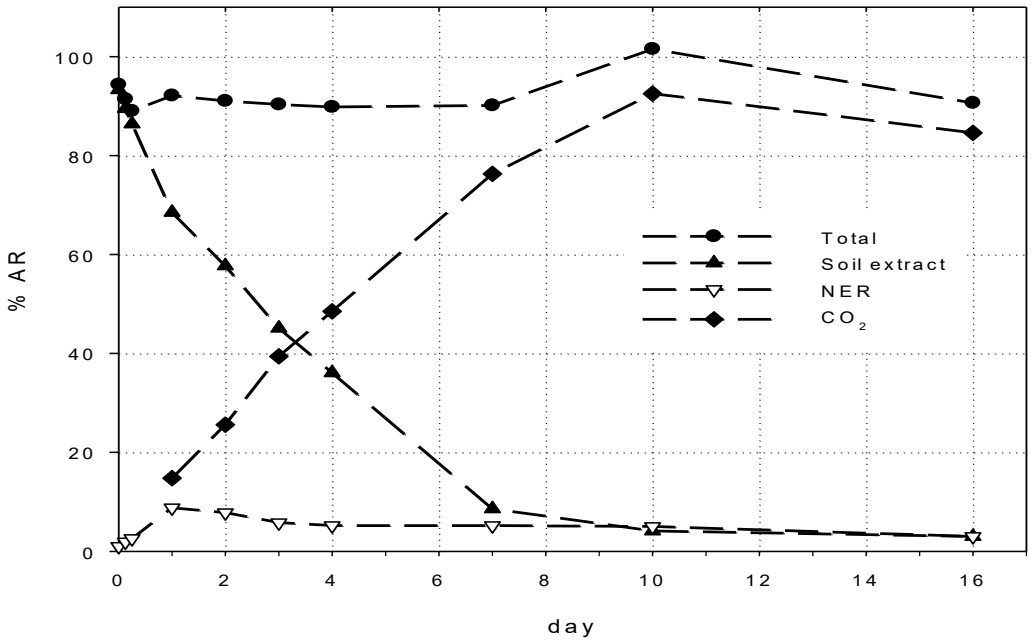

Figure A7_2_1-3: Soil 2.3: Distribution of applied radioactivity and mineralisation

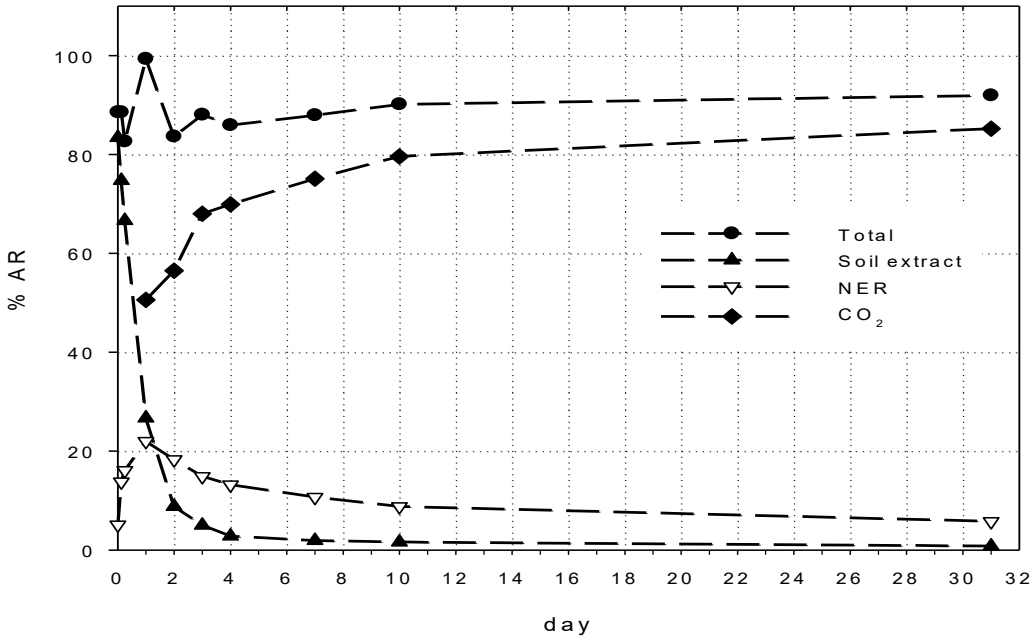

Figure A7_2_1-3: Soil 2.4: Distribution of applied radioactivity and mineralization

Table A7_2_1-10: Distribution of radioactivity in the extract of soil 2.2

Exposure Day	Repl.	Total Activity % of AR	mv	Activity of Test Item and Metabolites % of AR	mv	Residue Activity % of AR	mv
0 (0.33 h)	4#	87.3*	**87.3**	60.9*	**60.9**	26.4	**26.4**
0 (4 h)	4#	70.4*	**70.4**	56.9*	**56.9**	13.5	**13.5**
1	1 2	48.3 50.8	**49.6**	41.6 42.5	**42.1**	6.7 8.3	**7.5**
2	1 2	36.2 37.6	**36.9**	33.1 34.6	**33.9**	3.1 3.0	**3.0**
3	1 2	27.3 27.4	**27.3**	27.5 28.9	**28.2**	-0.2 -1.5	**-0.8**
4	1 2	19.8 21.0	**20.4**	13.4 15.0	**14.2**	6.4 6.0	**6.2**
7	1 2	8.2 7.9	**8.0**	4.5 4.4	**4.4**	3.7 3.5	**3.6**
23	1 2	2.4 2.1	**2.3**	0.4 0.4	**0.4**	2.0 1.7	**1.8**

AR = Applied Radioactivity; mv = mean values

4 subsamples of the total applied soil batch

* mean values of 4 subsamples of the total applied soil batch

Table A7_2_1-11: Distribution of radioactivity in the extract of soil 5M

Exposure Day	Repl.	Total Activity % of AR	mv	Activity of Test Item and Metabolites % of AR	mv	Residue Activity % of AR	mv
0 (0.33 h)	4#	84.2*	**84.2**	59.4*	**59.4**	24.8	**24.8**
0 (2 h)	4#	65.7*	**65.7**	48.0*	**48.0**	17.7	**17.7**
0 (4 h)	4#	55.2*	**55.2**	42.1*	**42.1**	13.1	**13.1**
1	1 2	61.1 59.7	**60.4**	49.9 47.3	**48.6**	11.2 12.4	**11.8**
2	1 2	43.1 45.3	**44.2**	36.5 36.8	**36.6**	6.9 8.8	**7.9**
3	1 2	31.3 33.3	**32.3**	28.1 28.6	**28.3**	3.2 4.7	**4.0**
4	1 2	20.3 24.9	**22.6**	15.7 21.1	**18.4**	4.6 3.8	**4.2**
6	1 2	5.9 5.5	**5.7**	2.1 2.0	**2.1**	3.8 3.5	**3.6**
14	1 2	1.2 1.1	**1.2**	0.2 0.2	**0.2**	1.0 0.9	**1.0**
21	1 2	0.9 1.0	**1.0**	< LOD < LOD	**< LOD**	0.9 1.0	**1.0**

AR = Applied Radioactivity; mv = mean values

4 subsamples of the total applied soil batch

* mean values of 4 subsamples of the total applied soil batch

Table A7_2_1-12: Distribution of radioactivity in the extract of soil 2.3

Exposure Day	Repl.	Soil % of AR	mv	Activity of Test Item and Metabolites % of AR	mv	Residue Activity % of AR	mv
0 (0.33 h)	4#	93.3*	**93.3**	62.3*	**62.3**	31.0	**31.0**
0 (2 h)	4#	89.5*	**89.5**	73.1*	**73.1**	16.4	**16.4**
0 (4 h)	4#	86.4*	**86.4**	72.3*	**72.1**	14.1	**14.1**
1	1 2	69.7 67.3	**68.5**	60.1 62.7	**61.4**	9.6 4.6	**7.1**
2	1 2	58.1 57.2	**57.7**	54.0 59.0	**56.5**	4.1 -1.8	**1.2**
3	1 2	44.8 45.2	**45.1**	36.9 41.5	**39.2**	7.9 3.7	**5.8**
4	1 2	36.7 35.5	**36.1**	28.5 27.6	**28.1**	8.2 7.9	**8.0**
7	1 2	9.2 8.0	**8.6**	3.0 2.4	**2.7**	6.2 5.6	**5.9**
10	1 2	4.0 4.1	**4.1**	0.4 0.4	**0.4**	3.6 3.7	**3.7**
16	1 2	2.4 3.6	**3.0**	0.2 0.3	**0.3**	2.2 3.3	**2.7**

AR = Applied Radioactivity; - = not determined; mv = mean values

4 subsamples of the total applied soil batch

* mean values of 4 subsamples of the total applied soil batch

Table A7_2_1-13: Distribution of radioactivity in the extract of soil 2.4

Exposure Day	Repl.	Soil % of AR	mv	Activity of Test Item and Metabolites % of AR	mv	Residue Activity % of AR	mv
0 (0.33 h)	4#	83.4*	**83.4**	63.0*	**63.0**	20.4	**20.4**
0 (2 h)	4#	74.7*	**74.7**	59.1*	**59.1**	15.6	**15.6**
0 (4 h)	4#	66.6*	**66.6**	50.6*	**50.6**	16.0	**16.0**
1	1 2	27.3 26.1	**26.7**	21.0 22.5	**21.8**	6.3 3.6	**4.9**
2	1 2	8.8 8.8	**8.8**	3.7 4.8	**4.3**	5.1 4.0	**4.5**
3	1 2	4.8 5.3	**5.0**	1.7 1.8	**1.7**	3.1 3.5	**3.3**
4	1 2	2.6 3.1	**2.8**	0.2 0.4	**0.3**	2.4 2.7	**2.6**
7	1 2	1.8 1.9	**1.9**	< LOD < LOD	< LOD	1.8 1.9	**1.9**
10	1 2	1.6 1.7	**1.6**	< LOD < LOD	< LOD	1.6 1.7	**1.7**
31	1 2	0.8 0.8	**0.8**	< LOD < LOD	< LOD	0.8 0.8	**0.8**

AR = Applied Radioactivity; - = not determined; mv = mean values

AR = Applied Radioactivity; - = not determined; mv = mean values

4 subsamples of the total applied soil batch

* mean values of 4 subsamples of the total applied soil batch

Table A7_2_1-14: Soil 2.2: Transformation of SONC969 Saltidin, [carboxyl-^{14}C]- and ^{14}C-Saltidin acid

Exposure Day RRT	Repl.	Soil Extract % of ARs SONC969 Saltidin, [carboxyl-^{14}C]- 1	mv	^{14}C- Saltidin acid (M1) 0.96	mv	M2 1.12	mv
0 h*	-	**96.4**	**96.4**	**3.6**	**3.6**	-	-
0 (0.33h)	1 2	**65.1** **59.2**	**62.1**	**31.1** **32.7**	**31.9**	< LOD < LOD	< LOQ
0 (4h)	1 2	0.6 1.1	0.9	**83.0** **86.1**	**84.6**	**2.5** **2.4**	**2.4**
1	1 2	*0.3* *0.3*	*0.3*	**62.1** **63.5**	**62.8**	**1.8** **1.9**	**1.8**
2	1 2	< LOD < LOD	< LOD	**49.6** **51.9**	**50.8**	1.5 1.6	1.5
3	1 2	< LOD < LOD	< LOD	**40.9** **43.1**	**42.0**	1.5 1.5	1.5
4	1 2	< LOD < LOD	< LOD	**20.8** **23.2**	**22.0**	0.6 1.0	0.8
7	1 2	< LOD < LOD	< LOD	**6.6** **6.5**	**6.5**	< LOD < LOD	< LOQ
23	1 2	< LOD < LOD	< LOD	0.7 0.7	0.7	< LOD < LOD	< LOQ

* Stock solution prior to application; - = not detected ; mv = mean values

ARs = Applied Radioactivity related to SONC969 Saltidin, [carboxyl-^{14}C]- and ^{14}C-Saltidin acid

Assignment of character style:

bold = values > LOQ, normal = values < LOQ, *italics* = values < LOD, calculated with ½ LOD

Table A7_2_1-15: Soil 5M: Transformation of SONC969 Saltidin, [carboxyl-^{14}C]- and ^{14}C-Saltidin acid

Exposure Day RRT	Repl.	Soil Extract % of AR_S SONC969 Saltidin, [carboxyl-^{14}C]- 1	mv	^{14}C- Saltidin acid (M1) 0.96	mv	M2 1.12	mv
0 h*	-	**94.2**	**94.2**	**5.8**	**5.8**	-	-
0 (0.33h)	1 2	**83.0** **79.3**	**81.1**	**9.1** **9.9**	**9.5**	- -	-
0 (2h)	1 2	**28.5** **25.2**	**26.8**	**45.4** **44.4**	**44.9**	1.2 **1.7**	1.5
0 (4h)	1 2	**2.4** **2.9**	**2.6**	**59.0** **60.5**	**59.8**	**2.0** **1.7**	**1.8**
1	1 2	*0.3* *0.3*	*0.3*	**73.5** **69.8**	**71.6**	**2.4** **2.2**	**2.3**
2	1 2	< LOD < LOD	< LOQ	**54.2** **54.2**	**54.2**	**1.9** 1.5	**1.7**
3	1 2	< LOD < LOD	< LOQ	**41.4** **42.2**	**41.8**	1.4 1.4	1.4
4	1 2	< LOD < LOD	< LOQ	**23.0** **31.0**	**27.0**	0.9 1.1	1.0
6	1 2	< LOD < LOD	< LOQ	**2.9** **2.8**	**2.9**	< LOD < LOD	< LOQ
14	1 2	< LOD < LOD	< LOQ	*0.3* *0.3*	*0.3*	< LOD < LOD	< LOQ
21	1 2	< LOD < LOD	< LOQ	< LOD < LOD	< LOQ	< LOD < LOD	< LOQ

* Stock solution prior to application; - = not detected; mv = mean values

AR_S = Applied Radioactivity related to SONC969 Saltidin, [carboxyl-^{14}C]- and ^{14}C-Saltidin acid

Assignment of character style:

bold = values > LOQ, normal = values < LOQ, *italics* = values < LOD, calculated with ½ LOD

Table A7_2_1-16: Soil 2.3: Transformation of SONC969 Saltidin, [carboxyl-^{14}C]- and ^{14}C-Saltidin acid

Exposure Day / RRT	Repl.	Soil Extract % of ARS: SONC969 Saltidin, [carboxyl-^{14}C]- 1	mv	^{14}C- Saltidin acid (M1) 0.96	mv	M2 1.12	mv	M3 1.22	mv	M4 0.57	mv
0 h*	-	**93.9**	**93.6**	**6.1**	**6.1**	-	-	-	-	-	-
0 (0.33h)	1	**57.2**	**56.5**	**23.0**	**26.4**	-	-	**14.8**	**14.7**	-	-
	2	**55.7**		**29.8**		-		**14.7**		-	
0 (2h)	1	1.2	1.3	**91.6**	**93.1**	**2.5**	**2.6**	< LOD	< LOQ	-	-
	2	1.5		**94.6**		**2.6**		< LOD		-	
0 (4h)	1	*0.3*	*0.3*	**94.4**	**95.5**	**2.4**	**2.5**	< LOD	< LOQ	1.0	**1.4**
	2	*0.3*		**96.6**		**2.6**		< LOD		**1.8**	
1	1	< LOD	< LOQ	**80.4**	**80.3**	**2.0**	**2.0**	< LOD	< LOQ	**2.0**	**2.2**
	2	< LOD		**80.2**		**2.0**		< LOD		**2.4**	
2	1	< LOD	< LOQ	**64.5**	**67.1**	**1.8**	**1.9**	< LOD	< LOQ	< LOD	< LOQ
	2	< LOD		**69.7**		**2.1**		< LOD		< LOD	
3	1	< LOD	< LOQ	**39.9**	**43.9**	0.9	1.0	< LOD	< LOQ	< LOD	< LOQ
	2	< LOD		**48.0**		1.2		< LOD		< LOD	
4	1	< LOD	< LOQ	**30.6**	**30.2**	0.5	0.7	< LOD	< LOQ	< LOD	< LOQ
	2	< LOD		**29.9**		1.0		< LOD		< LOD	
7	1	< LOD	< LOQ	**4.6**	**4.2**	< LOD	< LOQ	< LOD	< LOQ	< LOD	< LOQ
	2	< LOD		**3.7**		< LOD		< LOD		< LOD	
10	1	< LOD	< LOQ	0.7	0.6	< LOD	< LOQ	< LOD	< LOQ	< LOD	< LOQ
	2	< LOD		0.6		< LOD		< LOD		< LOD	
16	1	< LOD	< LOQ	*0.3*	0.4	< LOD	< LOQ	< LOD	< LOQ	< LOD	< LOQ
	2	< LOD		0.5		< LOD		< LOD		< LOD	

* Stock solution prior to application; - = not detected; mv = mean values

ARS = Applied Radioactivity related to SONC969 Saltidin, [carboxyl-^{14}C]- and ^{14}C-Saltidin acid

Assignment of character style:

bold = values > LOQ, normal = values < LOQ, *italics* = values < LOD, calculated with ½ LOD

Table A7_2_1-16 cont: Soil 2.3: Transformation of SONC969 Saltidin, [carboxyl-^{14}C]- and ^{14}C-Saltidin acid

Exposure Day / RRT	Repl.	Soil Extract % of ARs M5 0.65	mv	M6 0.67	mv	M7 0.70	mv	M8 0.71	mv	M9 0.63	mv
0 h*	-	1.7	**1.7**[1)]	1.6	**1.6**[1)]	-	-	-	-	-	-
0 (0.33h)	1	-	-	-	-	-	-	-	-	-	-
	2	-		-		-		-		-	
0 (2h)	1	-	-	**6.7**	**6.9**	-	-	**1.7**	**1.8**	-	-
	2	-		**7.1**		-		**1.8**		-	
0 (4h)	1	-	**1.5**	**7.8**	**5.7**	-	**0.8**	**2.2**	**1.1**	-	-
	2	**2.9**		**3.7**		**1.6**		< LOD		-	
1	1	**3.1**	**3.3**	**4.2**	**5.5**	**1.7**	**1.3**	0.5	**1.5**	-	-
	2	**3.5**		**6.7**		0.9		**2.5**		-	
2	1	**7.0**	**6.9**	**8.2**	**8.5**	< LOD	< LOQ	**3.1**	**3.4**	-	0.7
	2	**6.7**		**8.8**		< LOD		**3.6**		1.5	
3	1	**4.9**	**5.1**	**8.2**	**7.6**	< LOD	0.3	**2.8**	**2.4**	1.1	1.0
	2	**5.3**		**7.0**		0.6		**2.0**		1.0	
4	1	**5.9**	**3.0**	**5.4**	**7.4**	1.2	0.6	< LOD	0.9	1.0	1.3
	2	< LOD		**9.5**		< LOD		1.3		1.5	
6	1	< LOD	< LOQ	< LOD	< LOQ	< LOD	< LOQ	< LOD	< LOQ	< LOD	< LOQ
	2	< LOD		< LOD		< LOD		< LOD		< LOD	
10	1	< LOD	< LOQ	< LOD	< LOQ	< LOD	< LOQ	< LOD	< LOQ	< LOD	< LOQ
	2	< LOD		< LOD		< LOD		< LOD		< LOD	
16	1	< LOD	< LOQ	< LOD	< LOQ	< LOD	< LOQ	< LOD	< LOQ	< LOD	< LOQ
	2	< LOD		< LOD		< LOD		< LOD		< LOD	

***** Stock solution prior to application;- = not detected; mv = mean values

[1)] related to total activity of the stock solution

ARs = Applied Radioactivity related to SONC969 Saltidin, [carboxyl-^{14}C]- and ^{14}C-Saltidin acid

Assignment of character style:

bold = values > LOQ, normal = values < LOQ, *italics* = values < LOD, calculated with ½ LOD

Table A7_2_1-17: Soil 2.4: Transformation of SONC969 Saltidin, [carboxyl-^{14}C]- and ^{14}C-Saltidin acid

Exposure Day RRT	Repl.	Soil Extract % of AR_S SONC969 Saltidin, [carboxyl-^{14}C]- 1	mv	^{14}C- Saltidin acid (M1) 0.96	mv	M2 1.12	mv	M3 1.22	mv	M4 0.57	mv
0 h*	-	**93.6**	**93.6**	**6.4**	**6.4**	-	-	-	-	-	-
0 (0.33h)	1 2	**57.1** **55.6**	**56.4**	**29.6** **29.7**	**29.7**	- -	-	**5.7** **7.1**	**6.4**	- -	-
0 (2h)	1 2	**4.5** **4.1**	**4.3**	**76.0** **80.2**	**78.1**	**2.3** **2.5**	**2.4**	< LOD < LOD	< LOD	**2.0** 1.6	**1.8**
0 (4h)	1 2	0.9 1.1	1.0	**69.5** **68.1**	**68.8**	**2.1** **2.1**	**2.1**	< LOD < LOD	< LOD	**2.1** **2.4**	**2.3**
1	1 2	*0.3* *0.3*	*0.3*	**28.9** **30.9**	**29.9**	0.7 1.0	0.9	< LOD < LOD	< LOD	0.9 0.9	0.9
2	1 2	< LOD < LOD	< LOD	**4.7** **6.3**	**5.5**	< LOD < LOD	< LOD	< LOD < LOD	< LOD	0.5 0.5	0.5
3	1 2	< LOD < LOD	< LOD	**2.2** **2.4**	**2.3**	< LOD < LOD	< LOD	< LOD < LOD	< LOD	< LOD < LOD	< LOD
4	1 2	< LOD < LOD	< LOD	*0.3* 0.6	0.4	< LOD < LOD	< LOD	< LOD < LOD	< LOD	< LOD < LOD	< LOD
7	1 2	< LOD < LOD	< LOD	< LOD < LOD	< LOD	< LOD < LOD	< LOD	< LOD < LOD	< LOD	< LOD < LOD	< LOD
10	1 2	< LOD < LOD	< LOD	< LOD < LOD	< LOD	< LOD < LOD	< LOD	< LOD < LOD	< LOD	< LOD < LOD	< LOD
31	1 2	< LOD < LOD	< LOD	< LOD < LOD	< LOD	< LOD < LOD	< LOD	< LOD < LOD	< LOD	< LOD < LOD	< LOD

* Stock solution prior to application;- = not detected; mv = mean values

AR_S = Applied Radioactivity related to SONC969 Saltidin, [carboxyl-^{14}C]- and ^{14}C-Saltidin acid

Assignment of character style:

bold = values > LOQ, normal = values < LOQ, *italics* = values < LOD, calculated with ½ LOD

Table A7_2_1-18: Kinetic data of SONC969 Saltidin, [carboxyl-^{14}C]- and ^{14}C-Saltidin acid

Endpoint / Statistic	Soil			
	2.2	**5M**	**2.3**	**2.4**
Model	**Single First Order (SFO)**	**Single First Order (SFO)**	**Single First Order (SFO)**	**Single First Order (SFO)**
C_0	**96.5** ± 2.13	**93.5** ± 3.96	**90.7** ± 3.54	**93.1** ± 1.90
Initial value for fitting	*96.0*	*94.0*	*94.0*	*93.0*
K_P (1/d)	**1.337** ± 0.0937	**0.5296** ±0.0581	**1.224** ± 0.1220	**1.475** ± 0.0859
Initial value for fitting	*0.8*	*1.0*	*1.0*	*0.8*
K_{M1} (1/d)	**0.0128** ± 0.00065	**0.0107** ± 0.00135	**0.0130** ± 0.000854	**0.0483** ±0.00297
Initial value for fitting	*0.05*	*0.03*	*0.03*	*0.05*
ffM1 (as a fraction)	**0.9188** ± 0.0318	**0.8376** ± 0.0676	**1.202** ± 0.06349	**0.9071** ± 0.0301
Initial value for fitting	*0.9*	*0.9*	*0.9*	*0.9*
SONC969 Saltidin, [carboxyl-^{14}C]				
χ^2 error	**0.5**	**9.2**	**8.7**	**1.4**
t-Test (P=0.05)	Passed	Passed	Passed	Passed
^{14}C-Saltidin Acid				
χ^2 error	**7.9**	**17.9**	**10.4**	**9.4**
t-Test (P=0.05)	Passed	Passed	Passed	Passed

Table A7_2_1-19: DT values for SONC969 Saltidin, [carboxyl-^{14}C]- and ^{14}C-Saltidin acid

Endpoint	Soil			
	2.2	5M	2.3	2.4
DTX values in hours				
SONC969 Saltidin, [carboxyl-^{14}C]-				
DT_{50}	0.52	1.31	0.57	0.47
DT_{90}	1.72	4.35	1.88	1.56
^{14}C-Saltidin acid				
DT_{50}	54.2	64.5	53.2	14.3
DT_{90}	180	214	177	47.6
DTX values in days				
SONC969 Saltidin, [carboxyl-^{14}C]-				
DT_{50}	0.022	0.055	0.024	0.020
DT_{90}	0.072	0.181	0.078	0.065
^{14}C-Saltidin acid				
DT_{50}	2.26	2.69	2.22	0.596
DT_{90}	7.50	8.92	7.38	1.98

