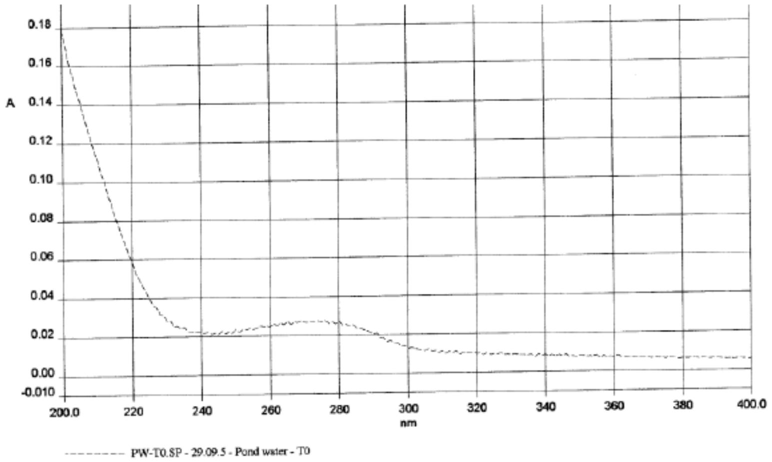
Section A7.1.1.1.2 Phototransformation in water including identity of transformation products
Annex Point IIA VII.7.6.2.2

			Official use only
		1 REFERENCE	
1.1	**Reference**	*Adam, D., 2007, Phototransformation of 2-(n-octyl)-4-[4,5-^{14}C]-isothiazolin-3-one (^{14}C-OIT) in water - direct photolysis,* [redacted]	
1.2	**Data protection**	*Yes*	
1.2.1	Data owner	*THOR GmbH* [redacted]	
1.2.2	Company with letter of access	*None*	
1.2.3	Criteria for data protection	*Data submitted on existing a.s. for the purpose of its entry into Annex I.*	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	*Yes* *OECD Guideline for Testing of Chemicals, Proposal for a new Guideline: Phototransformation of Chemicals in Water – Direct and Indirect Photolysis, Draft Document, August 2000.* *GCPF/ECPA –Comments on the Draft Proposal for a New OECD Test Guideline on:* *"Phototransformation of Chemicals in Water – Direct and Indirect Photolysis", 30 October 2000.* *JMAFF Agchem Test Guidelines 12 Nohsan N. 8147, 24 November 2000, revised 26 June 2001: Photodegradation in water (2-6-2)* *Pesticide Assessment Guidelines, Subdivision N, Chemistry: Environmental Fate, EPA-540/9-82-021, Section 161-2: Photodegradation Studies in Water, U.S. Environmental Protection Agency, October 18, 1982.* *FIFRA Accelerated Reregistration, Phase 3 Technical Guidance, EPA 540/09-90-078, December 1989.* *Environmental Chemistry and Fate Guideline for Registration of Pesticides in Canada.*	
2.2	**GLP**	*Yes*	
2.3	**Deviations**	*No*	X
		3 MATERIALS AND METHODS	
3.1	**Test material**	*2-(n-octyl)-4-[4,5-^{14}C]isothiazolin-3-one (^{14}C-OIT)*	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Radiochemical Purity	[redacted]	
3.1.3	Radiolabelling	[redacted]	
3.1.4	UV/VIS absorption spectra and absorbance value	*UV-absorption spectrum of ^{14}C-OIT in pond water before irradiation (0.473 mg/L)*	



3.1.5 Specific Radioactivity [redacted]

3.2 Reference substances *None.* X

3.3 Test solution *See table A7_1_1_2-1*

3.4 Testing procedure

3.4.1 Test system *See table A7_1_1_2-2*

3.4.2 Properties of light source *See table A7_1_1_2-2*

3.4.3 Determination of irradiance *The measurement of the intensity of artificial irradiance was performed with a radiometer (see Table A7_1_1_1_2-2).*

3.4.4 Temperature *25°C*

3.4.5 pH

Buffer pH values	Duplicate	Irradiation/Incubation Time in days								
		0	0.08	0.17	0.63	1	2	4	8	15
Irradiated solution	A	7.07	7.05	7 06	7.05	7.10	7.04	7 09	7.09	7.04
	B	7.07	7.05	7 05	7.05	7.07	7.05	7.10	7.11	7.05
Dark control solution		7.10	7.07	7 06	7.06	7.10	7.06	7 05	7.09	7.05

natural water pH values	Duplicate	Irradiation/Incubation Time in days								
		0	0.08	0.17	0.63	1	2	4	8	15
Irradiated solution	A	8.40	8.40	8 39	8.52	8.54	7.95	8 65	8.61	8.62
	B	8.40	8.44	8.41	8.59	8.53	8.50	8.75	8.51	8.70
Dark control solution		8.39	8.41	8.43	8.53	8.49	8.55	8.77	8.63	8.76

3.4.6 Duration of the test *15 days*

3.4.7 Number of replicates *Duplicates (irradiated samples) and single samples for dark controls.*

3.4.8 Sampling *Duplicate irradiated samples and dark controls were taken after 0, 2, 4 and 15 hours and 1, 2, 4, 8 and 15 days of irradiation/incubation. Dark* *controls were taken at the same sampling intervals.*