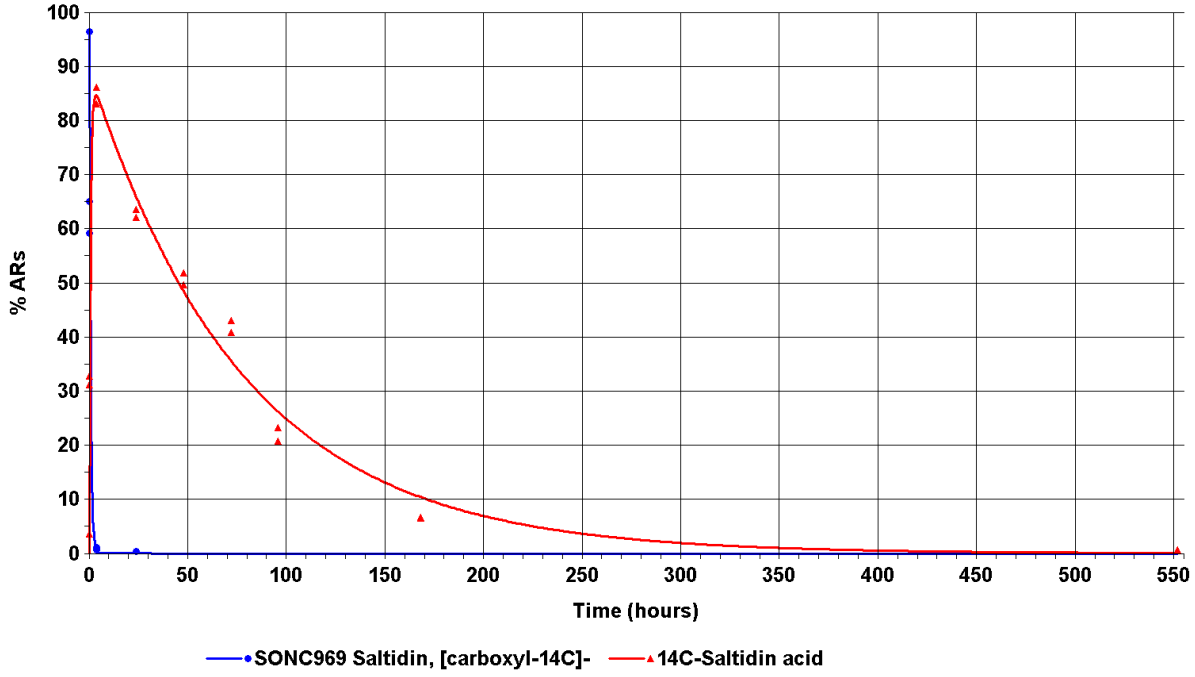

Figure A7_2_1-5: Soil 2.2: Kinetic fit of the degradation of SONC969 Saltidin, [carboxyl-^{14}C]- and ^{14}C-Saltidin acid in soil

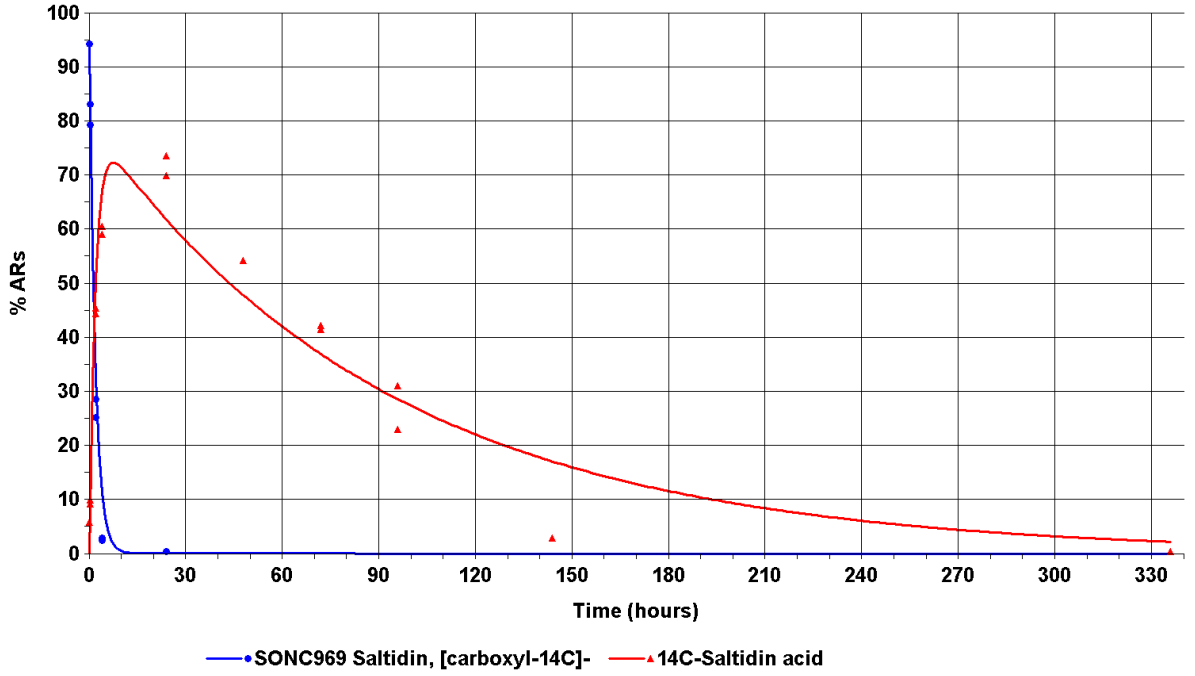

Figure A7_2_1-6: Soil 5M: Kinetic fit of the degradation of SONC969 Saltidin, [carboxyl-^{14}C]- and ^{14}C-Saltidin acid in soil

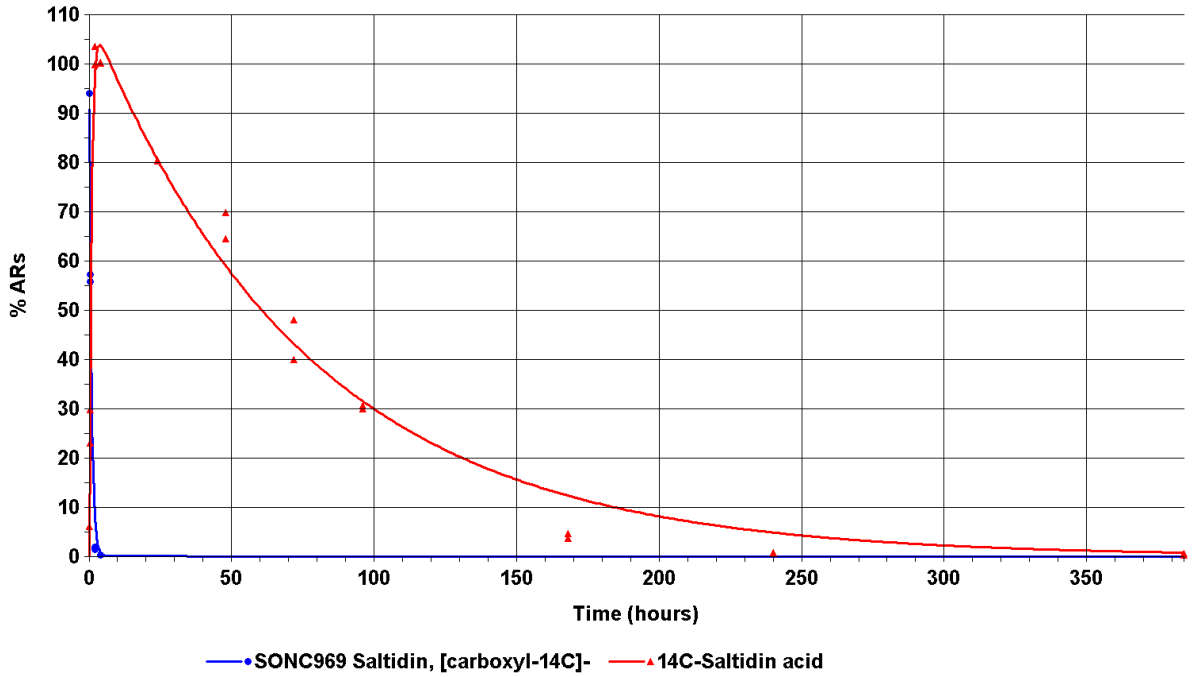

Figure A7_2_1-7: Soil 2.3: Kinetic fit of the degradation of SONC969 Saltidin, [carboxyl-^{14}C]- and ^{14}C-Saltidin acid in soil

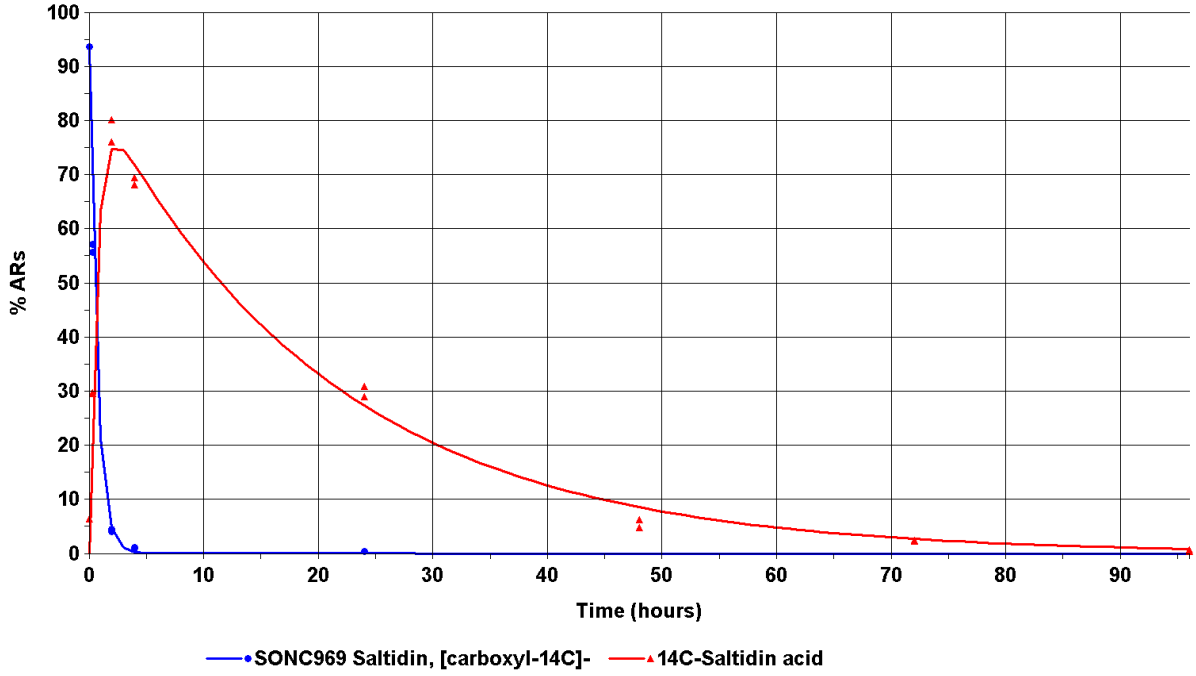

Figure A7_2_1-8: Soil 2.4: Kinetic fit of the degradation of SONC969 Saltidin, [carboxyl-^{14}C]- and ^{14}C-Saltidin acid in soil

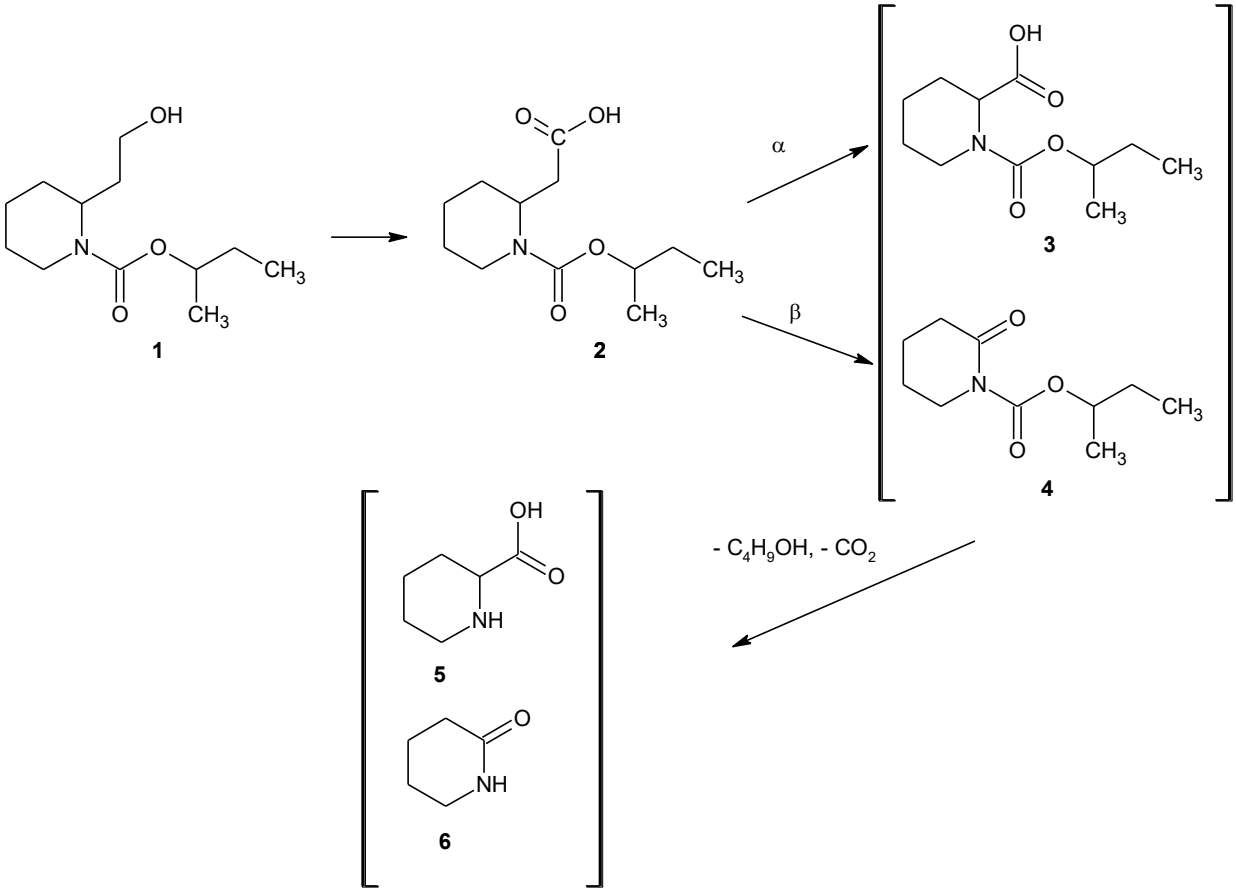

Figure A7_2_1-9: Proposed metabolic pathway of Saltidin in soil under aerobic conditions

Section A7.3.1 — Phototransformation in air (estimation method), including identification of breakdown products

Annex Point: IIIA, VII.5

Official use only

1 REFERENCE

1.1	**Reference**	Beiell, U., 2005, Icaridin (KBR 3023): Calculation of photodegradation, Dr. Knoell Consult GmbH, Mannheim, Germany, unpublished report, 2005-02-18.
1.2	**Data protection**	Yes
1.2.1	Data owner	LANXESS Deutschland 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**	Not applicable since the degradation behaviour of Icaridin in air was calculated.
2.2	**GLP**	Not applicable since the degradation behaviour of Icaridin in air was calculated.
2.3	**Deviations**	No

3 MATERIALS AND METHODS

To assess the degradation behaviour of Icaridin (KBR 3023) in air, its degradation rate in the atmosphere resulting from attack of hydroxyl radicals is calculated using the AOPWIN Program, version 1.91, 2000. The calculation is based on the structural formula. Based on the resulting degradation rate the degradation half-life of Icaridin can be estimated considering a mean OH concentration of 0.5×10^6 molecules per cm^3 as a 24 hour-average.

4 RESULTS

A tropospheric half-life of about 6.87 hours was estimated for Icaridin. The degradation rate was $56.08*10^{-12}$ $cm^3*molecule^{-1}*s^{-1}$.

5 CONCLUSION

5.1	**Conclusion**	The calculation indicates a rapid degradation of Icaridin when potentially entering the atmosphere. Hence, air will not be an environmental compartment of concern for the compound used in repellents.
5.1.1	Reliability	2

Section A7.3.1 Phototransformation in air (estimation method), including identification of breakdown products

Annex Point: IIIA VII.5

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 2007
Materials and Methods	To assess the atmospheric transformation of Icaridin (KBR 3023), its degradation rate in the atmosphere resulting from oxidation by hydroxyl radicals (OH) was calculated using the AOPWIN Program, version 1.91, 2000. Based on the resulting degradation rate the atmospheric transformation half-life of Icaridin was estimated applying a mean atmospheric OH concentration of 0.5 x 10^6 molecules per cm^3 as a 24 hour-average.
Results and discussion	A tropospheric half-life by oxidation by OH radicalof 6.87 hours was estimated for Icaridin. The half-life is based upon an atmospheric transformation rate of $56.08*10^{-12}$ $cm^3*molecule^{-1}*s^{-1}$.
Conclusion	The oxidation of organic compounds by OH radical in the troposphere is only one several potential atmospheric transformation routes. To quantify the atmospheric transformation solely by oxidation by OH radicals is considered conservative.
Reliability	2
Acceptability	acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section 7.3.2 Annex Point IIIA 12.3	Fate and behaviour in air, further studies	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [X]**	**Technically not feasible [ ] Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	Further studies on the fate and behaviour of Icaridin in air were not performed, because the air is not a compartment at risk for the compound. Due to its repellent effect the compound has a certain volatilization potential when applied to the skin surface of humans. However, the tropospheric half-life is very short (6.87 hours) and volatile Icaridin amounts cannot be considered to pose a risk, neither for humans nor for other environmental compartments. According to the risk assessment Icaridin will not exhale from the water phase due to its very low Henry constant.	
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	April 2007	
Evaluation of applicant's justification	Applicant's justification is OK	
Conclusion	Applicant's justification is acceptable	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA VII.7.1 *Oncorhynchus mykiss*

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1996): KBR 3023 techn. – Acute Toxicity (96 hours) to Rainbow Trout (*Oncorhynchus mykiss*) in a Static Test. [redacted], Report No. DOM 96024, 1996-09-02.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Lanxess Deutschland 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**	Yes OECD Guideline 203 (1992), Directive 92/69/EEC, Method C.1 (1992) U.S.-EPA FIFRA § 72-1 (1982)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Icaridin (KBR 3023); as given in section 2 of dossier	
3.1.1	Lot/Batch number	Batch number: 898446008	
3.1.2	Specification	As given in section 2 of dossier	
3.1.3	Purity	97.9%	
3.1.4	Composition of Product	-	
3.1.5	Further relevant properties	Water solubility: about 8.2 g/l (Krohn, 1996)	
3.1.6	Method of analysis	HPLC with UV detection (limit of quantification: 0.02 mg/l)	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	See table A7_4_1_1-1	
3.3	**Reference substance**	No	
3.3.1	Method of analysis for reference substance	-	
3.4	**Testing procedure**		

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA VII.7.1 *Oncorhynchus mykiss*

3.4.1	Dilution water	See table A7_4_1_1-2
3.4.2	Test organisms	See table A7_4_1_1-3
3.4.3	Test system	See table A7_4_1_1-4 To reach well equilibrated aquaria the test substance was added three days before start of testing by the way of intensive mixing with an ultra-turrax. Immediately before the start of the test, water samples were taken from the centre of the aquaria for analytical determination of the active ingredient concentration.
3.4.4	Test conditions	See table A7_4_1_1-5
3.4.5	Duration of the test	96 hours
3.4.6	Test parameter	Mortality; Sublethal and behavioural responses (observations)
3.4.7	Sampling	Observations for mortality and sublethal responses were made after 4 hours and then daily (after 24, 48, 72 and 96 h). Dead individuals were removed at each observation period. Dissolved oxygen and pH values were measured daily in each aquarium, water temperature was measured in the control aquarium and recorded hourly with a data logger.
3.4.8	Monitoring of TS concentration	Yes, analytical measurements were performed at day 0 and at day 4. In case 100% mortality was reached in test concentrations prior to the end of the test, the analytical determinations were made at that time.
3.4.9	Statistics	Statistical analysis of results for 24, 48, 72 and 96 – hour LC_{50} values and their corresponding 95% confidence limits was obtained by employing a computerized program: Stephan, C.E. (1982): US-EPA, Environmental Research Laboratory, Duluth, MN. Personal communication to Dr. Lowell Bahner, Chairman, ASTM Task Group on Calculating LC_{50}. The program estimated the LC_{50} using one of three statistical techniques moving average, binomial probability or probit analysis. The appropriate method was determined on the basis of data characteristics: Stephan, C.E. (1977): Methods for Calculating an LC_{50}. In: Mayer, FL. & Hamelink, J.L. (Eds.): Aquatic Toxicology and Hazard Evaluation, ASTM STP 634, American Society for Testing and Materials, Philadelphia, PA, pp. 65-84.
		4 RESULTS
4.1	**Limit Test**	Not performed
4.1.1	Concentration	-
4.1.2	Number/ percentage of animals showing adverse effects	-

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA VII.7.1 *Oncorhynchus mykiss*

4.1.3	Nature of adverse effects	-
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	Nominal concentrations: 0, 51.8, 86.5, 144, 240 and 400 mg/l
4.2.2	Actual concentrations of test substance	Measured concentrations (mean values): 0, 50.1, 85.6, 145, 240 and 397 mg/l. Under the test conditions the test substance was stable, resulting in mean measured values between 97% and 101% of nominal. All reported results are based on mean measured concentrations of the test substance.
4.2.3	Effect data (Mortality)	See table A7_4_1_1-6 and table A7_4_1_1-7
4.2.4	Concentration / response curve	The mortality increases from 0% to 100% between doses of 85.6 mg/l (0% mortality) and 240 mg/l (100% mortality). A concentration/ response curve is given in the report (p. 14)
4.2.5	Other effects	In the lowest tested concentration at 50.1 mg test substance/l all fish showed the following symptoms: darkened coloration, quiescent- marked by a state of inactivity or low activity and viscous excretion from intestins. See table A7_4_1_1-6 for detailed description of observed responses.
4.3	**Results of controls**	
4.3.1	Number/ percentage of animals showing adverse effects	There were neither mortalities nor symptoms of intoxication in the control group.
4.3.2	Nature of adverse effects	-
4.4	**Test with reference substance**	Not performed
4.4.1	Concentrations	-
4.4.2	Results	-

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	A 96 - hour static (*Oncorhynchus mykiss*) study was conducted in accordance with OECD Guideline 203 (1992), Directive 92/69/EEC, Method C.1 (1992) and U.S.-EPA FIFRA Guideline § 72-1 (1982) in order to estimate the acute toxicity of dichlofluanid to rainbow trout (*Oncorhynchus mykiss*).
5.2	**Results and**	A 96 – hour LC_{50} value was calculated to be 173 mg/l with 95%

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA VII.7.1 *Oncorhynchus mykiss*

	discussion	confidence limits ranging from 145 to 240 mg/l. Since several symptoms of intoxication were observed at the lowest test concentration (50.1 mg a.i./l) a NOAEL value of (< 50.1 mg/l) was derived. The lowest lethal concentration (LLC) was 145 mg/l. The minimum concentration causing 100% mortality (96 h-LC_{100}) was 240 mg/l. All results are based on the measured test concentrations of the test substance.
5.2.1	96h-LC_0	< 50.1 mg/l
5.2.2	96h-LC_{50}	173 mg/l
5.2.3	96h-LC_{100}	240 mg/l
5.3	**Conclusion**	The validity criteria are summarised in table A7_4_1_1-8. The test is considered as valid. The results are used in the environmental risk assessment.
5.3.1	Other Conclusions	-
5.3.2	Reliability	1
5.3.3	Deficiencies	None

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	07 03 07
Materials and Methods	The temperature of the test system (10.9-11.4 °C) was slightly lower than the temperature recommended by the OECD guideline at test on Rainbow Trout (13-17 °C). Acclimation of fish to test temperature was 48 hrs. According to the OECD guideline and the Directive 9h2/69/EEC the acclimation period should be at least 7 days. None of these deviations are assessed to significantly interfere with the interpretation of the test result.
Results and discussion	The lowest concentration of test substance (50.1 mg/l) caused no mortality but abbreviations of behavioural were observed after 24 hours. At the highest concentration (397 mg/l) 100 % mortality was observed. The choice of concentration interval therefore successfully illustrates the effect of Icaridin (KBR 3023) on fish. LC_{50} = 173 mg/l.
Conclusion	The validity criteria of the guidelines are fulfilled and the test is considered valid.
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7_4_1_1-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	No, since test substance has a water solubility of about 8.2 g/l (Krohn, 1996)
Vehicle	None, since test substance has a water solubility of about 8.2 g/l (Krohn, 1996)
Concentration of vehicle	-
Vehicle control performed	-
Other procedures	The suitability of test water for aquatic tests is examined by breeding of *Daphnia magna* in water from the same source (ASTM Guideline E 729-1988). The test water is analysed periodically for undesired impurities.

Table A7_4_1_1-2: Dilution water

Criteria	Details
Source	Reconstituted water prepared by adding salt stock solutions to demineralised water to yield ionic concentrations according to ISO.
Alkalinity	
Hardness	40-60 mg/l
pH	6.9 – 7.2
Oxygen content	Dissolved oxygen 87-102%
Conductance	< 0.2 µS/cm
Holding water different from dilution water	No

Table A7_4_1_1-3: Test organisms

Criteria	Details
Species/strain	Rainbow trout (*Oncorhynchus mykiss*)
Source	Test fish were obtained as green eggs from Dr. Rosengarten, Oesede-Georgsmarienhütte, Germany hatched in the test facility of Bayer AG.
Wild caught	No
Age/size	The green eggs and milt were delivered on 1996-01-10. Mean body wet weight at the beginning of the test was 1.7 (± 0.5) g and mean body standard length was 4.8 (± 0.5) cm. The biomass loading was 0.85 g fish/l test medium.
Kind of food	During the acclimation period fish were fed a commercial trout diet (Brutfutter FB50, Kronen-Fischkraftfutter, Wesel, Germany).
Amount of food	Fish were not fed 48 h before and during the study.
Feeding frequency	Daily
Pretreatment	All fish were held in culture tanks on a 16/8 h light/dark photoperiod and observed for at least 14 days prior to testing. 48 hours before initiation of test, trout were placed in the temperature acclimation unit and held without food during this time. No further pre-teatment of the fish used for the test
Feeding of animals during test	No

Table A7_4_1_1-4: Test system

Criteria	Details
Test type	Static
Renewal of test solution	No renewal of the test solution.
Volume of test vessels	40 l
Volume/animal	2 l
Number of animals/vessel	20
Number of vessels/ concentration	One vessel
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_1-5: Test conditions

Criteria	Details
Test temperature	10.9 – 11.4 °C (daily means)
Dissolved oxygen	87 – 102%
pH	6.9 – 7.2
Adjustment of pH	No
Aeration of dilution water	Yes; Test water was aerated to oxygen saturation with air
Intensity of irradiation	-
Photoperiod	Laboratory environment was maintained on a 16-hour daylight photoperiod

Table A7_4_1_1-6: Cumulative mortality and behavioural observations

Dose[1] (mg test substance/l)	Exposure time									
	4 h		24 h		48 h		72 h		96 h	
	Dead	Obs.	Dead	Obs.	Dead	Obs.	Dead	Obs.	Dead	Obs.
Control	0	0	0	0	0	0	0	0	0	0
50.1	0	0	0	20 DF, H, BO, SD	0	20 DF, AP, BO, SD	0	20 DF, AP, SD	0	20 DF, AP, SD
85.6	0	20 DF, H, BO	0	20 DF, SD, BO, TS, OB	0	20 DF, AP, BO, SD, OB	0	20, DF, SD, BO, H	0	20, DF, SD, BO
145	0	20 DF, H, BO, TS, OB, AT	0	20 DF, AT, SD, BO, TS, SR	0	20 DF, BO, SD, BA, TS, H, SR	2	20, DF, BO, SD, BA, TS, H, SR	3	20, DF, SD, BO, TS, SR
240	0	20 DF, BO, SR	20	20	+	+	+	+	+	+
397	20	20	+	+	+	+	+	+	+	+

[1] Effect data are based on measured concentrations

Abbreviations of behavioural observations

AP	Quiescent – marked by state of inactivity or abnormally low activity
BO	On bottom of aquarium lying
DF	Darkened coloration
AT	Labored respiration
SR	Fish lying on side or on back
TS	Loss of equilibrium - turned laterally, more or less, from the normal body orientation
H	Hyperactive-exaggerated response to stimulus or disturbance
OB	At water surface – rising and remaining unusually long at the surface
SD	Viscous excretion from intestins
+	No observations, all fish dead

+

Table A7_4_1_1-7: Calculated LC_{50} values (based on mean measured concentrations)

Exposure period (h)	LC_{50} (mg test substance/l) [1]	95 % C.I. (mg test substance/l)	Method of statistical calculation
24 h	187	145 – 240	Binominal Probability Method
48 h	187	145 – 240	Binominal Probability Method
72 h	177	145 – 240	Binominal Probability Method
96 h	173	145 - 240	Binominal Probability Method

[1] Effect data are based on measured concentrations

Table A7_4_1_1-8: Validity criteria for acute fish test according to OECD Guideline 203

	fulfilled	Not fulfilled
Mortality of control animals <10%	**X**	
Concentration of dissolved oxygen in all test vessels > 60% saturation	**X**	
Concentration of test substance ≥ 80% of initial concentration during test	**X**	

Criteria for poorly soluble test substances		**X**

Section A7.4.1.2 Acute toxicity to invertebrates

Annex Point IIA VII.7.2 *Daphnia magna*

			Official use only
		1 REFERENCE	
1.1	**Reference**	Heimbach, F. (1996): Acute Toxicity of KBR 3023 (tech.) to Water Fleas (*Daphnia magna*). Bayer AG, Crop Protection, Institute for Environmental Biology. Leverkusen, Germany, Report No. HBF/Dm 162 (unpublished), Date: 1996-07-08	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Lanxess Deutschland 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**	Yes, OECD Guideline 202, Part I: The 24h-EC_{50} Acute immobilisation Test (1984), US-EPA, Pesticide Assessment Guidelines, Series 72-2: Acute Toxicity Test for Freshwater Aquatic Invertebrates	
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	Batch No. 898 446 008	
3.1.2	Specification	As given in section 2 of dossier	
3.1.3	Purity	97.9%	
3.1.4	Composition of Product	-	
3.1.5	Further relevant properties	Water solubility of Icaridin: about 8.2 g/l (Krohn, 1996)	
3.1.6	Method of analysis	HPLC with UV Detection (Method No. 00445; Limit of Quantification: 0.02 mg/l). Report about analytical methods and analytical results (Bayer AG, Report No. MR-538/96, Date: 1996-07-04) is attached to the original report.	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not relevant since Icaridin has a water solubility of about 8.2 g/l (Krohn, 1996).	
3.3	**Reference**	Not in the frame of this study. However, an acute toxicity test was carried under same conditions with the reference substance $K_2Cr_2O_7$,	

	substance	reagent grade with test concentrations of 0.75, 1.00, 1.33, 1.78, 2.37 and 3.16 mg/l. Reference: Heimbach, F. (1996): Results on the toxicity to water fleas of the reference substance potassium dichromate, Bayer AG, Institute of Environmental Science, Leverkusen, Report No. HBF/Dm 155, Date: 1996-03-15.
3.3.1	Method of analysis for reference substance	-
3.4	**Testing procedure**	
3.4.1	Dilution water	See table A7_4_1_2-2
3.4.2	Test organisms	See table A7_4_1_2-3
3.4.3	Test system	See table A7_4_1_2-4
3.4.4	Test conditions	See table A7_4_1_2-5
3.4.5	Duration of the test	48 hours
3.4.6	Test parameter	Mortality and behavioural observation
3.4.7	Sampling	Mortality and behavioural observation was performed at 24 and 48 hours; pH and dissolved oxygen concentration of test samples (control, low, middle and high concentrations of test substance) were controlled at 0 and 48 hours
3.4.8	Monitoring of TS concentration	Yes, analytical measurements of test substance at 0 and 48 hours; see table A7_4_1_2-6
3.4.9	Statistics	Statistical analysis was obtained by employing a computerized program. The LC_{50} values were calculated using the moving average method.
		4 RESULTS
4.1	**Limit Test**	Not performed
4.1.1	Concentration	-
4.1.2	Number/ percentage of animals showing adverse effects	-
4.1.3	Nature of adverse effects	-
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	Nominal concentrations: 0, 10, 18, 32, 56 and 100 mg/l
4.2.2	Actual concentrations of test substance	Measured concentrations (mean values) at day 0 and at day 2 are given in table A7_4_1_2-6). The mean measured concentrations analysed at the beginning and at the

		end of the test were 101.7 to 104.5% of the nominal concentrations (average 103.2 %. These results indicate that the test concentrations prepared for this test correspond to nominal concentrations and that the test substance was stable for the duration of the study. At day 2, following concentrations were measured: 10.5, 18.3, 33.3, 57.9 and 103 mg Icaridin/l.
4.2.3	Effect data (Immobilisation)	See table A7_4_1_2-7 and table A7_4_1_2-8. As in the highest concentration (analysed concentration: 103 mg Icaridin/l) no mortality was observed after 24 and 48 h, an EC_{50} calculation could not be performed and the EC_{50} (24 and 48 h) was stated to be > 103 mg Icaridin/l (based on mean measured concentrations).
4.2.4	Concentration / response curve	Since in the highest test substance concentration no mortality was observed, plotting of a dose-response curve is not possible.
4.2.5	Other effects	No other effects observed
4.3	**Results of controls**	No mortality occurred in the controls
4.4	**Test with reference substance**	For $K_2Cr_2O_7$, a 24-h EC_{50} of 1.72 mg/l (confidence limits 1.55 to 1.91 mg/l) was determined.
4.4.1	Concentrations	$K_2Cr_2O_7$ concentrations: 0.75, 1.00, 1.33, 1.78, 2.37 and 3.16 mg/l.
4.4.2	Results	For $K_2Cr_2O_7$, a 24-h EC_{50} of 1.72 mg/l (confidence limits 1.55 to 1.91 mg/l) was determined
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	Acute toxicity test to *Daphnia magna* was performed in accordance with OECD Guideline 202, Part I (The 24h-EC_{50} Acute immobilisation Test, 1984) and US-EPA, Pesticide Assessment Guidelines, Series 72-2 (Acute Toxicity Test for Freshwater Aquatic Invertebrates). The test animals were exposed under static conditions to measured Icaridin concentrations of 10.5, 18.3, 33.3, 57.9 and 103 mg/l. After 24 and 48 hours, the inability to swim and/or the immobility of the animals was determined.
5.2	**Results and discussion**	As in the highest concentration (analysed concentration: 103 mg Icaridin/l) no mortality was observed after 24 and 48 h, an EC_{50} calculation could not be performed and the EC_{50} (24 and 48 h) was stated to be > 103 mg Icaridin/l (based on mean measured concentrations). No mortality occurred in the controls. The mean measured concentrations showed that the test concentrations prepared for this test correspond to nominal concentrations and that the test substance was stable for the duration of the study.
5.2.1	NOEC	103 mg Icaridin/l after 24 and 48 h
5.2.2	EC_{50}	> 103 mg Icaridin/l after 24 and 48 h
5.2.3	EC_{100}	> 103 mg Icaridin/l after 24 and 48 h
5.3	**Conclusion**	The validity criteria are summarised in table A7_4_1_2-8. All validity

Section A7.4.1.2 Acute toxicity to invertebrates

Annex Point IIA VII.7.2 *Daphnia magna*

		criteria are fulfilled by the study.
5.3.1	Reliability	1
5.3.2	Deficiencies	Yes It must be noted that the LC_{50} value was calculated instead of the EC_{50} value. Therefore the EC_{50} value based on immobilisation is lower than 0.42 mg/l after 48 hours. Information is incomplete about test organism. No concentration/response curve available

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	07 03 07
Materials and Methods	Acute toxicity test to *Daphnia magna* was performed in accordance with OECD Guideline 202, Part I (The 24h-EC_{50} Acute immobilisation Test, 1984) and US-EPA, Pesticide Assessment Guidelines, Series 72-2 (Acute Toxicity Test for Freshwater Aquatic Invertebrates). The test animals were exposed under static conditions to measured Icaridin concentrations of 10.5, 18.3, 33.3, 57.9 and 103 mg/l. After 24 and 48 hours, the immobilisation was determined. The temperature was only measured in one control after 48 hours more measurements are preferred.
Results and discussion	As in the highest concentration (analysed concentration: 103 mg Icaridin/l) no mortality/immobility was observed after 24 and 48 h, an EC_{50} calculation could not be performed and the EC_{50} (24 and 48 h) was stated to be > 103 mg Icaridin/l (based on mean measured concentrations). No mortality occurred in the controls. The mean measured concentrations showed that the test concentrations prepared for this test correspond to nominal concentrations and that the test substance was stable for the duration of the study.
Conclusion	All validity criteria are fulfilled by the study (see below). Immobilisation of control animals <10% Control animals not staying at the surface Concentration of dissolved oxygen in all test vessels >3 mg/l Concentration of test substance ≥ 80% of initial concentration during test
Reliability	1
Acceptability	Acceptable
Remarks	The comment from the applicant in 5.3.2 about the LC50 is not correct. In the study report it is clearly stated that a EC50 is determined on the basis of immobilisation. Thus, the text in table A7_4_1_2-7 and 2-8 should preferably be corrected.
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7_4_1_2-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	Not relevant since Icaridin has a water solubility of about 8.2 g/l (Krohn, 1996).
Vehicle	-
Concentration of vehicle	-
Vehicle control performed	-
Other procedures	-

Table A7_4_1_2-2: Dilution water

Criteria	Details
Source	The test medium is prepared using deionised water and adding mineral salts and vitamins. Test and breeding water was prepared as "M7-medium" as documented in the "Original Draft" of an EEC *Daphnia magna* Pilot Ring Test. The "M7-medium" is similar to the "M4-medium", which is described in: Elendt, B.P. & W.R. Bias (1990): Trace nutrient deficiency in *Daphnia magna* cultered in standard medium for toxicity testing. Water Research, 24, pp. 1157-1167.
Alkalinity ($CaCO_3$)	50 mg/l (3 ° dH)
Hardness ($CaCO_3$)	196 mg/l (11 ° dH)
pH	8.0
Ca / Mg ratio	-
Na / K ratio	-
Oxygen content	8.5 – 8.9 mg/l
Conductance	580 µS/cm
Holding water different from dilution water	No

Table A7_4_1_2-3: Test organisms

Criteria	Details
Strain	*Daphnia magna*, strain from the Bundesgesundheitsamt (Federal Health Agency), Berlin, Germany; the clone was classified as genotype No 2 (Dr. Bradley, University of Sheffield, Report, Date: 1988-02-03) and later renamed as "type B" according to Baird, D.J. et al. (1991): A comparative study of genotype sensitivity to acute stress using clones of *Daphnia magna* Strauss. Ecotoxic. Environ. Safety, 21, pp. 257-265.
Source	The strain has been maintained in own laboratory (Bayer AG, Crop Protection, Institute for Environmental Biology. Leverkusen, Germany) for more than ten years.
Age (at start of the study)	< 24 – hours old
Breeding method	The first instars used in the test were obtained by repeated carefully screening of adults (14-21 d old, only parents of the same age (± 12 h) were used). For this purpose plastic screens with 0.6 and 0.2 mm mesh (according to DIN 4195) were used.
Kind of food	The animals were fed with an aqueous suspension of single cell green algae (*Scenedesmus subspicatus*) and occasionally some commercial ornamental fish food (trade name TetraMin®).
Amount of food	During the test the water fleas were not fed.
Feeding frequency	-
Pre-treatment	-
Feeding of animals during test	During the test the water fleas were not fed. During the holding period daphnids were fed with the above named kind of food.

Table A7_4_1_2-4: Test system

Criteria	Details
Renewal of test solution	The test was performed under static conditions
Volume of test vessels	100 ml glass beakers according to DIN 12332; each test vessel contained 50 ml of test solution
Volume/animal	5 ml
Number of animals/vessel	10
Number of vessels/ concentration	3 replicates per concentration
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_2-5: Test conditions

Criteria	Details
Test temperature	20 ± 1 °C
Dissolved oxygen	8.5 – 8.9 mg/l
pH	8.0
Adjustment of pH	No
Aeration of dilution water	Yes; the dilution water was aerated and tempered to 20 °C in an in-house preparation apparatus. During the test the test solutions were not arated.
Quality/Intensity of irradiation	The light intensity was about 700 lux
Photoperiod	16:8 light-dark cycle (16-h daylight photoperiod)

Table A7_4_1_2-6: Measured test substance concentrations in test solutions

Nominal conc. (mg a.s./l)	Analysed conc. day 0 (mg a.s./l)	% of nominal conc.	Analysed conc. day 2 (mg a.s./l)	% of day 0 analysis	Mean measured conc. (mg a.s./l)	% of nominal conc.
Control	< LOD*	--	< LOD	--	--	--
10	10.4	104.0	10.5	101.0	10.5	104.5
18	18.3	101.7	18.3	100.0	18.3	101.7
32	33.3	104.1	33.3	100.0	33.3	104.1
56	58.2	103.9	57.5	98.8	57.9	103.3
100	102	102.0	103	101.0	103	102.5
Average (%)		**103.1**		**100.1**		**103.2**

* LOD = Limit of detection (0.02 mg/l)

Table A7_4_1_2-7: Mortality data (after 24 and 48 h) and test conditions (after 48 h)

Test Substance Concentration (measured)* [mg/l]	Mortality of *Daphnia*				Test conditions		
	Number		Percentage		Oxygen [mg/l]	pH	Temperature [°C]
	24 h	48 h	24 h	48 h	48 h	48 h	48 h
Control	0	0	0	0	8.5	8.0	20.0**
10.5	0	0	0	0	8.6	8.0	-
18.3	0	0	0	0	8.6	8.0	-
33.3	0	0	0	0	8.5	8.0	-
57.9	0	0	0	0	8.5	8.0	-
103	0	0	0	0	8.5	8.0	-

* Test substance concentrations are mean measured concentrations
** Temperature was only measured in one control beaker at the end of the study

Table A7_4_1_2-8: Effect data (measured concentrations)*

	LC_{50}	95 % c.l.	LC_0	LC_{100}
24 h [mg/l]	> 103	-	> 103	> 103
48 h [mg/l]	> 103	-	> 103	> 103

* Since no mortality was observed in any test substance concentration, an EC_{50} calculation was not possible.

Table A7_4_1_2-9: Validity criteria for acute daphnia immobilisation test according to OECD Guideline 202

	fulfilled	Not fulfilled
Immobilisation of control animals <10%	**X**	
Control animals not staying at the surface	**X**	
Concentration of dissolved oxygen in all test vessels >3 mg/l	**X**	
Concentration of test substance ≥ 80% of initial concentration during test	**X**	

Criteria for poorly soluble test substances	**Not applicable**	

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA VII.7.3 *Scenedesmus Subspicatus*

			Official use only
		1 REFERENCE	
1.1	**Reference**	Anderson, J.P.E. (1996): Influence of KBR 3023 Technical on the Growth of the Green Alga, *Scenedesmus subspicatus*. Bayer AG, Institute for Environmental Biology, Leverkusen, Germany, Report No. 107689 (AJO/146496), Date: 1996-09-02.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Lanxess Deutschland 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**	Yes, the test was performed in accordance with following guidelines: Directive 79/831/EEC, Annex V, Method C.3 (Algal Inhibition Test, Revised Version No. L 383 A/179; 1992-12-29); OECD guideline No. 201 (Alga Growth Inhibition Test, 1984); ISO Guideline No. 8692 (Water Quality – Fresh Water Algal Growth Inhibition Test with *Scenedesmus subspicatus*; 1989-11-15).	
2.2	**GLP**	Yes	
2.3	**Deviations**	None	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Icaridin (KBR 3023), technical ingredient	
3.1.1	Lot/Batch number	Batch number: 898446008	
3.1.2	Specification	As given in section 2 of dossier	
3.1.3	Purity	97.9%	
3.1.4	Composition of Product	-	
3.1.5	Further relevant properties	Water solubility of Icaridin: about 8.2 g/l (Krohn, 1996)	
3.1.6	Method of analysis	HPLC with UV Detection (Method No. 00445; Limit of Quantification: 0.02 mg/l). Report about analytical methods and analytical results (Bayer AG, Report No. MR-539/96, Date: 1996-07-04) is attached to the original report.	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not applicable since water solubility of Icaridin is about 8.2 g/l (Krohn, 1996)	
3.3	**Reference substance**	Yes, Potassium dichromate ($K_2Cr_2O_7$)	
3.3.1	Method of analysis	No data	

	for reference substance	
3.4	**Testing procedure**	
3.4.1	Culture medium	Two nutrient solutions were used: **Nutrient solution 1**, which was used to grow stock cultures of algae, is described in: Bringmann, G. and R. Kuehn (1980): Comparison of the toxicity thresholds of water pollutants to bacteria, algae and protozoa in the Cell Multiplication Inhibition Test. *Water Research, 14, pp. 231-241*. **Nutrient solution 2**, which was used for all other tests with the algae, was prepared - with slight modifications - according to: - Directive 79/831/EEC, Annex V, Method C.3; 1992-12-29); -OECD guideline No. 201 (Alga Growth Inhibition Test, 1984); - ISO Guideline No. 8692 (1989).
3.4.2	Test organisms	See table A7_4_1_3-1
3.4.3	Test system	See table A7_4_1_3-2
3.4.4	Test conditions	See table A7_4_1_3-3
3.4.5	Duration of the test	96 hours
3.4.6	Test parameter	Effects of Icaridin on the growth of the green alga *Scenedesmus subspicatus*: - The Icaridin concentration at which there was 50% inhibition of growth of biomass (E_bC_{50}) and - The Icaridin concentration at which there was 50% inhibition of the growth rate (E_rC_{50}) Also detected were the lowest concentration at which there was an observable effect (LOEC) and the concentration at which there was no observed effect (NOEC).
3.4.7	Sampling	Samples to determine the number of algae/ml suspension were taken at 24, 48 and 72 hours. pH of test medium was controlled at the beginning of the test and after 24, 48 and 72 hours.
3.4.8	Monitoring of TS concentration	Yes; Analytical concentrations of test substance in the test medium were determined for all nominal KBR 3023 tech. concentration levels (control, 5.60, 10.0, 18.0, 32.0, 56.0 and 100 mg/ml). The investigations with cell-free samples were made at the beginning of the test. Because growing algal cells can adsorb, incorporate and/or metabolise the active ingredient under study, concentrations were not determined at the end of the experiment.
3.4.9	Statistics	The EC_{50} values for growth of biomass (E_bC_{50}) and for algal growth rate (E_rC_{50}) were calculated using probit analyses after: Finney, D.J. (1952): Statistical Methods in Biological Assay, London. The slopes of the regression lines were calculated following Litchfeld and Wilcoxon (1949): A simplified method of evaluating dose-effect experiments. *J. Pharmacology, 31, pp. 99-113.* Calculations were carried out using commercial software (Ratte, H.T.

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA VII.7.3 *Scenedesmus Subspicatus*

(1993): Easy Assay, Algae Growth Inhibition. SPiRiT Aachen, Aachen Germany).

The NOEC and LOEC values were calculated by an analysis of variance (Dunnett's-Test).

4 RESULTS

4.1	**Limit Test**	Not performed
4.1.1	Concentration	-
4.1.2	Number/ percentage of animals showing adverse effects	-
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	Nominal concentrations: 5.6, 10.0, 18.0, 32.0, 56.0 and 100.0 mg/l
4.2.2	Actual concentrations of test substance	Measured concentrations of KBR 3023 tech. (average of two determinations) at beginning of test (Day 0)

Nominal concentration (mg/l)	Measured concentration (mg/l)	% of nominal concentration
5.60	5.18	95
10.0	9.29	95
18.0	16.3	93
32.0	29.4	94
56.0	51.7	94
100.0	92.1	94
Average		**94**

Analytical analyses showed good agreement between mean measured concentrations (94%) and nominal concentrations. So the nominal concentrations were used for all calculations.

4.2.3	Growth curves	A growth curve (number of cells vs. time) is given in the report (p. 17)
4.2.4	Concentration / response curve	Growth inhibition curves (effect of test substance on amount of algal biomass vs. test substance concentration as well as effect of test substance on the algal growth rate vs. test substance concentration, respectively) are plotted in the report (pp. 18-19).
4.2.5	Cell concentration data	See table A7_4_1_3-4
4.2.6	Effect data (cell multiplication inhibition)	See table A7_4_1_3-5, Table A7_4_1_3-6 and Table A7_4_1_3-7

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA VII.7.3 *Scenedesmus Subspicatus*

4.2.7	Other observed effects	-
4.3	**Results of controls**	See table A7_4_1_3-5 and Table A7_4_1_3-6
4.4	**Test with reference substance**	Performed
4.4.1	Concentrations	Control, 0.10, 0.18, 0.32, 0.56, 1.00 and 1.80 mg/l
4.4.2	Results	The 72 h-EC_{50} value of potassium dichromate determined for algal growth rate was 1.15 mg/.l
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	The influence of KBR 3023 tech. on the growth of the green alga *Scenedesmus subspicatus* was investigated in a 72 h hours static test according to following guidelines: Directive 79/831/EEC, Annex V, Method C.3 (Algal Inhibition Test, Revised Version No. L 383 A/179; 1992-12-29); OECD guideline No. 201 (Alga Growth Inhibition Test, 1984); ISO Guideline No. 8692 (Water Quality – Fresh Water Algal Growth Inhibition Test with Scenedesmus subspicatus; 1989-11-15). The test shows no significant deviations from the guidelines.
5.2	**Results and discussion**	
5.2.1	NOEC	NOE_bC = 56 mg KBR 3023 tech./l, equivalent to 54.8 mg Icaridin/l; NOE_rC = 56 mg/l KBR 3023 tech./l, equivalent to 54.8 mg Icaridin/l
5.2.2	EC_{50}	E_bC_{50} = 73.0 mg KBR 3023 tech./l, equivalent to 71.5 mg Icaridin/l; E_rC_{50} = 89.2 mg KBR 3023 tech./l, equivalent to 87.3 mg Icaridin/l;
5.3	**Conclusion**	Validity criteria are summarised in table A7_4_1_3-8. Dose – response relationship: a clear dose – response relationship can be derived from the cell concentration data.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA VII.7.3 *Scenedesmus Subspicatus*

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 07
Materials and Methods	The duration of the test proceeds the normal test duration (72 hours) by 24 hours. The final analysis was conducted after 72 hours. Concentration of test substance was not determined at the end of the test which is recommended by the OECD guideline. This was due to possible incorporation, adsorption and/or metabolisation of the active ingredient. The initial cell concentration was 10,000 cells/ml nutrient solution, which is slightly higher than the concentration recommended by the OECD guideline (2,000-5,000 cells/ml). In the study the continuous illumination is 8000 Lux ± 20 %. According o the OECD guideline the illumination should not vary more than 15 %. The pH in the control varies 2.08 pH values in 72 hours (8.09 to 10.17). According to the guidelines a deviation of 1.5 pH values is acceptable. The growth rates of both the control and all the test concentrations are declining during the study. This could be caused by the high pH. The EC_{50} would probably have been a little lower if the control had been growing exponentially.
Results and discussion	In the study a NOE_rC of 54.8 mg Icaridin/l and a E_rC_{50} of 87.3 mg Icaridin/l was found. The latter is not far from the highest test concentration which suggests that the concentration interval is in the higher end.
Conclusion	The validity criteria of the guidelines are fulfilled and the test is considered valid.
Reliability	2
Acceptability	Acceptable. Despite the increase in pH, the study is accepted, since algae is not the most sensitive aquatic group.
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7_4_1_3-1: Test organisms

Criteria	Details
Species	Green alga *Scenedesmus subspicatus*
Strain	SAG 86/81 (Collection of Algal Cultures, Institute for Plant Physiology, Goettingen, Germany
Source	Stock cultures of the alga were prepared once a week under sterile conditions in the test laboratory.
Laboratory culture	Yes
Method of cultivation	**Stock cultures** of the alga were grown at 23 ± 2 °C under 16 h light/day in cotton plugged, 300 ml Erlenmeyer flasks containing 50 ml nutrient solution 1 (see Point 3.4.1; Culture medium) **Pre-cultures** of the alga were inoculated with 10,000 cells/ml. These were grown in 200 ml nutrient solution 2 (see Point 3.4.1; Culture medium) for three days in an incubator and then used to prepare treated and control cultures for growth inhibition tests. **Test cultures and the cell-free culture media** used for quantitative analyses were prepared by mixing the appropriate quantities of the following components in the following order: - sterile, deionized water - 10-fold concentrated nutrient solution - stock solution of test substance. After mixing the medium was divided into two parts. One part was used for the growth inhibition tests by inoculating it with enough 3-day old pre-culture to give a density of 10,000 cells/ml. A second part was used for quantitative analyses was mixed with sterile deionised water (instead of algal pre-culture). All operations were done under sterile conditions.
Pre-treatment	-
Initial cell concentration	Test started with a biomass of 10,000 (= 1 x 10^4) cells per ml nutrient solution

Table A7_4_1_3-2: Test system

Criteria	Details
Volume of culture flasks	The medium was divided into 150 ml aliquots and these were poured into 300 ml Erlenmeyer flasks
Culturing apparatus	Incubation was performed under standardised conditions according to the mentioned guidelines
Light quality	Continuous illumination at 8000 Lux (± 20%); Illumination in the incubator was provided by 2 banks of light containing 3 fluorescent lamps each (Osram L 140W/20 Sa). A dimmer was used to maintain light at the intensity stipulated in the guidelines
Procedure for suspending algae	In the incubator, culture flasks were suspended by their necks from plastic discs supported in the middle by a central pole. By intermittent turning of the pole (6.5 thrusts per revolution, 3 revolutions per minute), sedimentation of the cells and test substance was prevented and exposure of individual flasks to light was made more uniform.
Number of vessels/ concentration	Control: 6 flasks; Each test substance concentration: 3 flasks
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_3-3: Test conditions

Criteria	Details
Test temperature	23 ± 2 °C
pH	At the beginning of the test the control cultures had a pH of 8.09; after 72 h, the rapid growth of the algal cells had changed the pH value to 10.17. Although slightly higher (0.58) than suggested in the guidelines, it did not interfere with interpretation of the test results
Aeration of dilution water	No data
Light intensity	8000 Lux
Photoperiod	Continuous illumination in the incubator (24 h/day)

Table A7_4_1_3-4: Cell numbers (value x 10^4) at different test substance concentrations during test (average* values) and Standard Deviations

Nominal concentration	After 24 h		After 48 h		After 72 h	
	Average Cell Number	Standard Deviation	Average Cell Number	Standard Deviation	Average Cell Number	Standard Deviation
Control	11.40	0.35	62.40	8.71	213.5	6.02
5.60	12.72	0.66	66.75	5.91	222.0	7.21
10.0	12.93	0.82	65.97	3.45	214.2	2.51
18.0	12.93	1.36	66.40	3.10	216.0	2.61
32.0	12.31	0.62	62.50	2.04	210.0	2.64
56.0	11.77	0.82	54.40	7.90	204.0	5.06
100.0	5.26	0.54	5.60	0.84	10.35	0.81

* Number of samples: control: 6 vessels; each test substance concentration level: 3 vessels

Table A7_4_1_3-5: Areas under the growth curves ("biomass integrals") of *Scenedesmus subspicatus* at different test substance concentrations, their % deviation from controls (= 100%) and calculated t-Values of the Dunnett's-Test*

	After 24 h			After 48 h			After 72 h		
Nominal concen-tration	**Area A**	**Inhibition %**	**Dunnett t**	**Area A**	**Inhibition %**	**Dunnett t**	**Area A**	**Inhibition %**	**Dunnett T**
Control	125	0.0	-	986	0.0	-	4302	0.0	-
5.60	141	-12.8	-0.11	1070	-8.5	-0.57	4511	-4.9	-1.38
10.0	143	-14.7	-0.13	1066	-8.0	-0.57	4404	-2.4	-0.71
18.0	143	-14.7	-0.13	1071	-8.6	-0.61	4435	-3.1	-0.93
32.0	136	-8.8	-0.08	1009	-2.3	-0.17	4255	1.1	0.33
56.0	129	-3.6	-0.03	899	8.8	0.63	3977	7.6	2.26
100.0	51	59.1	0.50	157	84.1	5.63**	261	93.9	26.6**

* Significance level = 5%

** Significantly different from the control

Table A7_4_1_3-6: Growth rates in *Scenedesmus subspicatus* cultures at different test substance concentrations, their % deviation from controls (= 100%) and calculated t-Values of the Dunnett's-Test*

	After 24 h			After 48 h			After 72 h		
Nominal concentration	**Growth Rate**	**Inhibition**	**Dunnet**	**Growth Rate**	**Inhibition**	**Dunnett**	**Growth Rate**	**Inhibition**	**Dunnett**
	r	**%**	**t**	**r**	**%**	**t**	**R**	**%**	**T**
Control	2.42	0.0	-	2.06	0.0	-	1.78	0.0	-
5.60	2.53	-4.5	-7.34	2.09	-1.8	-2.44	1.80	-0.7	-0.84
10.0	2.55	-5.2	-8.38	2.09	-1.5	-2.10	1.79	-0.1	-0.07
18.0	2.55	-5.1	-8.23	2.09	-1.7	-2.32	1.79	-0.2	-0.25
32.0	2.50	-3.2	-5.16	2.06	-0.2	-0.30	1.78	0.3	0.36
56.0	2.45	-1.3	-2.08	1.99	3.3	4.59**	1.77	0.9	0.99
100.0	1.65	32.0	52.04	0.85	58.6	80.86**	0.53	70.1	81.20**

* Significance level = 5%

** Significantly different from the control

Table A7_4_1_3-7: Summary of the results from a 72 h growth inhibition test with KBR 3023 tech. and *Scenedesmus subspicatus*

Inhibition-Parameter	Endpoint	Value (mg KBR 3023 tech./l
Biomass (72 h)	E_bC_{50}	73.0 mg KBR 3023 tech., equivalent to 71.5 mg Icaridin/l
	LOE_bC	between 56.0 and 100.0 mg KBR 3023 tech., equivalent to 54.8 and 97.9 mg Icaridin/l
	NOE_bC	56.0 mg KBR 3023 tech., equivalent to 54.8 mg Icaridin/l;
Growth Rate (72 h)	E_rC_{50}	89.2 mg KBR 3023 tech., equivalent to 87.3 mg Icaridin/l
	LOE_rC	between 56.0 and 100.0 mg KBR 3023 tech., equivalent to 54.8 and 97.9 mg Icaridin/l
	NOE_rC	56.0 mg KBR 3023 tech., equivalent to 54.8 mg Icaridin/l

Table A7_4_1_3-8: Validity criteria for algal growth inhibition test according to OECD Guideline 201

	fulfilled	Not fulfilled
Cell concentration in control cultures increased at least by a factor of 16 within 3 days	X	
Concentration of test substance ≥ 80% of initial concentration during test	X	

Criteria for poorly soluble test substances	Not applicable	-

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA VII.7.4

			Official use only
		1 REFERENCE	
1.1	**Reference**	Mueller, G. (1997): Investigation of the Ecological Properties of KBR 3023. Influence on Microbial Activity. Bayer AG, Institute of Environmental Analysis, Leverkusen, Germany, Report No. 610 N/96 (unpublished), Date: 1997-01-14	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Lanxess Deutschland 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**	Yes; Commission Directive 88/302/EEC, Part C: Biodegradability: Test for Inhibition of Oxygen Consumption. This test method is in most parts identical with OECD Guideline No. 209	
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes, Some reporting deficiencies: No data about the test system. Information incomplete about culture medium and test organism.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Icaridin (KBR 3023)	
3.1.1	Lot/Batch number	Batch No.: 898446008	
3.1.2	Specification	As given in Section 2 of dossier	
3.1.3	Purity	97.9 %	
3.1.4	Composition of Product	-	
3.1.5	Further relevant properties	Water solubility of Icaridin: about 8.2 g/l (Krohn, 1996)	
3.1.6	Method of analysis	Test substance concentrations are not confirmed by analytical method.	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not applicable since the water solubility of Icaridin is about 8.2 g/l (Krohn, 1996)	
3.3	**Reference substance**	Yes, 3,5-Dichlorophenol	
3.3.1	Method of analysis	Reference substance concentrations are not confirmed by analytical	

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA VII.7.4

	for reference substance	method
3.4	**Testing procedure**	
3.4.1	Culture medium	Synthetic medium
3.4.2	Inoculum / test organism	See Table A7_4_1_4-1
3.4.3	Test system	The defined quantity of activated sludge is mixed with synthetic medium and a respiratory rate is measured. This rate is compared to those measured in test preparations with various concentrations of the test substance. No further information included in report
3.4.4	Test conditions	See Table A7_4_1_4-2
3.4.5	Duration of the test	Incubation time: 30 minutes with permanent aeration
3.4.6	Test parameter	Respiration inhibition
3.4.7	Analytical parameter	Oxygen measurement
3.4.8	Sampling	The oxygen concentration was measured in the controls and in every concentration of the test and reference substance at the beginning and at the end of the test period. pH-values and temperature were determined in the controls and in every test concentration of test and reference substance during the test period.
3.4.9	Monitoring of TS concentration	No
3.4.10	Controls	Two controls without test substance are included in the test design. A physico-chemical oxygen consumption control with a test substance concentration of 10000 mg/l was carried out, since some substances can also consume oxygen by chemical reactivity.
3.4.11	Statistics	An EC_{50} value is calculated from determinations at different concentrations using statistical methods (probit analysis).
		4 RESULTS
4.1	**Preliminary test**	Not performed
4.1.1	Concentration	-
4.1.2	Effect data	-
4.2	**Results test substance**	
4.2.1	Initial concentration of test substance	Nominal concentrations: 320, 560, 1000, 1800 and 3200 mg/l
4.2.2	Actual concentrations of test substance	The test substance concentrations are not confirmed by analytical methods

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA VII.7.4

4.2.3	Growth curves	No graph available
4.2.4	Cell concentration data	Not reported
4.2.5	Concentration/ response curve	Concentration/response curves (inhibition vs. concentration) are given in the report on page 17 (test substance) and on page 20 (reference substance)
4.2.6	Effect data	EC_{50} = 1110 mg/l
4.2.7	Other observed effects	-
4.3	**Results of controls**	No physico – chemical oxygen consumption has been determined at 10000 mg/l test substance concentration.
4.4	**Test with reference substance**	Performed with 3,5-Dichlorophenol
4.4.1	Concentrations	2.5, 5, 10, 20 and 40 mg/l
4.4.2	Results	EC_{50} = 6.7 mg/l

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	To assess the toxicity of Icaridin (KBR 3023) to bacteria, a test was investigated according to the Commission Directive 88/302/EEC, Part C. This method is in most parts identical with OECD guideline No. 209. Activated sludge was exposed to Icaridin (KBR 3023) at different concentrations. The respiration rate of each mixture was determined after aeration periods of 30 minutes. The test shows no significant deviations from the OECD guideline No. 209.
5.2	**Results and discussion**	50 % inhibition of respiration was determined at EC_{50} = 1110 mg/l Icaridin (KBR 3023). No physico – chemical oxygen consumption has been determined at 10000 mg/l test substance concentration. Therefore lower concentrations of the test substance cause no physico – chemical oxygen consumption (deduced values). At nominal test concentrations of 320 – 3200 mg/l, inhibition of respiration in activated sludge was observed between 19.3 % and 83.0 % (see Table A7_4_1_4-3)
5.2.1	EC_{20}	-
5.2.2	EC_{50}	1110 mg/l
5.2.3	EC_{80}	-
5.3	**Conclusion**	All validity criteria of the test method were met: * respiratory rate of the two controls differs less than 15% * respiratory rate of the controls is < 60 mg O_2/l·h * EC_{50} of the reference substance 3.5-Dichlorophenol is in the range 5–30 mg/l

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA VII.7.4

		A dose – response relationship can be seen from the test.
5.3.1	Reliability	2
5.3.2	Deficiencies	Yes, Some reporting deficiencies: No data about the test system. Information incomplete about culture medium and test organism.

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA VII.7.4

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	10 March 2007
Materials and Methods	Biodegradability test according to Commission Directive 88/302/EEC, Part C: for Inhibition of Oxygen Consumption. This test method is in most parts identical with OECD guideline 209. The respiration rate of each mixture was determined after aeration periods of 30 minutes. Concentrations of test substance and reference substance are not confirmed by analytical methods and results are based on nominal concentration. Incomplete information about culture medium and test organism No significant derivations from the OECD Guideline No. 209.
Results and discussion	50 % inhibition of respiration was determined at EC_{50} = 1110 mg/l Icaridin (KBR 3023). No physical – chemical oxygen consumption has been determined at 10000 mg/l test substance concentration. Therefore lower concentrations of the test substance cause no physical – chemical oxygen consumption (deduced values). A dose – response relationship can be seen from the test. At nominal test concentrations of 320 – 3200 mg/l, inhibition of respiration in activated sludge was observed between 19.3 % and 83.0 %.
Conclusion	All validity criteria of the test method were met: Respiratory rate of the two controls differs less than 15%, respiratory rate of the controls is < 60 mg O_2/l·h, and EC50 of the reference substance 3.5-Dichlorophenol is in the range 5–30 mg/l. In addition a dose – response relationship can be seen from the test.
Reliability	2
Acceptability	The test is acceptable despite the incomplete information about culture medium and test organism, and despite the missing confirmation of test substance and reference substance concentration by analytical methods.
Remarks	Non
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7_4_1_4-1: Inoculum/Test organism

Criteria	Details
Nature	Activated sludge (mixed population of aquatic micro-organisms)
Species	Mixed population
Strain	-
Source	Laboratory-scale sewage treatment plant, treating predominantly domestic sewage
Sampling site	Aeration tank of the waste water treatment plant (South Wupper area water authority, Germany)
Laboratory culture	Yes
Method of cultivation	No data
Preparation of inoculum for exposure	No data
Pre-treatment	None
Initial cell concentration	Test concentration of the activated sludge: 400 mg/l

Table A7_4_1_4-2: Test conditions

Criteria	Details
Test temperature	19.2 -19.6 °C
pH	7.0 – 7.1 7.2 (physico-chemical oxygen consumption control)
Aeration of dilution water	No data
Suspended solids concentration	Because of strong respiration of the activated sludge, only 400 mg sewage sludge/l were used.

Table A7_4_1_4-3: Test results of test substance (based on nominal concentrations) and controls

Test Compound [mg/l]	Respiratory Rate test substance [mg O_2/l h]	Phys.-chem. Oxygen consumption [mg O_2/l h]	Respiratory Rate minus Phys.-chem. Oxygen consumption [mg O_2/l h]	Inhibition [%]
320	28.5	0.0*	28.5	19.3
560	25.2	0.0*	25.2	28.6
1000	20.0	0.0*	20.0	43.3
1800	13.2	0.0*	13.2	62.6
3200	6.0	0.0*	6.0	83.0

Control	Respiratory rate [mg O_2/l·h]
Control 1	35.3
Control 2	36.0
Control, mean	34.5

*: Phys.-chem. Oxygen consumption determined at 10,000 mg/l test substance

Section A7.4.2 Bioconcentration in aquatic organisms (fish)

Annex Point IIA, VII.7.5 *Lepomis macrochirus*

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (2000): Bioconcentration: Flow-through Fish Test of KBR 3023. [redacted] Report No. 746 A/98 BA, 2000-10-17.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Lanxess Deutschland 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**	Yes, OECD Guideline 305 (June 1996)	
2.2	**GLP**	Yes	
2.3	**Deviations**	- Characterisation of test substance (Batch-No., Purity) included only in German version of report - Keeping conditions of fish not mentioned (only SOP No. given) - Insufficient description of test system and test procedure - Test duration was shortened since equilibration was observed after short exposure time. - Results are not summarised accurately.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Icaridin	
3.1.1	Lot/Batch number	Batch No.: 898711001	
3.1.2	Specification	As given in section 2of dossier	
3.1.3	Purity	98.3%	
3.1.4	Further relevant properties	Water solubility of Icaridin: about 8.2 g/l (Krohn, 1996)	
3.1.5	Radiolabelling	No	
3.1.6	Method of analysis	HPLC (Bayer AG, Method No. 2030-4000104-98D), Detection limits: Test medium: 3 µg/l; Fish: 100 µg/kg wet weight	
3.2	**Reference substance**	No	
3.2.1	Method of analysis for reference substance	-	

Section A7.4.2 Bioconcentration in aquatic organisms (fish)

Annex Point IIA, VII.7.5 *Lepomis macrochirus*

3.3 Testing/estimation procedure

3.3.1 Test system/ performance

Test animals

The Zebra fish (*Brachydanio rerio*) used in this study were obtained from Bio Internattional B.V. (Netherlands). Food: Tetra Min fish food, ground, 2.4% of wet weight, applicated in 6 portions daily.

Test system

The test was run under constant flow-through conditions. The stock solution of the test substance was dosed into a mixing chamber with a volume of 0.8 l by means of a computer-diluting system, while the synthetic fresh water was pumped into the mixing chamber. From this mixing chamber, the test solution was directly introduced into the test vessel. The control vessel received synthetic fresh water only, the flow rate being the same as in the test vessel. The flow rate as well as the diluter system were checked prior to the test and during the test.

The exposure system consisted of one aquarium for each of the test concentrations (0.1 and 1.6 mg/l) and one control aquarium. The acclimatisation phase (7 days) without fish served to optimise the test system for the following main test.

Preparation of the test substance

To produce the stock solution (100 mg/l), the test substance was weighted into water and treated for 30 min. on a magnetic stirrer. It was renewed at 24 h intervals.
Detailed information not given in report.

Test procedure

Uptake phase:
The uptake phase (52 h) was initiated by transferring groups of 10 randomly selected and previously acclimated fish (length 2.5 - 3.5 cm) to the test chamber. The initial loading was 1 - 5 g fish (wet weight)/l.

Depuration phase:
44 hours

Sampling

Fish:
During the uptake phase fish samples were taken 4 h, 22 h, 28 h, 46 h and 52 h after test start. During the depuration phase (beginning 52 h after test initiation) analysis was carried out in fish samples 53 h, 70 h, 77 h and 96 h after test begin. Weight end length of the fish was detected at the end of the test. Lipid content of fish was measured at test start and at the end of the test.

Water:
Water samples were taken and analysed at the same time intervals as fish (i.e. 4 h, 22 h, 28 h, 46 h, 52 h, 53 h, 70 h, 77 h and 96 h after test begin).

Chemical and physical test parameters

Water quality parameters of dissolved oxygen and pH were measured initially and throughout the study in the control and exposure chambers. The temperature was also recorded during the test.

Section A7.4.2 Bioconcentration in aquatic organisms (fish)

Annex Point IIA, VII.7.5 *Lepomis macrochirus*

3.3.2	Estimation of bioconcentration	Steady-State Bioconcentration factor for whole fish were determined by the following calculation: $BCF = \frac{\text{Test substance concentration in test medium (Cw) [µg/l]}}{\text{Test substance concentration in fish (Cf) [µg/kg]}}$ Statistics Arithmetic means were calculated. No information given about use of a computer program.
		4 RESULTS
4.1	**Experimental data**	
4.1.1	Mortality/behaviour	Fish showed no abnormal behaviour throughout the test.
4.1.2	Lipid content	Lipid content at start of uptake phase (test begin): 11.00 % of wet weight (control fish); Lipid content at end of depuration phase (end of test): 8.96 % of wet weight (control fish), 7.86 and 7.17 % of wet weight for the test concentrations of 0.1 and 1.6 mg Icaridin/l, respectively.
4.1.3	Concentrations of test material during test	See table A7_4_2-1
4.1.4	Bioconcentration factor (BCF)	See table A7_4_2-2 **Steady-State-BCF referring to wet weight:** 1.8 / 0.9 for Icaridin concentrations of 0.1 / 1.6 mg/l, respectively. **Steady-State-BCF referring to lipid content of wet weight:** 19 / 10 for Icaridin concentrations of 0.1 / 1.6 mg/l, respectively. The BCF determinations during the uptake phase yielded more or less coherent data: 1.3, 2.8, 2.0, 1.6 and 1.2 in the nominal concentration level of 0.1 mg Icaridin/l; 0.9, 1.1, 0.8, 1.0 and 0.8 in the higher dosage (1.6 mg Icaridin/l). These results clearly demonstrate, that Icaridin (KBR 3023) does not have a potential for bioaccumulation.
4.1.5	Uptake and depuration rate constants	Rate constants for the uptake and depuration of Icaridin in fish were not calculated since the bioaccumulation potential of Icaridin is low.
4.1.6	Depuration time	During the examined depuration period (44 h) the measured Icaridin concentration in fish tissue decreased below the detection limit (100 µg/kg wet weight). See table A7_4_2-1 for detailed concentration values.
4.1.7	Metabolites	No metabolites identified
4.1.8	Other Observations	-
4.2	**Estimation of**	The calculated Bioconcentration factor is based on measurements.

Section A7.4.2 Bioconcentration in aquatic organisms (fish)

Annex Point IIA, VII.7.5 *Lepomis macrochirus*

bioconcentration

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The potential for Bioconcentration of Icaridine was investigated in a flow-through fish test according to OECD Guideline 305 (June 1996). The test was performed with test substance concentration levels of 0.1 mg/l and 1.6 mg/l.
5.2	**Results and discussion**	**Steady-State-BCF referring to wet weight:** 1.8 / 0.9 for Icaridin concentrations of 0.1 / 1.6 mg/l, respectively. **Steady-State-BCF referring to lipid content of wet weight:** 19 / 10 for Icaridin concentrations of 0.1 / 1.6 mg/l, respectively. The results clearly demonstrate, that Icaridin (KBR 3023) does not have a potential for bioaccumulation.
5.3	**Conclusion**	The results clearly demonstrate, that Icaridin (KBR 3023) does not have a potential for bioaccumulation.
5.3.1	Reliability	2
5.3.2	Deficiencies	-Characterisation of test substance (Batch-No., Purity) included only in German version of report - Keeping conditions of fish not mentioned (only SOP No. given) - Insufficient description of test system and test procedure - Test duration was shortened since equilibration was observed after short exposure time. - Results are not summarised accurately.

Section A7.4.2 Bioconcentration in aquatic organisms (fish)

Annex Point IIA, VII.7.5 *Lepomis macrochirus*

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*07 03 07*
Materials and Methods	The uptake phase was significantly reduced as it is stated that equilibrium was reached early. The water to fish ratio (1-5 g ww/l) is higher than recommended by the OECD guideline (0.1-1 g ww/l). This is acceptable as the concentration of test substance is maintained within ± 20% deviation and the concentration of dissolved oxygen does not fall below 60% saturation. There are 5 significant deviations noted in the study summery to which there is agreement. These are taken into account in the evaluation.
Results and discussion	The concentration of Icaridin (KBR 3023) in water and fish does not show a synonymous relation with uptake time. The results therefore demonstrate, that Icaridin (KBR 3023) does not have a potential for bioaccumulation.
Conclusion	The result demonstrates that Icaridin (KBR 3023) does not have potential for bioaccumulation.
Reliability	2
Acceptability	Acceptable
Remarks	Incomplete reporting and methodological deficiencies categorize the study with a reliability factor of 2.
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Findings	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7_4_2-1: Test results: Chemical analysis of Icaridin in water (C_w) and fish (C_f) during uptake and depuration phase of bioaccumulation study

Exposure time	Icaridin concentrations in			
	Control-/Test medium (C_w)		Control-/Test Fish (C_f)	
	Single values	Arithmetic mean	Single values	Arithmetic mean
CONTROL				
4 h (uptake phase)	< 3 / < 3*	< 3	< 110** / < 170	< 110
22 h (uptake phase)	< 3 / < 3	< 3	< 100 / < 100	< 100
18 h (uptake phase)	< 3 / < 3	< 3	< 140 / < 100	< 100
46 h (uptake phase)	< 3 / < 3	< 3	< 100 / < 120	< 100
52 h (end of uptake phase)	< 3 / < 3	< 3	< 100 / < 210	< 100
53 h (depuration phase)	< 3 / < 3	< 3	< 100 / < 100	< 100
70 h (depuration phase)	< 3 / < 3	< 3	< 100 / < 110	< 100
77 h (depuration phase)	< 3 / < 3	< 3	< 100 / < 180	< 100
96 h (depuration phase)	< 3 / < 3	< 3	< 100 / < 100	< 100
NOMINAL CONCENTRATION: 0.1 mg Icaridin/l				
4 h (uptake phase)	96 / 98	97	< 100** / 120 / 150	123
22 h (uptake phase)	92 / 96	94	350 / 260 / 190	267
18 h (uptake phase)	88 / 91	90	150 / 220 / 160	177
46 h (uptake phase)	94 / 95	95	180 / 110 / 170	153
52 h (end of uptake phase)	95 / 95	95	140 / 100 / < 100	113
53 h (depuration phase)	< 3 / < 3*	< 3	140 / 100 / < 100	113
70 h (depuration phase)	< 3 / < 3	< 3	< 100 / < 100 / < 120	< 100
77 h (depuration phase)	< 3 / < 3	< 3	< 100 / < 120 / < 100	107
96 h (depuration phase)	< 3 / < 3	< 3	< 100 / < 100 / < 100	< 100
NOMINAL CONCENTRATION: 1.6 mg Icaridin/l				
4 h (uptake phase)	1480 / 1490	1485	1140 / 1610 / 1400	1383
22 h (uptake phase)	1460 / 1470	1465	1080 / 1860 / 1720	1553
18 h (uptake phase)	1440 / 1430	1435	1320 / 1170 / 1040	1177
46 h (uptake phase)	1460 / 1460	1460	1200 / 1610 / 1630	1480
52 h (end of uptake phase)	1450 / 1480	1465	1130 / 1370 / 1230	1243
53 h (depuration phase)	< 3 / < 3*	< 3	< 100** / 100 / 920	373
70 h (depuration phase)	< 3 / < 3	< 3	270 / 150 / 120	180
77 h (depuration phase)	< 3 / < 3	< 3	110 / < 100 / ***	105
96 h (depuration phase)	< 3 / < 3	< 3	< 100 / < 100 / < 100	< 100

*: detection limit in test medium: 3 µg/l
**: detection limit test fish: 100 µg/kg wet weight, for smaller fish: 110-210 µg/kg wet weight
***: Only two fish were analysed

Table A7_4_2-2:Test results: Calculated Steady-State Bioconcentration Factors (BCF)

	Steady-State BCF	
Icaridin concentration	**Referring to: Wet weight**	**Referring to: Lipid content of wet weight**
0.1 mg/l	1.8	19
1.6 mg/l	0.9	10

Section 7.4.3.1 Annex Point IIIA 12.1	Prolonged toxicity to an appropriate species of fish	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X] **Limited exposure [ ]**	**Technically not feasible [ ] Scientifically unjustified [ ]** **Other justification [X].**	
Detailed justification:	No study on prolonged fish toxicity with Icaridin has been submitted for the following reasons: A fish early-life stage (ELS) test according to OECD Guideline 210 is available for Icaridin providing a reliable NOEC and covering the risk of possible chronic exposure. Furthermore, due its mode of application, chronic exposure of fish to Icaridin, a.i. of Autan Pump Spray 20%, is highly improbable. The product Autan Pump Spray 20% is exclusively used as a skin applied insect repellent and hence a direct contamination of surface with Icaridin can be excluded when applied according to the recommended use. The main emission route will be to wastewater as the product is directly released with wastewater at washing and bathing after application or indirectly when substances that have been transferred to clothing are removed at washing. Thus, Icaridin will reach sewage treatment plants (STP) via wastewater, where degradation will occur during the retention time in the STP to a major degree. Therefore, chronic exposure of fish to Icaridin can be considered to be negligible.	
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	April 2007
Evaluation of applicant's justification	Applicant's justification is OK
Conclusion	Applicant's justification is acceptable
Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section 7.4.3.2 Annex Point IIIA XIII 2.2 — Effects on reproduction and growth rate on an appropriate species of fish

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (2003): Early-Life Stage Toxicity Test with Zebrafish (*Danio rerio*) under Flow-Through Conditions. [redacted], Project No. 020524BD, Study No. FSZ86881, Date: 2003-02-18.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Lanxess Deutschland 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**	Yes; OECD Guideline 210: "Fish, Early-Life Stage Toxicity Test" (1992)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 METHOD	
3.1	**Test material**	Icaridin (KBR 3023); as given in section 2 of dossier	
3.1.1	Lot/Batch number	Batch No. 898711001	
3.1.2	Specification	As given in section 2 of dossier	
3.1.3	Purity	98.5% of active substance	
3.1.4	Composition of Product	n.a.	
3.1.5	Further relevant properties		
3.1.6	Method of analysis	Reversed phase HPLC, UV-detection	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not applicable, since water solubility of Icaridine is about 8.2 g/l (Krohn, 1996)	
3.3	**Reference substance**	None	
3.3.1	Method of analysis for reference substance	-	
3.4	**Testing procedure**		
3.4.1	Dilution water	See table A7_4_3_2-1	

Section 7.4.3.2 Annex Point IIIA XIII 2.2 — Effects on reproduction and growth rate on an appropriate species of fish

3.4.2	Test organisms	See table A7_4_3_2-2
3.4.3	Handling of embryos and larvae (OECD 210/212)	15 adult females and 30 adult males of zebrafish were kept in a aquarium. About 15 minutes before start of artificial dawning (1 h) glass dishes covered with a stainless-steel mesh and provided with artificial plants, were introduced into the aquarium for 1 hour. At the end of the dawning the glass dishes were gently removed and about 800 eggs were immediately transferred to prepared test and dilution medium. After 2 hours eggs were checked for fertilization. Under a stereo microscope every embryo was checked for its blastomer phase. Eggs with only a 2 cell blastomer were regarded not to be fertilized. These eggs as well as coagulated eggs were discarded. On study day 5 more than 95% of the eggs in the controls had hatched (post-hatch day 0). On day 19 the fish were transferred from the crystallisation dishes to the aquaria.
3.4.4	Test system	See table A7_4_3_2-3
3.4.5	Test conditions	See table A7_4_3_2-4
3.4.6	Duration of the test	Experimental Phase: 32 days
3.4.7	Test parameter(s)	Biological parameters: Hatched eggs, fertilization success, fry growth, post hatch period, mortality (criteria varied according to life stage), further effects (abnormal appearance, behaviour), fish size, wet body weight, dry body weight Chemical and physical parameters: Temperature, dissolved oxygen, pH-value, conductivity, total hardness, acid and alkalinity capacity, TOC, residual chlorine
3.4.8	Examination / Sampling	Hatched eggs: number was determined daily until study day 7, Post hatch period: beginning was on study day 5, Further effects: were recorded by visual inspecting each replicate, Fish size: was measured at end of exposure (post hatch day 27), Wet body weight: measured at end of exposure, Dry body weight: Each single fish was dried at 60 °C for 3 days
3.4.9	Monitoring of TS concentration	Yes, at study days -1, 0, 7, 14, 21 and 28
3.4.10	Statistics	t-Test: was used to check control and solvent control data with a normal distribution for significant differences One-Way-Analysis of Variance: was carried out routinely for the determination of statistically significant differences ANOVA: Hatching data of day 4; swim up (days 5 and 6) and selected mortality data (day 32) Bonferroni's and William's Test: Hatching data of days 5 and 6 Dunnett's Test:

Section 7.4.3.2 Annex Point IIIA XIII 2.2 — Effects on reproduction and growth rate on an appropriate species of fish

		Dry weight and length These statistical analyses were conducted with conclusions of statistical significance based on a 95% confidence level.
		4 RESULTS
4.1	**Range finding test**	
4.1.1	Concentrations	No range finding test was carried out for this study.
4.1.2	Number/ percentage of animals showing adverse effects	-
4.1.3	Nature of adverse effects	-
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	Nominal concentration levels: 0.1, 0.32, 1.0, 3.2 and 10 mg test substance/l
4.2.2	Actual concentrations of test substance	Icaridine was applied at measured concentration levels of 0.10, 0.30, 0.92, 3.19 and 9.54 mg test substance/l. Additionally solvent control and control experiments were done. For details see table A7_4_3_2-5.
4.2.3	Effect data	See table A7_4_3_2-6 Egg fertilisation rate: > 90% (2 d after spawning of the eggs) Egg hatch, time to hatch: There were no significant differences between the pooled controls and the concentration levels throughout the hatching period. Swim up: No statistically significant differences were found for this parameter Survival: No significant differences between pooled controls to the concentration levels. Fry growth: The fish of the highest dosage (9.54 mg/l) were significantly smaller and had significantly less weight than the control fish. Larvae survival rate: about 95% Based on these results the following NOEC and LOEC values were determined (active ingredient): -Fry survival (day 32) NOEC ≥ 9.54 mg/l, LOEC > 9.54 mg/l, - Egg hatch (day 6): NOEC ≥ 9.54 mg/l, LOEC > 9.54 mg/l, - Time to hatch:(days 4 and 5): NOEC ≥ 9.54 mg/l, LOEC > 9.54 mg/l, - Time to swim-up: NOEC ≥ 9.54 mg/l, LOEC > 9.54 mg/l - Growth: (length and weight at day 32): NOEC = 3.19 mg/l, LOEC = 9.54 mg/l

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		Based on the findings stated above the overall NOEC (32 d) was determined as 3.19 mg test substance/l and the overall LOEC (32 d) was 9.54 mg test substance/l.
4.2.4	Concentration / response curve	Not included in report
4.2.5	Other effects	Morphological and behavioural effects: during post-hatch period disorders of co-ordination and distortion of spine were observed sporadically in the controls and all test levels. These effects were found not to be dose related.
4.3	**Results of controls**	
4.3.1	Number/ percentage of animals showing adverse effects	No adverse effects were visible
4.3.2	Nature of adverse effects	
4.4	**Test with reference substance**	Not performed
4.4.1	Concentrations	
4.4.2	Results	
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	The test was performed according to OECD Guideline 210 (Juli 1992). Eggs of the zebrafish (*Danio rerio*) were exposed under flow-through conditions for 32 days to determine the effects of Icaridine on different toxic endpoints (fry survival, egg hatch, time to hatch, time to swim-up and growth, other effects). Icaridine was applied at measured concentration levels of 0.10, 0.30, 0.92, 3.19 and 9.54 mg a.i./l. Additionally solvent control and control experiments were done.
5.2	**Results and discussion**	Based on the findings stated above the overall NOEC (32 d) was determined as 3.19 mg test substance/l and the overall LOEC (32 d) was 9.54 mg test substance/l.
5.2.1	NOEC	Based on these results the following NOEC values were determined (active ingredient): -Fry survival (day 32): NOEC ≥ 9.54 mg/l, - Egg hatch (day 6): NOEC ≥ 9.54 mg/l, - Time to hatch:(days 4 and 5): NOEC ≥ 9.54 mg/l, - Time to swim-up: NOEC ≥ 9.54 mg/l, - Growth: (length and weight at day 32): NOEC = 3.19 mg/l
5.2.2	LOEC	Based on these results the following LOEC values were determined (active ingredient): -Fry survival (day 32): LOEC > 9.54 mg/l, - Egg hatch (day 6): LOEC > 9.54 mg/l, - Time to hatch:(days 4 and 5): LOEC > 9.54 mg/l, - Time to swim-up: LOEC > 9.54 mg/l

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		- Growth: (length and weight at day 32): LOEC = 9.54 mg/l
5.3	**Conclusion**	Based on the findings stated above the overall NOEC (32 d) was determined as 3.19 mg test substance/l and the overall LOEC (32 d) was 9.54 mg test substance/l. The validity criteria can be considered as fulfilled. The validity criteria are summarised in table A7_4_3_2-7.
5.3.1	Other Conclusions	
5.3.2	Reliability	Reliability indicator 1
5.3.3	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	*08 03 07*
Materials and Methods	The post-hatch exposure period was 32 days, which is slightly longer than the 30 days suggested by OECD guideline 210. This deviation is assessed not to significantly interfere with the interpretation of the test result.
Results and discussion	For all the considered end points LOEC was determined as > 9.54 mg/l. NOEC was determined as ≥ 9.54 mg/l for all end points except growth, for which NOEC = 3.19. Based on the experimental measurements the overall NOEC was therefore determined as 3.19 mg/l (32 days) and the overall LOEC was 9.54 mg/l (32 days).
Conclusion	The validity criterion according to the OECD guideline 210 is considered to be fulfilled.
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7_4_3_2-1: Dilution water

Criteria	Details
Source	De-chlorinated tap water
Alkalinity ($CaCO_3$)	Mean Alkalinity capacity: K_B = 0.03 – 0.04 mmol/l; Mean Acid capacity: K_S = 0.61 – 0.63 mmol/l
Hardness ($CaCO_3$)	66 - 76 mg/l
pH	7.16 – 7.24
Oxygen content	91 - 93% (dissolved oxygen concentration)
Conductivity	171 ± 15.5 µS/cm
TOC Content	1.49 mg/l
Holding water different from dilution water	No

Table A7_4_3_2-2: Test organisms

Criteria	Details
Species	Zebra fish (*Danio rerio*): Fertilised eggs of a population of 15 adult females and 30 adult males were used in this study
Source	Aquarium am Aegi, Feldstrasse 7, D-30159 Hannover, Germany (certified desease free)
Wild caught	No
Age/size	The biological phase of the study was initiated with the introduction of 15 eggs per replicate.
Kind of food	*Tetrhymena pyriformis;* *Artema salina*; (Brine shrimp nauplii)
Amount of food	Ad libitum
Feeding frequency	Feeding started on study day 5 (post-hatch day 0). *Tetrhymena pyriformis* was fed twice on this day and at least 5 times daily on study days 6 to 8 (post- hatch days 1 to 3). Brine shrimp nauplii were fed at least two times daily from post- hatch day 1 up to the end of the test.
Post-hatch larvae exposure	32 days
Time to first feeding	Post-hatch day 0
Feeding of animals during test	Yes
Treatment for disease within 2 weeks proceeding test	No

Table A7_4_3_2-3: Test system

Criteria	Details
Test type	Flow-through
Renewal of test solution	Membrane piston pumps were used to maintain the dilution water flow. Study days 0-19: The mean flow rates were 1.17 ± 0.03 l/h in the controls and all test levels. These flow-through conditions resulted in about 40 changes of test water per day. Study days 19 to end: The mean flow rates were 5.87 ± 0.08 l/h in the controls and all test levels. These flow-through conditions resulted in about 40 changes of test water per day.
Volume of test vessels	Days 0-19: Crystallisation dishes provided with mesh coating fittings allowing flow-through of test media (inner diameter 13.5 cm, water height about 5 cm) were used. The volume of the test media in the dishes was about 0.7 l. Days 19 to test end: Glass aquaria with mesh coating fittings allowing flow-through of test media (12.5 cm x 14 cm x 21.5 cm) were used. The volume of the glass media was about 3.5 l. The aquaria were covered with glass plates after the transfer of the juveniles.
Volume/animal	Not applicable (growing animals)
Number of animals/vessel	15 eggs per test vessel
Number of vessels/ concentration	Four replicates per test concentration, four replicates of control and four replicates of solvent control (DMF)
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_3_2-4: Test conditions

Criteria	Details
Test temperature	25 °C ± 2 °C (11-15 °C from measurements of the water bath)
Dissolved oxygen	> 60% of air saturation value
pH	6 –8
Adjustment of pH	No
Aeration of dilution water	Yes, Aeration was carried out in the dilution water before splitting. No aeration was provided in the test vessels.
Intensity of irradiation	Light intensity: 600 – 900 lux, measured by a luxmeter
Photoperiod	The test chambers were positioned under regulated lighting to produce an overall photoperiod of 16 h light and 8 h dark.

Table A7_4_3_2-5 Mean measured concentrations of Icaridine (KBR 3023) in the test media

Study Day	Control	Solvent Control	Nominal Icaridine Concentration (mg/l)				
			0.10	**0.32**	**1.0**	**3.2**	**10.0**
-1	ND	ND	0.10	0.30	0.86	3.07	8.77
0	ND	ND	0.10	0.29	0.90	3.59	9.79
7	ND	ND	0.10	0.27	0.81	2.90	9.45
14	ND	ND	0.09	0.27	1.02	3.44	9.97
21	ND	ND	0.09	0.34	1.01	3.24	10.1
28	ND	ND	0.09	0.30	0.90	2.88	9.17
Mean	ND	ND	0.10	0.30	0.92	3.19	9.54
SD	ND	ND	0.006	0.026	0.083	0.290	0.510
Min.	ND	ND	0.09	0.27	0.81	2.88	8.77
Max.	ND	ND	0.10	0.34	1.02	3.59	10.1
Mean measured in % of nominal concentration			100	93.8	92.0	99.7	95.4

ND = Not detectable (Limit of quantification (LOQ) = 6 µg/l)
Min. = Minimum measured concentration
Max. = Maximum measured concentration

Table A7_4_3_2-6 Egg hatch (on days 4 – 6), Percent Swim-up of hatched larvae (on days 5 – 7), Standard length, wet weight, Dry weight and survival of fry (on day 32)

Parameter	Control	Solvent Control	Measured Icaridine Concentration (mg/l)				
			0.10	0.30	0.92	3.19	9.54
EGG HATCH IN % (Mean values)							
Study day 4	15	23	23	17	32	17	18
Study day 5	98	100	87	95	98	92	100
Study day 6	98	100	98	100	100	95	100
SWIM-UP OF HATCHED LARVAE IN % (Mean values)							
Study day 5	89	92	75	88	95	70	68
Study day 6	97	98	98	100	100	93	100
Study day 7	100	100	100	100	100	100	100
LENGTH (mm, Mean values)	21.7	22.3	22.0	22.1	21.4	21.4	20.4*
WET WEIGHT (mg; mean values)	79.9	90.0	-	-	-	-	-
DRY WEIGHT (mg; mean values)	20.6	23.2	22.8	22.3	19.4	20.0	16.9*
SURVIVAL (%; mean values)	95	95	97	95	98	90	90

*: Statistically significant difference from pooled controls (ά = 0.05) when tested with DUNNETT's test.

Table A7_4_3_2-7: Validity criteria for fish tests according to OECD Guidelines 210/212

	fulfilled	Not fulfilled
Concentration of dissolved oxygen > 60% saturation throughout the test	**yes**	
Difference of water temperature < 1.5% between test chambers or successive days at any time during test; temperature within range for specific test species	**yes**	
Overall survival of fertilized eggs in controls (and solvent controls) ≥ value, specified for the specific test species	**yes**	
Test substance concentrations maintained within ± 20% of mean measured values	**yes**	
No effect on survival nor any other adverse effect found in solvent control	**yes**	
Further criteria for poorly soluble test substances	**Not applicable**	

Section 7.4.3.3 Annex Point IIIA 12.2	Bioaccumulation in an aquatic organism	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X] **Limited exposure [ ]**	**Technically not feasible [ ] Scientifically unjustified [ ]** **Other justification [X].**	
Detailed justification:	No further study is submitted on bioaccumulation in aquatic organisms. Because the measured bioconcentration in fish is below 100 (see Section 7 of Doc. III-A 7.4.2), there is no indication for further testing on bioaccumulation in aquatic organisms.	
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	April 2007	
Evaluation of applicant's justification	Applicant's justification is OK	
Conclusion	Applicant's justification is acceptable	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section 7.4.3.4 Annex Point IIIA XIII 2.4 — Effects on reproduction and growth rate with an invertebrate species

			Official use only
		1 REFERENCE	
1.1	**Reference**	Dorgerloh, M. (2003): Influence of KBR 3023 (techn.) on Development and Reproductive Output of the Water Flea *Daphnia magna* in a Static Renewal Laboratory Test System. Bayer CropScience AG, BCS-Development, Ecotoxicology, Leverkusen, Germany Report No. DOM 22039, Date: 2003-09-22 (unpublished)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Lanxess Deutschland GmbH	
1.2.2	Companies with letter of access		
1.2.3	Criteria for data protection	Data submitted to the MS after 14 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. 211 (1998): *Daphnia magna* Reproduction Test; Method C.20 of Directive 92/69/EC; US-EPA Pesticide Assessment Guidelines, Subdivision E, §72-4 (October 1982): Aquatic Invertebrate Life-cycle Studies; US-EPA OPPTS Guideline 850.1300 (April 1996): Daphnid Chronic Toxicity Test – public Draft	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 METHOD	
3.1	**Test material**	KBR 3023 (techn.)	
3.1.1	Lot/Batch number	Batch number: 898711001	
3.1.2	Specification	As given in section 2 of dossier	
3.1.3	Purity	98.5 %	
3.1.4	Composition of Product		
3.1.5	Further relevant properties	Water solubility of Icaridin: about 8.2 g/l (Krohn, 1996)	
3.1.6	Method of analysis	HPLC-UV, Method No. 00445, analysis performed at Bayer AG, BIS, Dormagen, Germany, Date: 2002-05-16.	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not applicable since water solubility of Icaridin is about 8.2 g/l (Krohn, 1996)	
3.3	**Reference substance**	No	
3.3.1	Method of analysis	-	

	for reference substance	
3.4	**Testing procedure**	
3.4.1	Dilution water	See table A7_4_3_4-2
3.4.2	Test organisms	See table A7_4_3_4-3
3.4.3	Handling of offspring	See table A7_4_3_4-3
3.4.4	Test system	See table A7_4_3_4-4
3.4.5	Test conditions	See table A7_4_3_4-5
3.4.6	Duration of the test	21 days
3.4.7	Test parameter	Parent animals: Survival of parent animals, parent mobility, sublethal effects, body-length Offspring: Number and survival of offspring (alive, dead, immobilised), aborted eggs, sublethal effects Physicochemical parameters: Conductivity, total hardness and alkalinity of the dilution media, oxygen concentration, pH value, total chemical oxygen demand, environmental temperature, temperature of test media, light intensity,
3.4.8	Examination / Sampling	Parent animals were observed visually daily, with exception of days 3 and 4. Body length was determined at study termination The number of alive, dead and immobilized offspring produced by each parent animal, as well as the possible presence of aborted eggs and males, were counted and recorded for each test vessel separately. This was done daily from the appearance of the first brood (day 8) until study-termination at day 21. Physicochemical parameters: Conductivity, total hardness and alkalinity of the dilution media: Prior to test initiation, prior to test medium renewal and during test (once weekly in the control and highest test substance concentration); Oxygen concentration, pH value: In the freshly prepared test solutions and repeatedly in the aged media prior to renewal; Total chemical oxygen demand: Once weekly in the freshly prepared and in the aged test solutions Environmental temperature: Continuously during the test Temperature of test media: Once on every working day Light intensity: Three times during the course of the study
3.4.9	Monitoring of TS concentration	Yes, The concentration of the active ingredient was analysed in the control and in the test medium. Samples were taken also at day 0, 2, 9, 12, 19 and 21. On sampling days 2, 12 and 19, only four test concentrations were investigated (1.56, 6.24, 25 and 100 mg/l).
3.4.10	Statistics	All statistical procedures were carried out by using the ToxRat-

Section 7.4.3.4 Annex Point IIIA XIII 2.4 — Effects on reproduction and growth rate with an invertebrate species

Professional© software, Version 2.07 (2002) of the ToxRat Solutions GmbH, Germany.

Arc-sin Transformation:
the parental survival rates were initially transformed

Kolmogorroff-Smirnov Test:
Transformed mobility data and reproductive output were checked on normal distribution

Bartlett Test:
Transformed mobility data and reproductive output were checked on variance equality. If equality of variances was confirmed, parametric methods were used for subsequent analyses. Otherwise, non-parametric techniques have to be performed.

Parametric procedures:
ANOVA (for reproduction data), if significant differences among the means were indicated, multiple comparison procedures were performed at a 5% significance level (Dunnett's Multiple t-Test, Williams Multiple Sequential t-Test, Student t-Test)

Non-Parametric procedures:
Mann-Whitney-Wilcoxon U-Test was applicable. Alternatively, Bonferroni-Correction was available.

Dose-response relationship curve:
modelled by Probit-Analysis after Finney (Finney, D.J.: Statistical Methods in Biological Assays, Griffin, Weycombe, UK, 1978); fitted according to Maximum-Likelyhood principle

If requirements for linear regression methods (variance homogeneity, normal distribution) are not fulfilled and cannot be achieved by a suitable transformation, the used software package allows an automatically correction for heterogeneity (based on a goodness of fit measure).

4 RESULTS

4.1	**Range finding test**	Not performed
4.1.1	Concentrations	-
4.1.2	Number/ percentage of animals showing adverse effects	-
4.1.3	Nature of adverse effects	-
4.2	**Results test substance**	*Non-entry field*
4.2.1	Initial concentrations of test substance	Nominal concentrations of Icaridin (KBR 3023): 0.78, 1.56, 3.12, 6.24, 12.5, 25.0, 50.0 and 100 mg/l. the chosen test concentrations were based on the results of historical acute tests with the test substance on *Daphnia magna*.
4.2.2	Actual	Measured concentrations of Icaridin (KBR 3023) are given in Table

Section 7.4.3.4 Annex Point IIIA XIII 2.4 — Effects on reproduction and growth rate with an invertebrate species

	concentrations of test substance	A7_4_3_4-6. The active ingredient contents analysed in the freshly prepared test medium were between 94.2 and 102.6 % of the corresponding nominal concentrations (mean 98.8 %). The test concentrations used in the study therefore corresponded well with the nominal values. Weekly measurements of actual Icaridin concentrations during the test revealed analytical values between 94.9 and 99.6 % of the proposed nominal concentrations. In aged media the Icaridin concentrations ranged between 78.8 and 104.5 % (mean 96.8 %) of nominal. Therefore, all given results are based on nominal values. Icaridin could not be detected in the control samples. The lowest standard concentration of Icaridin used during analysis was 0.074 mg/l.
4.2.3	Effect data	The number of surviving parent water fleas are summarised in Table A7_4_3_4-7. There was no mortality higher than 20 % in the control, the solvent control and the test concentrations throughout the study period. Table A7_4_3_4-8 summarises the body length of the daphnids, measured at termination of the test. The total numbers of newborn offspring is summarised in Table A7_4_3_4-9, whereas the daily offspring per female parent is given in Table A7_4_3_4-10. Table A7_4_3_4-11 presents data on the neonates behaviour and the quality of reproductive output. As result from a 21-days lasting static-renewal exposure of KBR 3023 (Icaridin) to Daphnia magna, the following threshold concentrations have been evaluated: - for the total summarised offspring per surviving parent animals: NOEC = 50 mg a.s./l, LOEC = 100 mg a.s./l; - for the body length of the surviving parent animals: NOEC = 50 mg a.s./l, LOEC = 100 mg a.s./l; - for mortality of the parent animals: NOEC ≥ 100 mg a.s./l, LOEC > 100 mg a.s./l; - for the day of first offspring emergence: NOEC ≥ 100 mg a.s./l, LOEC > 100 mg a.s./l; - Additional assessments as made for neonates behaviour revealed the following results: NOEC ≥ 100 mg a.s./l, LOEC > 100 mg a.s./l; Thus, the chronic NOEC (21 days) is 50 mg a.s./l. This NOEC is based on a decreased final body length of parental animals exposed to the highest tested concentration of 100 mg a.s./l and a distinctly lower number of offspring from these parentals (both statistically significant on a 5% level of significance.
4.2.4	Concentration / response curve	Derivation of a concentration-response curve seems not to be reasonable, since significant effects were only observed at the highest tested concentration. In the original report, the following parameters were plotted against the test substance concentrations: Body length (Figure 1, p. 22), the total

		offspring (Figure 2, p. 23), average daily offspring (Figure 3, p. 24), time of first offspring emergence (Figure 4, p. 26).
4.2.5	Other effects	No
4.3	**Results of controls**	There was no mortality in the control and the solvent control higher than 20 %. The mean number of newborn water fleas per adult was 141 in the control and 142 in the solvent control.
4.4	**Test with reference substance**	
4.4.1	Concentrations	Not performed
4.4.2	Results	Not performed

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The aim of the study was to determine the influence of Icaridin (KBR 3023) on development, reproductive capacity and behaviour of Daphnia magna over 21 days under static-renewal exposure. The test was performed according to following guidelines: - OECD Guideline No. 211 (1998): Daphnia magna Reproduction Test; - Method C.20 of Directive 92/69/EC; - US-EPA Pesticide Assessment Guidelines, Subdivision E, §72-4 (October 1982): Aquatic Invertebrate Life-cycle Studies; - US-EPA OPPTS Guideline 850.1300 (April 1996): Daphnid Chronic Toxicity Test – public Draft The test shows no significant deviations from the guideline, except the deviations mentioned in point 2.3.
5.2	**Results and discussion**	As result from a 21-days lasting static-renewal exposure of KBR 3023 (Icaridin) to Daphnia magna, the following threshold concentrations have been evaluated: - for the total summarised offspring per surviving parent animals: NOEC = 50 mg a.s./l, LOEC = 100 mg a.s./l; - for the body length of the surviving parent animals: NOEC = 50 mg a.s./l, LOEC = 100 mg a.s./l; - for mortality of the parent animals: NOEC ≥ 100 mg a.s./l, LOEC > 100 mg a.s./l; - for the day of first offspring emergence: NOEC ≥ 100 mg a.s./l, LOEC > 100 mg a.s./l; - Additional assessments as made for neonates behaviour revealed the following results: NOEC ≥ 100 mg a.s./l, LOEC > 100 mg a.s./l; Thus, the chronic NOEC (21 days) is 50 mg a.s./l. This NOEC is based on a decreased final body length of parental animals exposed to the highest tested concentration of 100 mg a.s./l and a distinctly lower number of offspring from these parentals (both statistically significant on a 5% level of significance.

Section 7.4.3.4 Effects on reproduction and growth rate with an invertebrate species
Annex Point IIIA XIII 2.4

5.2.1	NOEC (21 d)	For the total summarised offspring per surviving parent animals: NOEC = 50 mg a.s./l, LOEC = 100 mg a.s./l; For the body length of the surviving parent animals: NOEC = 50 mg a.s./l For mortality of the parent animals: NOEC ≥ 100 mg a.s./l; For the day of first offspring emergence: NOEC ≥ 100 mg a.s./l; Additional assessments as made for neonates behaviour revealed the following results: NOEC ≥ 100 mg a.s./l
5.2.2	LOEC (21 d)	For the total summarised offspring per surviving parent animals: LOEC = 100 mg a.s./l; For the body length of the surviving parent animals: LOEC = 100 mg a.s./l; For mortality of the parent animals: LOEC > 100 mg a.s./l; For the day of first offspring emergence: LOEC > 100 mg a.s./l; Additional assessments as made for neonates behaviour revealed the following results: LOEC > 100 mg a.s./l
5.2.3	EC_{50} (EC_x)	Not determined
5.3	**Conclusion**	The active ingredient contents analysed in the freshly prepared test medium were between 94.2 and 102.6 % of the corresponding nominal concentrations (mean 98.8 %). The test concentrations used in the study therefore corresponded well with the nominal values. Weekly measurements of actual Icaridin concentrations during the test revealed analytical values between 94.9 and 99.6 % of the proposed nominal concentrations. In aged media the Icaridin concentrations ranged between 78.8 and 104.5 % (mean 96.8 %) of nominal. Therefore, all given results are based on nominal values. There was no mortality in the control higher than 20 % which is regarded as natural rate. The recorded pH values and oxygen concentrations indicated that the water quality required by the guideline (pH 7.5 – 8.5, oxygen content 2 mg/l) was maintained throughout the study period.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	08 03 07
Materials and Methods	The test was performed according to following guidelines: - OECD Guideline No. 211 (1998): Daphnia magna Reproduction Test; - Method C.20 of Directive 92/69/EC; - US-EPA Pesticide Assessment Guidelines, Subdivision E, §72-4 (October 1982): Aquatic Invertebrate Life-cycle Studies; - US-EPA OPPTS Guideline 850.1300 (April 1996): Daphnid Chronic Toxicity Test – public Draft The influence of Icaridin on development, reproductive capacity and behaviour of Daphnia magna over 21 days under static-renewal exposure was studied. The test shows no significant deviations from the guideline, therefore the materials and methods are acceptable.
Results and discussion	Results from the 21-days static-renewal exposure of KBR 3023 (Icaridin) to Daphnia magna: - offspring per surviving parent animals: NOEC = 50 mg a.s./l, LOEC = 100 mg a.s./l; - body length of the surviving parent animals: NOEC = 50 mg a.s./l, LOEC = 100 mg a.s./l; - mortality of the parent animals: NOEC ≥ 100 mg a.s./l, LOEC > 100 mg a.s./l; - the day of first offspring emergence: NOEC ≥ 100 mg a.s./l, LOEC > 100 mg a.s./l; - Additional assessments as made for neonates behaviour revealed the following results: NOEC ≥ 100 mg a.s./l, LOEC > 100 mg a.s./l; Thus, the chronic NOEC (21 days) is 50 mg a.s./ l (statistically significant on a 5% level of significance). The analysed concentrations specified in Table A7_4_4-6 is different from Table 11 in the study report, the aged Day 2 concentration is 78.8% instead of 98.4%. As the deviation from the nominal concentration is greater than ± 20 per cent (78.8 %), results should be expressed in terms of the time-weighted mean (TWM) instead of nominal concentration. However, at the concentration of 50 mg/l no aged test solutions were analyzed and therefore it is not possible to calculate the TWM. As the deviation is very close to 20 % (21.2 %) the NOEC of 50 mg/l are accepted.
Conclusion	There was no mortality in the control and the solvent control higher than 20 %. The mean number of offspring per surviving parent animals was 141 in the control and 142 in the solvent control and therefore the test meets the validity criterion of a mean of ≥ 60 offspring per parent animal surviving. Based on offspring per surviving parent animals and body length of the surviving parent animals, NOEC (21 days) is 50 mg a.s./ l (statistically significant on a 5% level of significance).

Reliability	1
Acceptability	The study fulfils the specified OECD guideline and is considered acceptable.
Remarks	Table A7_4_4-6 should be corrected.
Date	**COMMENTS FROM ...** *(specify)*
Materials and Methods	*Give date of comments submitted*
Results and discussion	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7_4_3_4-1: Preparation of TS Solution for Poorly Soluble or Volatile Test Substances

Criteria	Details
Dispersion	Not applicable since water solubility of Icaridin is about 8.2 g/l (Krohn, 1996)
Vehicle	Not applicable since water solubility of Icaridin is about 8.2 g/l (Krohn, 1996)
Concentration of vehicle	-
Vehicle control performed	-
Other procedures	-

Table A7_4_3_4-2: Dilution Water

Criteria	Details
Source	The water is supplied by the central waterworks of the Bayer CropScience Research Centre, Monheim. The test medium is prepared using deionised water and adding mineral salts and vitamins. Test and breeding water was prepared as "M7-medium" as documented in the "Original Draft" of an EEC *Daphnia magna* Pilot Ring Test. The "M7-medium" is similar to the "M4-medium", which is described in: Elendt, B.P. & W.R. Bias (1990): Trace nutrient deficiency in *Daphnia magna* cultered in standard medium for toxicity testing. Water Research, 24, pp. 1157-1167.
Salinity	-
Hardness	196 mg/l $CaCO_3$ (corresponding to 11° dH)
pH	8.2 (day 0, control, freshly prepared solution)
Ca / Mg ratio	Ca:Mg ratio amounts to 7:1
Na / K ratio	Na:K ratio amounts to 6:1
Oxygen content	8.3 mg/l (day 0, control, freshly prepared solution)
Conductance	580 µS/cm (= µmhos/cm)
TOC	-
Holding water different from dilution water	No

Table A7_4_3_4-3: Test Organisms

Criteria	Details
Strain / Clone	*Daphnia magna*, strain from the Bundesgesundheitsamt (Federal Health Agency), Berlin, Germany; the clone was classified as genotype No 2 (Dr. Bradley, University of Sheffield, Report, Date: 1988-02-03) and later renamed as "type B" according to: Baird, D.J. et al. (1991): A comparative study of genotype sensitivity to acute stress using clones of *Daphnia magna* Strauss. Ecotoxic. Environ. Safety, 21, pp. 257-265.
Source	The strain has been maintained in own laboratory (Bayer CropScience AG, Crop Protection, Monheim, Germany) for more than 15 years.
Age	The parent animals were of the same age (± 12 hours), between 21 and 28 days old and from the 4th brood or later. The culture showed no delay in first offspring emergence.
Breeding method	The first instars used in the test were obtained by repeated carefully screening of adults (21-28 d old, only parents of the same age (± 12 h) were used). For this purpose plastic screens with 0.6 and 0.2 mm mesh (according to DIN 4195) were used. The parents were kept in 2-litre containers (50 to 100 daphnids per container) in an climate-controlled environment under following conditions: 16:8 hour light:dark cycle, 20 ± 1 °C
Kind of food	The animals were fed with an aqueous suspension of single cell green algae (*Scenedesmus subspicatus*). Furthermore, during breeding procedure the daphnids were fed occasionally with some commercial ornamental fish food (trade name TetraMin®) as a supplement to the algae diet.
Amount of food	The daphnids were fed daily with 0.2 mg TOC (total organic carbon) per test vessel with 100 ml. This corresponds to 1 x 10E8 cells/litre.
Feeding frequency	Over the whole exposure period, the water fleas were fed daily, except the first weekend (study days 3 and 4). On day 2 the three-fold amount was fed for the first weekend.
Pre-treatment	No
Feeding of animals during test	Yes, See above

Table A7_4_3_4-4: Test System

Criteria	Details
Test type	Static-renewal conditions with at least three renewals of test medium per week.
Renewal of test solution	During the course of the study, all test units were refilled with freshly prepared test-solutions 2, 5, 7, 9, 12, 14, 16 and 19 days after start of exposure to meet the recommended renewal period of max. 3 days.
Volume of test vessels	250 ml glass beakers according to DIN 12332; each test vessel contained 100 ml of test solution (corresponding to a fluid level of approximately 4 cm height).
Volume/animal	100 ml
Number of animals/vessel	One daphnia (parental animal) was transferred into each test beaker
Number of vessels/concentration	10 vessels (replicates) per concentration and 10 control vessels
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_3_4-5: Test Conditions

Criteria	Details
Test temperature	The animals in the test containers were exposed to a temperature of 20 ± 1 °C in a climatic chamber. Initial water temperature of the freshly prepared test solutions ranged between 19.5 and 19.6 °C.
Dissolved oxygen	Control: 8.3 mg/l (day 0), 8.6 mg/l (day 19); Lowest concentration: 8.3 mg/l (day 0), 8.5 mg/l (day 19); Highest concentration: 8.3 mg/l(day 0 and day 19)
pH	Control: 8.2 (day 0), 8.1 (day 19); Lowest concentration: 8.2 (day 0), 8.1 (day 19); Highest concentration: 8.1 (day 0 and day 19)
Adjustment of pH	No
Aeration of dilution water	Yes, before use, the basis solution was aerated
Quality/Intensity of irradiation	About 1,500 lux, maintained by fluorescent-tubes (daylight visual spectrum)
Photoperiod	16:8 hour light-dark cycle

Table A7_4_3_4-6: Analysed Concentrations of KBR 3023 (Icaridin) in Test Solutions

Nominal Conc. [mg/l]	Analysed Concentrations of KBR 3023 (Icaridin)						Time-weighted Concentrations	
	Week 1 samples		Week 1 samples		Week 1 samples			
	Day 0 (fresh)	Day 2 (aged)	Day 9 (fresh)	Day 12 (aged)	Day 19 (fresh)	Day 21 (aged)	mg a.s./l	% of nominal
Control	<0.074*	<0.074	<0.074	<0.074	<0.074	<0.074	-	-
0.78	0.800	-	0.790	-	0.736	-	-	-
1.56	1.57	1.39	1.59	1.63	1.47	1.54	1.54	98.9
3.12	3.09	-	3.16	-	3.03	-	-	-
6.24	6.18	6.25	6.30	6.27	6.09	6.11	6.21	99.6
12.5	12.4	-	12.5	-	12.3	-	-	-
25	24.8	24.9	24.7	24.7	24.4	24.6	24.69	98.7
50	49.2	-	49.5	-	49.0	-	-	-
100	97.5	98.4	98.4	98.5	96.5	96.7	94.88	94.9

*: Lowest standard concentration used during analyses

Table A7_4_3_4-7: Survival and Sublethal Affection of Parental Water Fleas

Study day	Alive parental animals at test-termination								
	Water Control	**0.78 mg a.s./l**	**1.56 mg a.s./l**	**3.12 mg a.s./l**	**6.24 mg a.s./l**	**12.5 mg a.s./l**	**25 mg a.s./l**	**50 mg a.s./l**	**100 mg a.s./l**
0	10	10	10	10	10	10	10	10	10
1	10	10	10	10	10	10	10	10	10
2	10	10	10	10	10	10	10	10	10
3	10	10	10	10	10	10	10	10	10
4	10	10	10	10	10	10	10	10	10
5	10	10	10	10	10	10	10	10	10
6	10	10	10	10	10	10	10	10	10
7	10	10	10	10	10	10	10	10	10
8	10	10	10	10	10	10	10	10	10
9	10	10	10	10	10	10	10	10	10
10	No recordings								
11	No recordings								
12	10	10	10*	10	10	10	10	10	10
13	10	10	10	10	10	10	10	10	10
14	10	10	10	10	10	10	10**	10	10
15	10	10	10	10	10	10	10	10	10
16	10	10	10	10	9	10	9	10**	10
17	No recordings								
18	10	10	10	10	9	10	9	9	10
19	10	10	10	10	9	10	9	9	10
20	10	10	10	10	9	10	9	9	10
21	10	10	10	10	9	10	9	9	10
Total	**100 %**	**100 %**	**100 %**	**100 %**	**90 %**	**100 %**	**90 %**	**90 %**	**100 %**

*: Animals lie at the bottom
**: Frequency of antennae movements clearly decreased

Table A7_4_3_4-8: Parental Body Length at Study Termination

Nominal Test Concentr. [mg a.s./l]	Sample Size (n)	Mean [mm]	Standard Deviation	Variation Coefficient (%)	% of Control	SIG* (p = 0.05)
Control	10	4.4	0.2	4.0	-	No
0.78	10	4.5	0.1	2.7	100.4	No
1.56	10	4.4	0.2	4.7	98.7	No
3.12	10	4.4	0.2	4.2	99.1	No
6.24	9	4.4	0.1	1.9	99.7	No
12.5	10	4.4	0.2	4.7	99.9	No
25	9	4.5	0.1	2.3	102.3	No
50	9	4.4	0.1	3.2	99.8	No
100	10	4.1	0.2	5.8	92.0	**Yes**

*: Denotes statistically significant difference from control (Boniferroni t-test procedure for inhomogeneous variances)

Table A7_4_3_4-9: Total Number of Alive Offspring per Surviving Parental Female

Nominal Test Concentr. [mg a.s./l]	Sample Size (n)	Mean	Standard Deviation	Variation Coefficient (%)	% of Control	SIG* (p = 0.05)
Control	10	121.8	16.9	13.9	-	No
0.78	10	103.3	16.8	16.3	84.8	No
1.56	10	103.3	15.8	15.3	84.8	No
3.12	10	105.9	10.4	9.8	86.9	No
6.24	9	109.1	20.4	18.7	89.6	No
12.5	10	102.4	16.6	16.2	84.1	No
25	9	103.6	28.0	27.0	85.0	No
50	9	106.8	18.7	17.5	87.7	No
100	10	74.4	26.3	35.4	61.1	**Yes**

*: Denotes statistically significant difference from control (Boniferroni t-test procedure for inhomogeneous variances)

Table A7_4_3_4-10: Daily Offspring per Surviving Parental Female

Nominal Test Concentr. [mg a.s./l]	Sample Size (n)	Mean	Standard Deviation	Variation Coefficient (%)	% of Control
Control	10	9.2	1.4	14.8	-
0.78	10	8.1	1.6	19.7	88.5
1.56	10	7.7	1.1	15.0	83.4
3.12	10	8.1	0.8	9.4	88.4
6.24	9	8.6	1.3	15.0	93.9
12.5	10	8.2	1.0	12.8	89.0
25	9	8.2	1.8	22.4	89.2
50	9	8.4	1.2	14.6	91.3
100	10	6.0	1.9	31.7	65.8

*: Denotes statistically significant difference from control (Boniferroni t-test procedure for inhomogeneous variances)

Table A7_4_3_4-11: Summarised Data on Neonates Behaviour

Nominal Test Concentr. [mg a.s./l]	Total Repro-ductive Output	Alive Offspring					Premature Mortality	
		Un-affected	Discoor-dinated Move-ments	Laying on the Ground	Total Alive Offspring	% Alive Offspring	Dead Offspring	Aborted eggs
Control	1223	1218	0	0	1218	100	0	5
0.78	1042	1033	0	0	1033	99	0	9
1.56	1041	1033	0	0	1033	99	0	8
3.12	1061	1059	0	0	1059	100	0	2
6.24	1039	1037	0	0	1037	100	0	2
12.5	1028	965	0	59	1024	100	0	4
25	981	978	0	0	978	100	0	3
50	1006	1003	0	0	1003	100	0	3
100	746	744	0	0	744	100	0	2

*: Denotes statistically significant difference from control (Boniferroni t-test procedure for inhomogeneous variances)

Section 7.4.3.5.1 Annex Point IIIA 13.2	Effects on sediment dwelling organisms	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [...]**	**Technically not feasible [ ] Scientifically unjustified [ ]** **Other justification [X].**	
Detailed justification:	A test on sediment-dwelling organisms is not submitted as it not a data requirement for biocidal actives used as repellents (PT19, see TNsG, chapter 2.5). Furthermore, an exposure of sediment dwelling organisms to Icaridin, a.i. of Autan Pump Spray 20%, from its application is highly improbable due to the following reasons: The main emission route will be to wastewater as the product is directly released with wastewater at washing and bathing after application or indirectly when substances that have been transferred to clothing are removed at washing. Thus, Icaridin will reach sewage treatment plants (STP) via wastewater, where degradation will occur during the retention time in the STP to a major degree. The available data for Icaridin (log H = -3.04; log Pow = 2.23) indicate that residues will mainly be present in the water phase (99%). Therefore, the sediment compartment is not a major concern and exposure of sediment dwelling organisms can be regarded to be not relevant.	
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	April 2007
Evaluation of applicant's justification	applicant's justification is OK
Conclusion	applicant's justification is acceptable
Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section 7.4.3.5.2 Annex Point IIIA 13.2	Aquatic plant toxicity	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [...]**	**Technically not feasible [ ] Scientifically unjustified [ ]** **Other justification [X].**	
Detailed justification:	A test on aquatic plant toxicity was not submitted and is not required for biocidal actives used as repellents (PT19, see TNsG, chapter 2.5). Furthermore, an exposure of aquatic plants to Icaridin, a.i. of Autan Pump Spray 20%, from its application is highly improbable. The product Autan Pump Spray 20% is exclusively used as a skin applied insect repellent and hence a direct contamination of surface with Icaridin can be excluded when applied according to the recommended use. The main emission route will be to wastewater as the product is directly released with wastewater at washing and bathing after application or indirectly when substances that have been transferred to clothing are removed at washing. Thus, Icaridin will reach sewage treatment plants (STP) via wastewater, where degradation will occur during the retention time in the STP to a major degree. Due to this lack of exposure no tests with aquatic plants are required in the context of the application of Icaridin as a skin applied insect repellent.	
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	April 2007
Evaluation of applicant's justification	Applicant's justification is OK
Conclusion	Applicant's justification is acceptable
Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A7.5.1.1 Annex Point IIA 7.4	Inhibition to microbial activity (terrestrial)	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [X]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [...]**	
Detailed justification:	An exposure of soil non-target micro-organisms to Icaridin, a.i. of Autan Pump Spray 20%, from its application is highly improbable due to the following reasons: The product Autan Pump Spray 20% is exclusively used as a skin applied insect repellent and hence a direct contamination of the soil with Icaridin can be excluded when applied according to the recommended use. The main emission route will be to wastewater as the product is directly released with wastewater at washing and bathing after application or indirectly when substances that have been transferred to clothing are removed at washing. Thus, Icaridin will reach sewage treatment plants (STP) via wastewater, where degradation will occur during the retention time in the STP to a major degree. The exposure route via sewage sludge treatment is of no concern since Icaridin will be predominately present in the water phase of a STP (99%). Another potential route of emission is those to the atmosphere, either due to the volatilisation of the compound from the skin surface or as a result of volatilisation from the sewage treatment plant. However, the short atmospheric half-life of Icaridin prevents the compound to be deposed to soils. Therefore, a contamination of soil regarding these pathways can also be neglected. It is justified not to perform a test on effects on nitrogen transformation or carbon mineralization in soil in the context of the application of Icaridin as a skin applied insect repellent and the resulting lack of exposure.	
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 FI
Date	April 2007
Evaluation of applicant's justification	Applicant's justification is OK
Conclusion	Applicant's justification is acceptable
Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A7.5.1.2 Earthworm, acute toxicity test

Annex Point IIIA XIII 3.2

Eisenia fetida andrei

			Official use only
		1 REFERENCE	
1.1	**Reference**	Lechelt Kunze, C. (2002): KBR 3023 (techn.): Acute Toxicity to Earthworms (*Eisenia fetida*), Bayer CropScience AG, Development – Environmental Biology, Monheim, Germany, Report No. LKC/Rg 408/02 (unpublished), Date: 2002-12-03.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	LANXESS Deutschland 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**	Yes OECD Guideline No. 207 (April 1984)	
2.2	**GLP**	Yes	
2.3	**Deviations**	None	
		3 METHOD	
3.1	**Test material**	KBR 3023 tech. (active ingredient: Icaridin)	
3.1.1	Lot/Batch number	Batch Number 898711001	
3.1.2	Specification	As given in section 2 of the dossier	
3.1.3	Purity	98.5 % of active substance (Icaridin)	
3.1.4	Composition of Product	Not applicable	
3.1.5	Further relevant properties	Water solubility of Icaridin: about 8.2 g/l (Krohn, 1996)	
3.1.6	Method of analysis	No data	
3.2	**Reference substance**	Yes; Chloroacetamide Reference: Heimbach, F. (2001): Bayer AG, Report No. HBF/Rg 395, Date: 2001-12-18.	
3.2.1	Method of analysis for reference substance	No data	
3.3	**Testing procedure**		
3.3.1	Preparation of the test substance	Stock solution: For the study 5000 mg of the test substance were weighted into 50 ml acetone p.a. and stirred on a magnetic stirrer for 20 minutes. Test solutions:	

Section A7.5.1.2 Earthworm, acute toxicity test

Annex Point IIIA XIII 3.2

Eisenia fetida andrei

		The test substance concentrations were prepared by mixing equivalent parts of the stock solution with some further acetone.
3.3.2	Application of the test substance	With a chromatographic sprayer 5 ml of the test solutions were sprayed into the test soil of each test container while mixing thoroughly with a domestic mixer. In addition, 50 ml of the deionised water required was mixed into the test soil in each test container. 500 g dry weight test soil (equivalent to 625 g wet weight) was prepared for each test container. The soils were aerated thereafter for about two minutes while still mixing with the mixer to allow evaporation of acetone.
3.3.3	Test organisms	See Table A7_5_1_2-2
3.3.4	Test system	See Table A7_5_1_2-3
3.3.5	Test conditions	See Table A7_5_1_2-4
3.3.6	Test duration	14 days
3.3.7	Test parameter	Mortality and weight alteration of the survivors
3.3.8	Examination	Seven days after the start of the study, the number of surviving earthworms was counted by emptying the soil out onto an inert surface and removing the earthworms by hand. The animals were then returned to the test container with the test soil. After 14 days, number and weight of surviving earthworms was determined as well as abnormal behaviour and symptoms observed. Earthworms which show no reaction upon being prodded with a blunt probe were considered dead.
3.3.9	Monitoring of test substance concentration	No
3.3.10	Statistics	The weight alterations of the test organisms were statistically evaluated by the U-Test of Wilcoxon, Mann & Whitney (Sachs, L. (1978): Angewandte Statistik, Springer Verlag, Heidelberg, New York). Probability level P = 0.05 (two sided) using the computer program "Easy Assay, Multiple Testing". Version 4.0 (Ratte, H.T. 1993-1996) If possible, the LC_{50} values and the 95 percent confidence limits were calculated by the Prohibit-Analysis according to "Maximum-Likelihood" Method (Finney, D.J. (1978): Statistical Methods in Biological Assays. Griffin, Weycombe, UK) using the computer program "Easy Assay, Critical Values". Version 3.0 (Ratte, H.T. 1992)
		4 RESULTS
4.1	**Filter paper test**	Not performed
4.1.1	Concentration	-
4.1.2	Number/ percentage of animals showing adverse effects	-
4.1.3	Nature of adverse effects	-
4.2	**Soil test**	

Section A7.5.1.2 Earthworm, acute toxicity test

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Eisenia fetida andrei

4.2.1	Initial concentrations of test substance	See Table A7_5_1_2-3
4.2.2	Effect data (Mortality)	For mortalities and weight alterations see Table A7_5_1_2-5; the ecotoxicological endpoints are reported in Table A7_5_1_2-6.
4.2.3	Concentration / effect curve	Regression curve (after Litchfield & Wilcoxon) for Icaridin was not calculated.
4.2.4	Other effects	The weight alterations of the surviving animals are given in Table A7_5_1_2-5
4.3	**Results of controls**	
4.3.1	Mortality	See Table A7_5_1_2-5
4.3.2	Number/ percentage of earthworms showing adverse effects	No adverse effects observed
4.3.3	Nature of adverse effects	No adverse effects observed
4.4	**Test with reference substance**	Yes; chloroacetamide
4.4.1	Concentrations	10, 18, 24, 32 and 56 mg/kg
4.4.2	Results	LC_{50} (14 days) = 21 mg/kg dry weight soil (95 % confidence limits 20-23 mg/kg). This value is within the concentration range normally determined in international ring studies

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Acute earthworm toxicity of KBR 3023 (a.i. Icaridin) was investigated according to OECD Guideline 207. The test animals were exposed to following concentrations of Icaridin: 1, 3.2, 10, 18, 32, 100, 316 and 1000 mg/kg dry weight soil After 14 days, the number of surviving animals and their weight alteration was determined as well as abnormal behaviour and symptoms observed.
5.2	**Results and discussion**	
5.2.1	LC_0	316 mg a.i./kg dry weight soil
5.2.2	LC_{50}	Approximately 1000 mg a.i./ kg dry weight soil
5.3	**Conclusion**	The mortality rate in the control was below 10 % which is regarded as the limit for natural mortality. The properties of the substrate are in agreement with the nominal values. The LC_{50} of the reference substance is within the usual range. The test conditions are therefore equivalent to the standard.
5.3.1	Other Conclusions	-

Section A7.5.1.2 Earthworm, acute toxicity test
Annex Point IIIA XIII 3.2

Eisenia fetida andrei

5.3.2	Reliability	1
5.3.3	Deficiencies	None

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	10 March 2007
Materials and Methods	Acute earthworm toxicity of KBR 3023 (a.i. Icaridin) was investigated according to OECD Guideline 207. The test animals were exposed to following concentrations of Icaridin: 1, 3.2, 10, 18, 32, 100, 316 and 1000 mg/kg dry weight soil After 14 days, the number of surviving animals and their weight alteration was determined as well as abnormal behaviour and symptoms observed. Test with reference substance chloroacetamide are made. Concentrations of test substance and reference substance are not confirmed by analytical methods and results are based on nominal concentration.
Results and discussion	LC_0 316 mg a.i./kg dry weight soil LC_{50} Approximately 1000 mg a.i./ kg dry weight soil. Since effects are only observed at the highest test concentration (1000 mg/kg dry weight soil) a concentration-effect curve is not established.
Conclusion	The mortality rate in the control was below 10 % which is regarded as the limit for natural mortality. The properties of the substrate are in agreement with the nominal values. The LC_{50} of the reference substance is within the usual range. The test conditions are therefore equivalent to the standard.
Reliability	2
Acceptability	It is acceptable that a concentration-effect curve is not established, since effects are only observed at the highest test concentration.
Remarks	Reliability indicator by applicants was 1
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7_5_1_2-1: Preparation of TS solution

Criteria	Details
Type and source of dilution water	deionised water
Alkalinity / Salinity	-
Hardness	-
PH	-
Oxygen content	-
Conductance	-
Holding water different from dilution water	No

Table A7_5_1_2-2: Test organisms

Criteria	Details
Species/strain	*Eisenia fetida Andrei*
Source of the initial stock	Strain of Prof. Graff, Federal German Biological Agency for Agriculture and Forestry (BBA), Brunswick, Germany
Culturing techniques	The animals are kept at 22 ± 2 °C, 70-90 % relative humidity, 12:12 hour light-dark cycle. The substrate consists of ca. 70 % by weight of natural soil, 25 % peat and 5 % straw (dry weight in each case). The animals are fed on ground, dried cattle manure at 14 day intervals. At the same time, the substrate is also replenished with water. The animals are transferred into fresh substrate at half-yearly intervals.
Age/weight	The adult worms used in the test were more than two months old. The average weight of the animals at test begin was 0.33 g. The weight of the individual earthworms was determined at the start of the study and after 14 days exposure (original Report, pp. 13/14, Tables 4 and 5).
Pre-treatment	On the day prior to the start of the study, the earth-worms were removed from the breeding substrate for acclimatisation and kept in the test substrate (without test substance) under the test conditions until the start of the study.

Table A7_5_1_2-3: Test system

Criteria	Details
Artificial soil test substrate	The test substrate consists of 69 % fine quartz sand (68 % of the sand has a particle size of 0.05-0.2 mm), 10 % dried, finely ground peat (sphagnum peat; pH 2-4), 20 % kaolin (kaolinite content of around 36 %, pH value ca. 7) and around 1 % calcium carbonate (pure) to adjust the pH value to 6 +/- 0.5. The substrate was first of all mixed dry from these components in a mixer, and moistened with water.
Test mixture	Not applicable
Size, volume and material of test container	1.5 litre preserving jars, covered with glass lids
Amount of artificial soil (kg)/ container	500 g dry weight (equivalent to 625 g wet weight)
Nominal levels of test concentrations	I. Pre-Test: Control, 0.1, 1, 10, 100 and 1000 mg test substance/kg dry weight substrate II. Main Test: Control, 1.0, 3.2, 10, 18, 32, 100, 316 and 1000 mg test substance/kg dry weight substrate
Number of replicates/concentration	4
Number of earthworms/test concentration	40
Number of earthworms/container	10
Light source	Constant light 400-800 lux
Test performed in closed vessels due to significant volatility of test substrate	No

Table A7_5_1_2-4: Test conditions

Criteria	Details
Test temperature	20 ±1 °C
Moisture content	Average moisture content in substrate [%] / [% of max. water capacity]: Start of study: 22.9 / 56.6; End of study: 24.2 / ---
pH	Average pH values: Start of study: 5.89; End of study: 6.07
Adjustment of pH	Yes; Around 1 % pure calcium carbonate was added to the test substrate to adjust the pH value to 6.0 ± 0.5
Light intensity / photoperiod	Constant light (400 – 800 lux)
Relevant degradation products	Degradation products were not investigated in this study.

Table A7_5_1_2-5: Mortality data, weight alteration of the test animals and results of the U-test (means of n = 4 test containers, each containing 10 earthworms)

Nominal Test Substance Concentration [mg Icaridin/kg dry weight soil]	Mortality	Weight alteration of the survivors	
	%	%	U-test (P = 0.05)
Control	0	+ 8 ± 2	
1.0	0	+ 8 ± 3	-
3.2	0	+ 8 ± 4	-
10	0	+ 7 ± 2	-
18	0	+ 8 ± 2	-
32	0	+ 7 ± 2	-
100	0	+ 6 ± 2	-
316	0	+ 5 ± 2	-
1000	58 ± 10	- 33 ± 2	+

*: Results of the U-test: + = weights of control and the test concentration do differ significantly;
- = weights of control and the test concentration do not differ significantly

Table A7_5_1_2-6: Individual data obtained in the study (number of surviving animals and mean weight of worms)

Nominal Test Substance Concentration [mg Icaridin/kg dry weight substrate]	Container Number	Number of surviving worms			Mean weight of worms (g)	
		Day 0	Day 7	Day 14	Day 0	Day 14
Control	1	10	10	10	0.32	0.35
	2	10	10	10	0.34	0.37
	3	10	10	10	0.33	0.35
	4	10	10	10	0.34	0.37
1.0	1	10	10	10	0.32	0.36
	2	10	10	10	0.33	0.35
	3	10	10	10	0.34	0.36
	4	10	10	10	0.35	0.37
3.2	1	10	10	10	0.34	0.38
	2	10	10	10	0.33	0.37
	3	10	10	10	0.33	0.35
	4	10	10	10	0.34	0.35
10	1	10	10	10	0.34	0.35
	2	10	10	10	0.34	0.36
	3	10	10	10	0.33	0.37
	4	10	10	10	0.33	0.35
18	1	10	10	10	0.33	0.36
	2	10	10	10	0.32	0.36
	3	10	10	10	0.33	0.35
	4	10	10	10	0.33	0.36
32	1	10	10	10	0.34	0.36
	2	10	10	10	0.33	0.36
	3	10	10	10	0.35	0.37
	4	10	10	10	0.34	0.37
100	1	10	10	10	0.33	0.35
	2	10	10	10	0.33	0.35
	3	10	10	10	0.33	0.36
	4	10	10	10	0.33	0.35
316	1	10	10	10	0.34	0.35
	2	10	10	10	0.35	0.36
	3	10	10	10	0.32	0.35
	4	10	10	10	0.34	0.35
1000	1	10	5	4	0.33	0.23
	2	10	5	5	0.34	0.22
	3	10	6	5	0.33	0.22
	4	10	3	3	0.36	0.25

Table A7_5_1_2-6: Effect data after 14 days (nominal concentrations)

		[mg Icaridin/kg dry weight soil]
LC_{50}		approximately 1000
LLC	**Lowest lethal conc.**	1000
LOEC	**Lowest observed effect concentration**	1000
NOEC (LC_0)	**No-observed-effect-concentration**	316

Table A7_5_1_2-7: Validity criteria for acute earthworm test according to OECD Guideline 207

	fulfilled	Not fulfilled
Mortality of control animals < 10%	**X**	

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			Official use only
		1 REFERENCE	
1.1	**Reference**	Spatz, B. (2002): Effects of KBR 3023 (technical) on Terrestrial (Non-Target) Plants: Seedling Emergence and Seedling Growth Test. IBACON GmbH, Rossdorf, Germany, Report No. 14671084, Date: 2002-11-04.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	LANXESS Deutschland 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**	Yes; OECD Guideline 208 (Proposal for Updating Guideline 208, Draft Document, July 2000): Seedling Emergence and Seedling Growth Test	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 METHOD	
3.1	**Test material**	Icaridin (KBR 3023)	
3.1.1	Lot/Batch number	Batch number 898 711 001	
3.1.2	Specification	As given in section 2 of dossier	
3.1.3	Purity	Purity 98.5 %	
3.1.4	Composition of Product	-	
3.1.5	Further relevant properties	Water solubility of Icaridin: about 8.2 g/l (Krohn, 1996)	
3.1.6	Method of analysis	-	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not applicable since water solubility of Icaridin is about 8.2 g/l (Krohn, 1996)	
3.3	**Reference substance**	No	
3.3.1	Method of analysis for reference substance		
3.4	**Testing procedure**		
3.4.1	Dilution water	See Table A7_5_1_3-2	
3.4.2	Test plants	See Table A7_5_1_3-3	

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3.4.3	Test system	See Table A7_5_1_3-4; After application of the test substance into the soil, pots were filled with the soil and sown into the contaminated soil.
3.4.4	Test conditions	See Table A7_5_1_3-5
3.4.5	Test duration	Exposure time: 21 days after application
3.4.6	Test parameter	Visual phytotoxicity (e.g. chlorosis, necrosis, abnormal growth); Growth stages; Shoot fresh weight; Height; Number of seedlings; Mortality. See Tables A7_5_1_3-4 and A7_5_1_3-5 for details.
3.4.7	Sampling	Visual phytotoxicity ratings: Days 0, 7, 14 and 21; Growth stages (BBCH): Day 21; Shoot fresh weight: Day 21; Height: Day 21; Number of seedlings: Day 7, 14 and 21; Mortality: Number of living and dead plants was recorded on day 21; See also Tables A7_5_1_3-4 and A7_5_1_3-5 for details.
3.4.8	Method of analysis of the plant material	Not applicable
3.4.9	Quality control	Yes (Test was performed according to GLP by certified laboratory)
3.4.10	Statistics	For all metric parameters controls were tested with Student-t Test for significant differences. For all numeric parameters controls were tested with Fischer Exact Test for significant differences. As controls were not statistically different, they were pooled. Fresh weight and height data were tested for normality by using Kolmogorrof-Smirnov-Test. Homogeneity was tested with Cochran-Test if data were not normally distributed. If the normal distribution was accepted, Bartlett Test was used for all data with $n > 10$ and Cochran Test for data with $n < 10$. If the data were normally distributed and homogeneous Dunnett Test was used and if they were normally distributed, homogenious and monotonous increasing or decreasing Williams Test was used for comparing treatment groups and control. If the data were not homogeneous Bonferroni U-Test was used. In order to determine the ECx values, a regression analysis (Probit analysis) was performed. For the germination and mortality data Fischer Exact Test was used. The significance level for all test was 0.05. Computer program used to perform the statistical analyses: ToxRat®, SPiRiT Solutions (2001-2002), Version 2.07 and SYSTAT Version 9.

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4 RESULTS

4.1	**Results test substance**	
4.1.1	Applied initial concentration	The dosages of the test substance were (nominal / weighted sample): 12.35 / 12.53 mg test substance/kg dry weight soil, 37.04 / 37.59 mg test substance/kg dry weight soil, 111 / 112.78 mg test substance/kg dry weight soil, 333 / 338.33 mg test substance/kg dry weight soil, 1000 / 1015 mg test substance/kg dry weight soil.
4.1.2	Phytotoxicity rating	See Table A7_5_1_3-6b Phytotoxicity at day 21: Phytotoxic effects were slight chlorosis and growth reduction. Phytotoxicity was observed from 37.04 mg a.i./kg soil and above (1-89 %). Growth stages were reduced from 333 mg test substance/kg soil.
4.1.3	Plant height	See Table A7_5_1_3-6b The most sensitive species in height was *Brassica napus* (EC_{50}: 438.44 mg a.i./kg soil) followed by *Glycine max* and *Avena sativa* with EC_{50} values of 580.62 and 738.49 mg a.i./kg soil.
4.1.4	Plant dry weights	For fresh weights see Table A7_5_1_3-6a. The most sensitive species in fresh weight was *Brassica napus* (EC_{50}: 97.79 mg a.i./kg soil) followed by *Avena sativa* and *Glycine max* with EC_{50} values of 103.82 and 353.34 mg a.i./kg soil.
4.1.5	Root dry weights	Not described
4.1.6	Root length	Not described
4.1.7	Number of dead plants	See Table A7_5_1_3-6a Most sensitive species for the parameter mortality was *Brassica napus* which showed mortality from 37.04 mg a.i./kg soil and above.
4.1.8	Effect data	See Table A7_5_1_3-7a (effect data based on results of the fresh weight) and Table A7_5_1_3-7b (effect data based on results of the shoot height).
4.1.9	Concentration / response curve	No plot of concentration/response curve given in report.
4.1.10	Other effects	None
4.2	**Results of controls**	
4.2.1	Number/ percentage of plants showing adverse effects	See Tables A7_5_1_3-6a and A7_5_1_3-6b
4.2.2	Nature of adverse effects	Not relevant
4.3	**Test with reference substance**	Not performed

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4.3.1	Concentrations	-
4.3.2	Results	-

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Test according to OECD Guideline 208 (Proposal for Updating Guideline 208, Draft Document, July 2000): Seedling Emergence and Seedling Growth Test. Test was performed for 21 days in a growth chamber under controlled test conditions with three plant species: *Brassica napa, Glycine max, Avena sativa.* Effective concentrations were calculated based on fresh weight and shoot height.
5.2	**Results and discussion**	
5.2.1	EC_{20}	Not described
5.2.2	EC_{50}	Based on fresh weight: *Brassica napa:* EC_{50} = 97.79 (32.35-284.6) mg a.i./kg soil; *Glycine max:* EC_{50} = 353.3 (233.4-834.1) mg a.i./kg soil; *Avena sativa:* EC_{50} = 130.8 (41.9-349.4) mg a.i./kg soil Based on height: *Brassica napa:* EC_{50} = 438.4 mg a.i./kg soil; *Glycine max:* EC_{50} = 580.6 mg a.i./kg soil; *Avena sativa:* EC_{50} = 738.5 (567.4-1105.2) mg a.i./kg soil.
5.2.3	EC_{80}	Not described
5.3	**Conclusion**	There is a clear dose-response relationship for all 3 plants. The validity criteria can be considered fulfilled according to the mentioned OECD guideline.
5.3.1	Reliability	1
5.3.2	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	10-Mar-07
Materials and Methods	Test according to OECD Guideline 208 (Proposal for Updating Guideline 208, Draft Document, July 2000): Seedling Emergence and Seedling Growth Test. Test was performed for 21 days in a growth chamber under controlled test conditions with three plant species: Brassica napa, Glycine max, Avena sativa. Effective concentrations were calculated based on fresh weight and shoot height. Concentrations of test substance are not confirmed by analytical methods and results are based on nominal concentration.
Results and discussion	Based on fresh weight: *Brassica napa:* EC_{50} = 97.79 (32.35-284.6) mg a.i./kg soil; *Glycine max:* EC_{50} = 353.3 (233.4-834.1) mg a.i./kg soil; *Avena sativa:* EC_{50} = 130.8 (41.9-349.4) mg a.i./kg soil Based on height: *Brassica napa:* EC_{50} = 438.4 mg a.i./kg soil; *Glycine max:* EC_{50} = 580.6 mg a.i./kg soil; *Avena sativa:* EC_{50} = 738.5 (567.4-1105.2) mg a.i./kg soil.
Conclusion	There is a clear dose-response relationship for all 3 plants. EC_{50} for plants is 97.79 mg/kg. NOEC is < 12.35 mg/kg as effects (shoot height) were observed at the lowest test concentration. The validity criteria can be considered fulfilled according to the mentioned OECD guideline.
Reliability	2
Acceptability	Acceptable
Remarks	Non
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7_5_1_3-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	Not applicable since water solubility of Icaridin is about 8.2 g/l (Krohn, 1996)
Vehicle	Not applicable since water solubility of Icaridin is about 8.2 g/l (Krohn, 1996)
Concentration of vehicle	-
Vehicle control performed	-
Other procedures	-

Table A7_5_1_3-2: Dilution water

Criteria	Details
Source	Not applicable, no dilution with water
Alkalinity / Salinity	
Hardness	
pH	
Oxygen content	
Conductance	
Holding water different from dilution water	

Table A7_5_1_3-3: Test plants

	Family	Species	Common name	Source (seed/plant)
Dicotyledonae	Brassicaceae	***Brassica napus***	Oilseed Rape	Source not mentioned in report
	Leguminoseae	***Glycine max***	Soybean	
Monocotyledonae	Gramineae	***Avena sativa***	Oat	Source not mentioned in report

Table A7_5_1_3-4: Test system

Criteria	Details
Test type	Test was performed in a growth chamber under controlled test conditions.
Container type	Commercial plastic flower pots Size of pots: 9 x 9 cm (for *Avena sativa*) or 16 cm diameter for *Brassica napa* and *Glycine max*)
Seed germination potential	Germination rate in the study (mean of controls): *Brassica napa*: 91 %, *Glycine max*: 95 %, *Avena sativa*: 94 %
Identification of the plant species	Each test unit was uniquely identified with at least study number, treatment and replicate number
Number of replicates	8 pots per treatment group were tested
Numbers of plants per replicate per dose	Each pot contained 5 seeds; in total 40 seeds per treatment group were tested.
Date of planting	Experimental starting date: 2002-08-16
Plant density	Each pot contained 5 seeds; Size of pots: 9 x 9 cm (for *Avena sativa*) or 16 cm diameter for *Brassica napa* and *Glycine max*)
Date of test substance application	Experimental starting date: 2002-08-16
High of plants at application	The test seeds were sowed in soil incorporated with the test item
Date of phytotoxicity rating or harvest	Visual phytotoxicity ratings (e.g. stunted growth, chlorosis, necrosis): Days 0, 7, 14 and 21; Growth stages: Were reported according to BBCH-Monograph-Growth stages (for day 21) Shoot fresh weight: was determined at the end of the test (Day 21). The plants of one pot represent one replicate. Experimental completion date: 2002-08-16
Dates of analysis	Not mentioned in report

Table A7_5_1_3-5: Test conditions

Criteria	Details
Test type	Terrestrial plants, Seedling emergence and seedling growth test according to OECD 208
Method of application	The test substance was solved in acetone and mixed with fine quartz sand under the fume hood. After the solvent was evaporated the quartz sand with the test substance was mixed into the soil until the sand was dispersed homogeneously (minimum 10 minutes). Treatment of the control: The same amount of untreated quartz sand was mixed into the soil. Treatment of the solvent control: The same amount of acetone was mixed with fine quartz sand and after evaporation of the organic solvent the sand was mixed into the soil.
Application levels	The dosages of the test substance were (nominal / weighted sample): 12.35 / 12.53 mg test substance/kg dry weight soil, 37.04 / 37.59 mg test substance/kg dry weight soil, 111 / 112.78 mg test substance/kg dry weight soil, 333 / 338.33 mg test substance/kg dry weight soil, 1000 / 1015 mg test substance/kg dry weight soil. The described Icaridin concentrations were tested on each tested species.
Dose rates	Dose rates: See above; Application scheme: 1. control, 2. solvent control, 3. test substance (increasing concentrations)
Substrate characteristics	The soil was delivered and analysed by LUFA Speyer, Germany, Soil Type (USDA): Sandy loam (LUFA soil 2.3); Particle size: All particles smaller than 0.2 mm; Organic carbon (%): 1.2 ± 0.2 %; pH 6.3 ± 0.2
Watering of the plants	The irrigation with tap water was done automatically with fibreglass-wicks connecting pot (soil) and water supply (bowl standing below each pot and containing maximum 500 ml water).
Temperature	The test plants were grown at 25 °C (range 24-27 °C) during daytime and 19 °C (range 18-19 °C) at night.
Thermoperiod	See above
Light regime	Light regime: 16 hours light : 8 hours dark; Light intensity: *Brassica napa, Avena sativa*: 13655 (5290-25000) lux, *Glycine max*: 7526 (5120-11270) lux
Relative humidity	Day: 62 % (range 52-81 %); Night 89 % (range 68-100 %)

Table A7_5_1_3-5: Test conditions (continued)

Criteria	Details
Wind volatility	Not mentioned in report
Observation periods and duration of test	Visual phytotoxicity ratings (e.g. stunted growth, chlorosis, necrosis): Days 0, 7, 14 and 21; Growth stages: Were reported according to BBCH-Monograph-Growth stages (for day 21) ; Shoot fresh weight: was determined at the end of the test (Day 21). The plants of one pot represent one replicate; Height: Height of each single plant was measured at day 21; Number of seedlings: At day 7, 14 and 21; Mortality: Number of living and dead plants was recorded on day 21; Test duration: 21 days
Pest control	Not applicable
Any other treatments and procedures	Fertilizer was given one to three times per week: 1 g/l Flory 9 (Euflor) + 0.05 g/l Sequestren (Ciba-Geigy)

Table A7_5_1_3-6a: Effective phytotoxicity after test termination (Part 1)

		Germination		Mortality		Fresh weight			
Species	**Treatment Group***	**(%)**	**Statistics**	**(%)**	**Statistics**	**(g)**	**SD****	**Effect (%)**	**Statistics**
		Day 21		**Day 21**		**Day 21**			
Brassica napus	Mean Controls	91	-	0	-	14.28	± 2	-	-
	12.35	98	n.s.[4]	0	n.s.[4]	12.58	± 2	11.96	n.s.[3]
	37.04	98	n.s.[4]	8	s.[4]	8.75	± 3	38.72	s.[3]
	111	85	n.s.[4]	3	n.s.[4]	8.16	± 1	42.85	s.[3]
	333	50	s.[4]	15	s.[4]	4.40	± 2	69.21	s.[3]
	1000	18	s.[4]	14	s.[4]	0.27	± 0	98.10	s.[3]
Avena sativa	Mean Controls	94	-	0	-	6.08	± 1	-	-
	12.35	100	n.s.[4]	0	n.s.[4]	6.83	± 0	-12.35	n.s.[1]
	37.04	88	n.s.[4]	0	n.s.[4]	4.65	± 1	23.59	s.[1]
	111	90	n.s.[4]	0	n.s.[4]	3.60	± 1	40.85	s.[1]
	333	68	s.[4]	0	n.s.[4]	1.63	± 1	73.15	s.[1]
	1000	15	s.[4]	17	s.[4]	0.06	± 0	99.04	s.[1]
Glycine max	Mean Controls	95	-	0	-	16.69	± 2	-	-
	12.35	95	n.s.[4]	0	n.s.[4]	15.85	± 1	5.04	n.s.[2]
	37.04	98	n.s.[4]	0	n.s.[4]	15.31	± 3	8.30	n.s.[2]
	111	90	n.s.[4]	0	n.s.[4]	10.94	± 3	34.42	s.[2]
	333	33	n.s.[4]	0	n.s.[4]	3.63	± 2	78.22	s.[2]
	1000	5	n.s.[4]	0	n.s.[4]	2.13	± 1	87.26	s.[2]

* : Nominal test substance concentrations (mg a.i./kg soil)
** : Standard Deviation
1 : Multiple comparison Dunnett Test, a = 0.05 (n.s. = not significant)
2 : Multiple comparison Williams Test, a = 0.05 (n.s. = not significant)
3 : Multiple comparison Bonferroni U-Test, a = 0.05 (n.s. = not significant)
4 : Multiple comparison Fisher Exact Test, a = 0.05 (n.s. = not significant)

Table A7_5_1_3-6b: Effective phytotoxicity after test termination (Part 2)

		Height				Phytotoxicity (%)			Growth Stage (BBCH)
Species	**Treatment Group***	**(mm) Day 21**	**SD****	**Effect (%)**	**Statistics**	**Day 7**	**Day 14**	**Day 21**	
Brassica napus	Mean Controls	121	± 16	-	-	0	0	3	-
	12.35	101	± 12	16.48	s.[3]	0	0	20	13-14
	37.04	83	± 25	31.02	s.[3]	0	0	11	(12) 13-14
	111	92	± 13	24.01	s.[3]	0	0	32	13 (14)
	333	88	± 17	26.77	s.[3]	0	0	84	13 (14)
	1000	27	± 34	77.89	s.[3]	0	1	84	10-12
Avena sativa	Mean Controls	406	± 30	-	-	0	0	0	-
	12.35	438	± 20	-7.82	s.[3]	0	0	0	13-14
	37.04	389	± 55	4.39	n.s.[3]	0	0	1	13 (14)
	111	349	± 23	14.07	s.[3]	0	0	5	13-14
	333	238	± 57	41.41	s.[3]	0	4	27	10-13
	1000	82	± 77	79.83	s.[3]	0	0	89	10-12
Glycine max	Mean Controls	342	± 36	-	-	0	0	0	-
	12.35	261	± 35	16.48	s.[1]	0	0	0	14
	37.04	239	± 40	31.02	s.[1]	0	3	1	14
	111	193	± 57	24.01	s.[1]	0	7	14	10-14
	333	140	± 82	26.77	s.[1]	0	74	54	10-12-14
	1000	195	± 78	77.89	s.[1]	0	75	45	13-14

* : Nominal test substance concentrations (mg a.i./kg soil)
** : Standard Deviation
*** : values in brackets were observed only seldom
1 : Multiple comparison Dunnett Test, a = 0.05 (n.s. = not significant)
3 : Multiple comparison Bonferroni U-Test, a = 0.05 (n.s. = not significant)

Table A7_5_1_3-7a: Summary of Effective concentrations *(based on fresh weight)*

		NOEC	LOEC	Statistical	EC_{25}	EC_{50}	Statistical
Species	**Confidence limit (c.l.)**	(mg a.i./kg soil)		**Analysis**	(mg a.i./kg soil)		**Analysis**
Brassica napus		12.35	37.04	3	28.46	97.79	4
	lower 95 % c.l.				n.d.	32.35	
	upper 95 % c.l.				66.30	284.59	
Avena sativa		12.35	37.04	1	50.76	130.82	4
	lower 95 % c.l.				n.d.	41.90	
	upper 95 % c.l.				104.35	349.44	
Glycine max		12.35	111.00	2	164.63	353.34	4
	lower 95 % c.l.				107.09	233.44	
	upper 95 % c.l.				253.52	834.07	

1 : Multiple comparison Dunnett Test, a = 0.05
2 : Multiple comparison Williams Test, a = 0.05 (n.s. = not significant)
3 : Multiple comparison Bonferroni U-Test, a = 0.05 (n.s. = not significant)
4 : Probit Analysis
n.d. : Not determined due to mathematical reasons

Table A7_5_1_3-7b: Summary of Effective concentrations *(based on shoot height)*

		NOEC	LOEC	Statistical	EC_{25}	EC_{50}	Statistical
Species	**Confidence limit (c.l.)**	(mg a.i./kg soil)		**Analysis**	(mg a.i./kg soil)		**Analysis**
Brassica napus		< 12.35	12.35	3	61.58	438.44	4
	lower 95 % c.l.				n.d.	n.d.	
	upper 95 % c.l.				n.d.	n.d.	
Avena sativa		37.04	111.00	3	404.45	738.49	4
	lower 95 % c.l.				318.03	567.41	
	upper 95 % c.l.				520.49	1105.19	
Glycine max		< 12.35	12.35	2	8.52	580.62	4
	lower 95 % c.l.				n.d.	n.d.	
	upper 95 % c.l.				n.d.	n.d.	

1 : Multiple comparison Dunnett Test, a = 0.05
2 : Multiple comparison Williams Test, a = 0.05 (n.s. = not significant)
3 : Multiple comparison Bonferroni U-Test, a = 0.05 (n.s. = not significant)
4 : Probit Analysis
n.d. : Not determined due to mathematical reasons

Section A7.5.2.1 Annex Point IIIA 13.3	Reproduction study with earthworms or other soil non-target macro-organisms	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [X]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [...]**	
Detailed justification:	A test on earthworm reproduction is not data requirement for biocidal actives used in PT 19 (see TNsG, chapter 2.5). Furthermore, a long-term exposure of earthworms or other soil non-target macro-organisms to Icaridin, a.i. of Autan Pump Spray 20% from its application is highly improbable due to the following reasons: The product Autan Pump Spray 20% is exclusively used as a skin applied insect repellent and hence a direct contamination of the soil with Icaridin can be excluded when applied according to the recommended use. The main emission route will be to wastewater as the product is directly released with wastewater at washing and bathing after application or indirectly when substances that have been transferred to clothing are removed at washing. Thus, Icaridin will reach sewage treatment plants (STP) via wastewater, where degradation will occur during the retention time in the STP to a major degree. The exposure route via sewage sludge treatment is of no concern since Icaridin will be predominately present in the water phase of a STP (99%). Another potential route of emission is those to the atmosphere, either due to the volatilisation of the compound from the skin surface or as a result of volatilisation from the sewage treatment plant. However, the short atmospheric half-life of Icaridin prevents the compound to be deposed to soils. Therefore, a contamination of soil regarding these pathways can be neglected and a long-term exposure of earthworms or other soil non-target macro-organisms has not to be considered. Due to this lack of exposure it is justified not to perform a long-term test on earthworms or other soil non-target macro-organisms in the context of the application of Icaridin as a skin applied insect repellent.	
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	April 2007
Evaluation of applicant's justification	applicant's justification is OK
Conclusion	applicant's justification is acceptable
Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A7.5.2.2 Annex Point IIIA 13.3	Long-term tests with terrestrial plants	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [X]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [...]**	
Detailed justification:	A specific long term test on terrestrial plants is not available for Icaridin, a.i. of Autan Pump Spray 20%. An agreed guideline for such a test is not available at the time being. However, a long-term exposure of terrestrial plants to Icaridin, a.i. of Autan Pump Spray 20%, from its application can be excluded due to the following reasons: The product Autan Pump Spray 20% is exclusively used as a skin applied insect repellent and hence a direct contamination of the soil with Icaridin can be excluded when applied according to the recommended use. The main emission route will be to wastewater as the product is directly released with wastewater at washing and bathing after application or indirectly when substances that have been transferred to clothing are removed at washing. Thus, Icaridin will reach sewage treatment plants (STP) via wastewater, where degradation will occur during the retention time in the STP to a major degree. The exposure route via sewage sludge treatment is of no concern since Icaridin will be predominately present in the water phase of a STP (99%). Another potential route of emission is those to the atmosphere, either due to the volatilisation of the compound from the skin surface or as a result of volatilisation from the sewage treatment plant. However, the short atmospheric half-life of Icaridin prevents the compound to be deposed to soils. Therefore, a contamination of soil regarding these pathways can be neglected and even for the case of a systemic active substance, which could indirectly affect terrestrial plants, a long-term exposure can be excluded. Due to this lack of exposure no long-term tests with terrestrial plants are required in the context of the application of Icaridin as a skin applied insect repellent.	
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	April 2007
Evaluation of applicant's justification	applicant's justification is OK
Conclusion	Applicant's justification is acceptable
Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A7.5.3.1.1 Annex Point IIIA XIII 1.1	Effects on birds: Acute oral toxicity	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X] **Limited exposure [X]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	An acute test on birds is not submitted as it not a data requirement for biocidal actives used in repellents (PT19, see TNsG, chapter 2.5) when they are not applied in the form of baits, granulates of powder. The product Autan Pump Spray 20% is exclusively used as a skin applied insect repellent and an exposure of the environment including soil, herbs and insects to Icaridin can be excluded when applied according to the recommended use. In general, birds may be exposed to a product by the consumption of contaminated feed like herbs or insects picked from the treated area. Thus, exposure of birds to Icaridin, a.i. of Autan Pump Spray 20%, from its mode of application is highly improbable. The main emission route will be to wastewater as the product is directly released with wastewater at washing and bathing after application or indirectly when substances that have been transferred to clothing are removed at washing. Thus, Icaridin will reach sewage treatment plants (STP) via wastewater, where degradation will occur during the retention time in the STP to a major degree. The exposure route via sewage sludge treatment is of no concern since Icaridin will be predominately present in the water phase of a STP (99%). Another potential route of emission is those to the atmosphere, either due to the volatilisation of the compound from the skin surface or as a result of volatilisation from the sewage treatment plant. However, the short atmospheric half-life of Icaridin prevents the compound to be deposed to soils. Therefore, a contamination of soil regarding these pathways can be neglected and even for the case of a systemic active substance, which could indirectly affect plants acting as food for birds, exposure can be considered negligible. Due to this lack of exposure no acute tests with birds are required in the context of the application of Icaridin as a skin applied insect repellent. Furthermore, a 5-day dietary study has been conducted with Bobwhite quails showing no adverse effects up to the highest concentration tested (i.e. 5000 mg Icaridin/kg feed). Thus, Icaridin can be considered as non-toxic to birds.	
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	April 2007	

Section A7.5.3.1.1 Annex Point IIIA XIII 1.1	Effects on birds: Acute oral toxicity
Evaluation of applicant's justification	Applicant's justification is OK
Conclusion	applicant's justification is acceptable
Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A7.5.3.1.2 Short-term toxicity on birds

Annex Point IIIA XIII 1.2

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1997): Five Day Dietary Toxicity of KBR 3023 on Bobwhite Quail (*Colinus virginianus*). [redacted] Report No. 107844 (unpublished), Date: 1997-03-20	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Lanxess Deutschland 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**	Yes, OECD guideline No. 205 (1984); US-EPA FIFRA Guideline 71-2	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	KBR 3023 (Icaridin)	
3.1.1	Lot/Batch number	Mixed Batch No. 898446008	
3.1.2	Specification	As given in section 2 of dossier	
3.1.3	Purity	97.6 %	
3.1.4	Composition of Product	-	
3.1.5	Further relevant properties	-	
3.1.6	Method of analysis	Active ingredient: Concentration, stability and homogeneity analysis data were obtained from the analytic laboratory of Bayer AG in Elberfeld, Germany. Homogeneity of the test substance in the diet was evaluated by collecting three samples each from respective batches. Test substance concentrations in the test diet were verified by analysing duplicate samples from the treatment group. Method used for analysis: Extraction with acetonitrile, analysed by HPLC with reversed C18 column and 200 nm UV detection (Analytical Report is attached to the original study).	
3.2	**Administration of the test substance**	Birds were exposed for five days to a nominal dietary concentration of 5000 mg a.i./kg feed. Diet preparation:	

Section A7.5.3.1.2 Short-term toxicity on birds

Annex Point IIIA XIII 1.2

The test compound was added in the respective amounts to a small quantity of the basal diet (quail diet, type: Altromin 0719) and premixed by a high-speed homogenizer. Subsequently, this premix was transferred into a 125-litre Lödige Mixer "MGT 125", the remaining aliquot of the test diet added and agitated at 3000 UPM for five minutes. No carrier was used.

The amount of test substance added and the corresponding quantity of feed were:

Nominal Dietary Concentration	KBR 3023 techn. [g]	Altromin 0719 [g]
0 mg/kg food	0.000	5000
5000 mg/kg food	25.615	4974.385

3.3	**Reference substance**	No
3.3.1	Method of analysis for reference substance	-
3.4	**Testing procedure**	*Non-entry field*
3.4.1	Test organisms	See table A7_5_3_1_2-1
3.4.2	Test system	See table A7_5_3_1_2-2
3.4.3	Diet	See table A7_5_3_1_2-2
3.4.4	Test conditions	See table A7_5_3_1_2-3
3.4.5	Duration of the test	9 days: 5 treatment days and 3 days post-exposure observation period
3.4.6	Test parameter	Mortality, toxic signs, body weight changes, feed consumption, post-mortem examinations.
3.4.7	Examination/ Observation	See table A7_5_3_1_2-2
3.4.8	Statistics	Bartlett´s test of equal variances was performed on the body mass data to determine if the dose groups have equal variances ($p \leq 0.001$). If the data variances were equal, subsequent analyses were conducted using parametric techniques; otherwise, non-parametric techniques were used. Parametric procedures: Body mass data were subject to a standard one-way analysis of variance (ANOVA) to assess significance. If significant differences between treatment means and controls ($p \leq 0.05$) were indicated, the means of the treatment groups were compared to that of the controls using the William's test. For the non-parametric procedures, the test of equality of means was performed using the Kruskal-Wallis test. If significant differences among the means were indicated, Dunn's Summed Rank test was used to determine which treatment groups differed significantly from the control. All statistical analysis, with the exception of LC_{50} calculations, were conducted using software package Statgraphics plus v5.5, supplied by

Section A7.5.3.1.2 Short-term toxicity on birds

Annex Point IIIA XIII 1.2

		STSC, Inc., Rockville, Maryland, USA.
		4 RESULTS
4.1	**Limit Test / Range finding test**	Yes
4.1.1	Concentration	5000 mg a.i./kg food
4.1.2	Number/ percentage of animals showing adverse effects	There were no treatment related mortalities, overt signs of toxicity or treatment related effects on body mass or feed consumption at the 5000 mg a.i./kg food test concentration. One bird displayed an impaired motility starting on day 6 and lasting until study termination. However due to absence of other typical signs of pesticide intoxication as e.g. apathy, diarrhea, ptosis, this motility impairment was considered as a genetically derived defect (morphological defect in leg muscle development). See Table A7_5_3_1_2-4
4.1.3	Nature of adverse effects	See Point 4.1.2
4.2	**Results test substance**	*Non-entry field*
4.2.1	Applied concentrations	Birds were exposed for five days to a nominal dietary concentration of 5000 mg a.i./kg feed. Analytical data showed KBR 3023 (Icaridin) measured concentrations in the diet to be 96.8 % at test start, 93.6 % at study day 1 (based on day 0) and 94.5 % at day 7 (based on day 0), respectively.
4.2.2	Effect data (Mortality)	There were no treatment related mortalities. LC_{50} > 5000 mg a.i./kg food, NOEC ≥ 5000 mg a.i./kg food
4.2.3	Body weight	No treatment related effects on body weight See Table A7_5_3_1_2-5
4.2.4	Feed consumption	See Table A7_5_3_1_2-6
4.2.5	Concentration / response curve	Not reasonable, since test was performed with one test concentration only (Limit test)
4.2.6	Other effects	There were no treatment related overt signs of toxicity or treatment related effects on body mass or feed consumption at the 5000 mg a.i./kg food test concentration. One bird displayed an impaired motility starting on day 6 and lasting until study termination. However due to absence of other typical signs of pesticide intoxication as e.g. apathy, diarrhea, ptosis, this motility impairment was considered as a genetically derived defect. See Table A7_5_3_1_2-7
4.3	**Results of controls**	No mortalities were observed in the control.
4.3.1	Number/ percentage of animals showing	-

Section A7.5.3.1.2 Short-term toxicity on birds

Annex Point IIIA XIII 1.2

	adverse effects	
4.3.2	Nature of adverse effects	-
4.4	**Test with reference substance**	Not performed
4.4.1	Concentrations	-
4.4.2	Results	-

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	A five-day dietary toxicity study according to OECD guideline No. 205 (1984) and US-EPA FIFRA Guideline 71-2 was conducted on 12-day old Northern bobwhite chicks (*Colinus virginianus*).with KBR 3023 (Icaridin). The test substance was administered ad libitum in the diet to groups of 10 birds. Two groups received KBR 3023 at nominal dietary concentrations of 5000 mg a.i./kg food. Two control groups (10 birds each) were maintained concurrently with the treatment groups. Birds were observed daily for mortality, abnormal behaviour and signs of toxicity. Body mass was measured at test initiation, at day 5 and at sacrifice. Feed consumption was measured daily for each group. Necropsis were performed on all animals surviving until sacrifice and all chicks that died during the test.
5.2	**Results and discussion**	There were no treatment related mortalities, overt signs of toxicity or treatment related effects on body mass or feed consumption at the 5000 mg a.i./kg food test concentration. One bird displayed an impaired motility starting on day 6 and lasting until study termination. However due to absence of other typical signs of pesticide intoxication as e.g. apathy, diarrhea, ptosis, this motility impairment was considered as a genetically derived defect. LC_{50} > 5000 mg a.i./kg feed, NOEC ≥ 5000 mg a.i./kg feed. Based on the results, KBR 3023 (Icaridin) can be considered as non-toxic to birds.
5.2.1	LC_{50}	LC_{50} > 5000 mg a.i./kg feed
5.3	**Conclusion**	Validity criteria for short-term avian toxicity test according to OECD Guideline 205 which are given in Table A7_5_3_1_2-8, can be considered as fulfilled. Dose-response relationship: Not applicable (Limit test)
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	10 March 2007
Materials and Methods	A five-day dietary toxicity study according to OECD guideline No. 205 (1984) and US-EPA FIFRA Guideline 71-2 was conducted on 12-day old Northern bobwhite chicks with KBR 3023 (Icaridin). GLP study. Birds were exposed for five days to a nominal dietary concentration of 5000 mg a.i./kg feed. Analytical data showed KBR 3023 (Icaridin) measured concentrations in the diet to be 96.8 % at test start, 93.6 % at study day 1 (based on day 0) and 94.5 % at day 7 (based on day 0), respectively. Two control groups (10 birds each) were maintained concurrently with the treatment groups. Birds were observed daily for mortality, abnormal behaviour and signs of toxicity. Body mass was measured at test initiation, at day 5 and at sacrifice. Feed consumption was measured daily for each group. Necropsis were performed on all animals surviving until sacrifice and all chicks that died during the test.
Results and discussion	There were no treatment related mortalities, overt signs of toxicity or treatment related effects on body mass or feed consumption at the 5000 mg a.i./kg food test concentration. LC_{50} > 5000 mg a.i./kg feed, NOEC ≥ 5000 mg a.i./kg feed. Based on the results, KBR 3023 (Icaridin) can be considered as non-toxic to birds.
Conclusion	Validity criteria OECD Guideline 205 was fulfilled. (Mortality of control animals < 10 %,, test substance concentration > 80 % of nominal concentration throughout the dosing period, and Lowest treatment level causing no compound-related mortality or other observable toxic effects Dose-response relationship: Not applicable (Limit test)
Reliability	1
Acceptability	Acceptable
Remarks	Non
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*

Remarks

Table A7_5_3_1_2-1: Test animals

Criteria	Details
Species/Strain	Northern bobwhite quail (*Colinus virginianus*)
Source	Barrett's Quail Farm (USA)
Age (in weeks), sex and initial body weight (bw)	Age: 12 days at study initiation; Sex: unknown; Body weight: 22.2 – 35.7 g at study initiation
Breeding population	Fertile eggs were obtained from Barrett's Quail Farm (USA) and placed in an incubator for 21 days. Eggs were candled on day o for cracks and all eggs cracked were discarded. On day 21, eggs were placed in a hatcher and allow ed to hatch. When hatched, chicks were placed into the test cages to get acclimatized to test cages and test conditions.
Amount of food	Food and water were available ad libitum, prior to and after the exposure period.
Age at time of first dosing	Age: 12 days
Health condition / medication	No data

Table A7_5_3_1_2-2: Test system

Criteria	Details
Test location	Indoor, steel cages
Holding pens	Stainless steel wire cages (70 x 46 x 20 cm high); cages were not changed during the study
Number of animals	40 (20 for dose groups, 20 for control groups)
Number of animals per pen [cm²/bird]	10 birds of unknown sex (322 cm²/bird)
Number of animals per dose	Two control groups, with 10 birds each, Two dose groups (5000 mg a.i./kg feed) with 10 birds each
Pre-treatment / acclimatisation	Birds were examined after hatching and periodically until used for tests. During the pre-test period they were observed daily on working days.
Diet during test	Full value diet for quail (Altromin 0719; supplier: Altromin GmbH, Lage, Germany, Batch No. 011296/0849) and water (Monheim City Municipal Water) were available, ad libitum, prior to and after the exposure period. Diet preparation: See Point 3.2 (Administration of the test substance) of this study summary. The amount of diet required for the entire study was prepared in one batch. The diet was kept deep-frozen at –18 °C in portions. Each day a freshly thawed portion was offered to the birds. The uneaten feed from the day before was weighed back and discarded.
Dosage levels (of test substance)	Birds were exposed for five days to a nominal dietary concentration of 5000 mg a.i./kg feed.
Replicate/dosage level	Two dose groups were investigated
Feed dosing method	Orally by feed
Dosing volume per application	Food was available ad libitum
Frequency, duration and method of animal monitoring after dosing	Birds were observed daily for mortality, abnormal behaviour and signs of toxicity. Body mass was measured at test initiation, at day 5 and at sacrifice. Feed consumption was measured daily for each group. Necropsis were performed on all animals surviving until sacrifice and all chicks that died during the test.
Time and intervals of body weight determination	Body mass was measured at test initiation, at day 5 and at sacrifice.

Table A7_5_3_1_2-3: Test conditions (housing)

Criteria	Details
Test temperature	Birds were maintained under indoor conditions with a controlled climate. Room temperature ranged between 20.5 and 22.9 °C
Shielding of the animals	No data
Ventilation	No data
Relative humidity	RH values ranged between 56.3 and 77.7 %
Photoperiod and lighting	14/10 hour light/dark cycle

Table A7_5_3_1_1-4: Mortality and toxic symptoms observed in Bobwhite chicks fed with KBR 3023 (Icaridin)

Test substance dosage level	No. of birds exhibiting toxic signs / No. of dead birds / No of dosed birds	Signs Noted
0 mg/kg feed	0 / 0 / 20	
5000 mg/kg feed	(1*) / 0 / 20	Impaired motility*

* On day 6, one bird showed impaired motility. Since there were no other signs typically observed in the context of pesticide intoxication, this impairment was not considered as related to treatment but to a morphological defect in leg muscle development.

Table A7_5_3_1_1-5: Temporal changes in mean body mass of Bobwhite chicks fed with diets containing KBR 3023 (Icaridin)

Nominal Test substance concentration	Mean Body Mass ± Standard deviation (SD) in [g]		
	Test Initiation	Test Day +5	Test Day +8
0 mg/kg feed (Control)	28.9 ± 3.1	43.6 ± 4.0	53.7 ± 4.8
5000 mg/kg feed	31.2 ± 2.9	45.3 ± 3.9	54.8 ± 6.0

Table A7_5_3_1_1-6: Mean feed consumption of Bobwhite chicks fed with diets containing KBR 3023 (Icaridin)

Nominal Test substance concentration	Feed consumption in g/bird/day							
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
0 mg/kg feed (Control)	8.6	10.9	9.5	11.7	8.6	12.1	10.7	9.9
5000 mg/kg feed	7.9	10.1	8.3	11.0	7.8	11.7	9.7	8.5

Table A7_5_3_1_1-7: Gross necropsy observations on the Bobwhite chicks subjected to a dietary treatment with KBR 3023 (Icaridin)

Findings	No. of animals with observations / No. of animals on study	
	0 mg/kg feed (Control)	**5000 mg/kg feed**
Sacrificed	20	20
No gross lesions	19 / 20	19 / 20
Spleen- anemic	1 / 20	1 / 20

Table A7_5_3_1_2-8: Validity criteria for short-term avian toxicity test according to OECD Guideline 205

	fulfilled	Not fulfilled
Mortality of control animals < 10 %	**X**	
Test substance concentration > 80 % of nominal concentration throughout the dosing period	**X**	
Lowest treatment level causing no compound-related mortality or other observable toxic effects	**X**	

Section A7.5.3.1.3 Annex Point IIIA 13.1.3	Effects on birds: Effects on reproduction	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [X]**	**Technically not feasible [ ] Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	An reproduction test on birds is not submitted as it not a data requirement for biocidal actives used in repellents (PT19, see TNsG, chapter 2.5) when they are not applied in the form of baits, granulates of powder. The product Autan Pump Spray 20% is exclusively used as a skin applied insect repellent and an exposure of the environment including soil, herbs and insects to Icaridin can be excluded when applied according to the recommended use. In general, birds may be exposed to a product by the consumption of contaminated feed like herbs or insects picked from the treated area. Thus, long-term exposure of birds to Icaridin, a.i. of Autan Pump Spray 20%, from its mode of application is highly improbable. The main emission route will be to wastewater as the product is directly released with wastewater at washing and bathing after application or indirectly when substances that have been transferred to clothing are removed at washing. Thus, Icaridin will reach sewage treatment plants (STP) via wastewater, where degradation will occur during the retention time in the STP to a major degree. The exposure route via sewage sludge treatment is of no concern since Icaridin will be predominately present in the water phase of a STP (99%). Another potential route of emission is those to the atmosphere, either due to the volatilisation of the compound from the skin surface or as a result of volatilisation from the sewage treatment plant. However, the short atmospheric half-life of Icaridin prevents the compound to be deposed to soils. Therefore, a contamination of soil regarding these pathways can be neglected and even for the case of a systemic active substance, which could indirectly affect plants acting as food for birds, long-term exposure can be excluded. Due to the lack of exposure described above an avian reproduction study is not required in the context of the application of Icaridin as a skin applied insect repellent.	
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	April 2007

Evaluation of applicant's justification	applicant's justification is OK
Conclusion	applicant's justification is acceptable
Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A7.5.4.1 Annex Point IIIA 13.3	Acute toxicity to honeybees and other beneficial arthropods, for example predators	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [X]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [...]**	
Detailed justification:	An exposure of bees and other beneficial arthropods to Icaridin, a.i. of Autan Pump Spray 20%, from its application is highly improbable due to the following reasons: The product Autan Pump Spray 20% is exclusively used as a skin applied insect repellent and an exposure of the environment to Icaridin can be excluded when applied according to the recommended use. The main emission route will be to wastewater as the product is directly released with wastewater at washing and bathing after application or indirectly when substances that have been transferred to clothing are removed at washing. Thus, Icaridin will reach sewage treatment plants (STP) via wastewater, where degradation will occur during the retention time in the STP to a major degree. The exposure route via sewage sludge treatment is of no concern since Icaridin will be predominately present in the water phase of a STP (99%). Another potential route of emission is those to the atmosphere, either due to the volatilisation of the compound from the skin surface or as a result of volatilisation from the sewage treatment plant. However, the short atmospheric half-life of Icaridin prevents the compound to be deposed to soils. Therefore, a contamination of soil regarding these pathways can be neglected and even for the case of a systemic active substance, which could indirectly affect plants and their pollen, exposure can be considered negligible. Merely some individual bees or other arthropods might be affected by getting in contact with residues of the product caused by unwary application. But obviously, this exposure by chance does not pose any hazard for natural communities. Due to the lack of exposure described above tests on honeybees or other beneficial arthropods are not required in the context of the application of Icaridin as a skin applied insect repellent.	
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 FI
Date	April 2007
Evaluation of applicant's justification	applicant's justification is OK

Conclusion	applicant's justification is acceptable
Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section 7.5.5 Annex Point IIIA 13.3	Bioconcentration, terrestrial / further studies	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ] Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	Due to the low potential of Icaridin to bioaccumulate (log P_{OW} < 3), no studies on bioconcentration of Icaridin in soil organisms were submitted. Furthermore, there is no data requirement for such data for biocidal actives used in PT 19 (according to TNsG, chapter 2.5).	
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	April 2007	
Evaluation of applicant's justification	applicant's justification is OK	
Conclusion	applicant's justification is acceptable	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A7.5.6 Annex Point IIIA 13.3	Effects on other terrestrial non-target organisms	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [X]**	**Technically not feasible [ ] Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	Tests on other terrestrial non-target organisms are not a data requirement for biocidal actives used in PT 19 (according to TNsG, chapter 2.5). The data presented in the dossier are regarded to allow a complete assessment of the product concerning the risk to non-target organisms and further data are not considered necessary. Furthermore, an exposure of other terrestrial non-target organisms to Icaridin, a.i. of Autan Pump Spray 20%, from its application is highly improbable due to the following reasons: The product Autan Pump Spray 20% is exclusively used as a skin applied insect repellent and hence a direct contamination of the environment with Icaridin can be excluded when applied according to the recommended use. The main emission route will be to wastewater as the product is directly released with wastewater at washing and bathing after application or indirectly when substances that have been transferred to clothing are removed at washing. Thus, Icaridin will reach sewage treatment plants (STP) via wastewater, where degradation will occur during the retention time in the STP to a major degree. The exposure route via sewage sludge treatment is of no concern since Icaridin will be predominately present in the water phase of a STP (99%). Another potential route of emission is those to the atmosphere, either due to the volatilisation of the compound from the skin surface or as a result of volatilisation from the sewage treatment plant. However, the short atmospheric half-life of Icaridin prevents the compound to be deposed to soils. Therefore, a contamination of soil regarding these pathways can also be neglected. Due to the lack of exposure described above further tests (e.g. field tests) on other terrestrial organisms are not required in the context of the application of Icaridin as a skin applied insect repellent.	
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	April 2007
Evaluation of applicant's justification	applicant's justification is OK

Conclusion	applicant's justification is acceptable
Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section 7.5.7.1 Annex Point IIIA 13.3	Effects on mammals: acute oral toxicity, short term toxicity, effects on reproduction	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X] **Limited exposure [X]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	Such studies are not a data requirement for the ecotoxicological assessment of biocidal actives used in PT 19 (according to TNsG, chapter 2.5). However, information on toxicology of Icaridin to mammals is provided in Section 6 of Doc. III-A 6: "Toxicological and Metabolic Studies". Furthermore, exposure of mammals to Icaridin, a.i. of Autan Pump Spray 20%, is highly improbable due to the following reasons: In general, mammals may be exposed to a biocidal product by the consumption of contaminated feed like herbs or insects picked from the treated area. The product Autan Pump Spray 20% however is exclusively used as a skin applied insect repellent and an exposure of the environment including soil, herbs and insects to Icaridin can almost be excluded. Even if a mammal might ingest small amounts of contaminated feed by accident, no adverse effects are expected due to the low intrinsic acute toxicity of Icaridin (LC_{50} 2236 mg/kg bw, see Doc. III-A Section 6.1.1). Thus, mammals are considered not to be at risk by the use of Autan Pump Spray 20%.	
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	April 2007	
Evaluation of applicant's justification	applicant's justification is OK	
Conclusion	applicant's justification is acceptable	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		