3.4.9 Analytical methods

<u>HPLC</u>:
Pre-column: [redacted]
Column: [redacted]
Column Temperature: [redacted]
Mobile Phase:
Solvent A: [redacted]
Solvent B: [redacted]
Gradient:

Time (min)	0	5	15	45	50	55	55.1	70
Solvent A (%)	100	100	40	40	0	0	100	100
Solvent B (%)	0	0	60	60	100	100	0	0

Flow: [redacted]
UV-Detection: [redacted]
14C-Detection: [redacted]

<u>TLC</u>:
[redacted]

3.5 Transformation products

Transformation products tested: Yes

3.5.1 Method of analysis for transformation products

<u>LCMS</u>:
MS Conditions
Instrument: [redacted]
Software: [redacted]
Ionisation Mode: [redacted]
Detection Mode: [redacted]
Scan Mode: [redacted]
Sheath Gas Pressure: [redacted]
Auxiliary Gas Pressure: [redacted]
Capillary temperature: [redacted]
Discharge current: [redacted]

4 RESULTS

4.1 Controls

Report the initial molar test substance concentration (C_0) and the final molar concentration (C_t) of the controls.

4.2 Photolysis data

Non-entry field

4.2.1 Concentration values

See Tables A7_1_1_2-3 through A7_1_1_2-6

4.2.2 Mass balance

The total mean recoveries from the irradiated and dark control buffer samples during the study were 96.7% ± 2.1% and 100.1% ± 2.1% of the applied radioactivity, respectively. Corresponding values for the natural pond system are 100.1% ± 1.5% and 100.1% ± 2.6%

4.2.3 Half-lives

Section A7.1.1.1.2 Phototransformation in water including identity of transformation products

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Calculated Half-life (DT_{50}) and DT_{90} in Days							
Compound	k-value (days ^{1})	Suntest *		Sunlight ** 50°N		Sunlight *** 30-40°N	
		DT_{50}	DT_{90}	DT_{50}	DT_{90}	DT_{50}	DT_{90}
Buffer pH7							
OIT	0 379	1 8	6 1	3 7	12 4	3 5	11 9
M8	0 341	2 0	6 7	4 1	13 6	3 9	13 0
Pond							
OIT	0 280	2 5	8 3	5 1	16 6	4 9	16 6
M7	0 104	6 6	22 1	13 4	44 8	12 9	43 0
M8	0 212	3 3	10 8	6 7	21 9	6 4	21 0

* *continuous irradiation*
** *natural summer sunlight at 50°N*
*** *natural summer sunlight at 30-40°N*

4.2.4 Kinetic order *First order* X

4.3 Specification of the transformation products *See table A7_1_1_2-3 through A7_1_1_2-6 and Figures A7_1_1_2-1 through A7_1_1_2-31.*

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The direct and indirect photochemical degradation of ^{14}C-OIT, i.e. 2-(n-octyl)-4-[4,5-^{14}C]isothiazolin-3-one, was investigated under simulated sunlight in sterilised buffer solution at pH 7 and sterilised natural pond water at about pH 8.

The photolysis apparatus (Suntest) was equipped with a [redacted]*. Filters were used to cut off ultraviolet light with a wavelength below 290 nm. For a representative range (300 nm to 400 nm) of the whole visual light spectrum, the intensity of light was determined to be 41.9 W/m^{2} at the surface of the photo-degradation vessels. The intensity of the lamp was slightly higher than that of natural summer sunlight measured at the test facility (latitude 47.5°N, August 29, 2006) and representing 40.2 W/m^{2}.*

For both systems (buffer solution and pond water), duplicate samples at an initial concentration of approximately 0.5 mg 2-(n-octyl)-4-[4,5-^{14}C]isothiazolin-3-one (^{14}C-OIT) per litre were prepared and continuously irradiated for a period of 15 days at a mean temperature of 25.1 ± 0.2°C. Control solutions were incubated under identical conditions but in the dark and at a temperature of 24.6 ± 0.0°C. Duplicate aliquots of the irradiated samples and dark controls were taken for analysis periodically over the incubation period. The 15 days of continuous Suntest irradiation corresponded to 30.5 natural summer sunlight days at latitude 50°N.

5.2 Results and discussion X

The total mean recoveries from the irradiated and dark control solutions during the 15-day incubation period were 96.7% ± 2.1% and 100.1% ± 2.1% of the initially applied radioactivity, respectively, for the buffered system. Corresponding values for the pond system were 100.1% ± 1.5% and 100.1% ± 2.6%.

2-(n-octyl)-4-[4,5-^{14}C]isothiazolin-3-one (^{14}C-OIT) was found to be rapidly photolysed in both systems. In sterile pH 7 buffer solution, the concentration of the test item in the irradiated samples decreased from 97.9% to 1.1% of the applied radioactivity (mean values), within 15 days of irradiation. The corresponding values for natural pond system were

98.0% and 2.5%.

Under irradiation the test item degraded to form a significant number of degradates. Some of them were further mineralised to $^{14}CO_2$*. The formation of radioactive carbon dioxide increased continuously amounting to 8.8% and 6.8% for the buffer and pond samples, respectively. The presence of* $^{14}CO_2$ *demonstrates that photodegradation involves cleavage of the isothiazolone ring. Besides 2-(n-octyl)-4-[4,5-*^{14}*C]isothiazolin-3-one (*^{14}*C-OIT), up to 10 radioactive fractions were detected, four of which accounted for more than 10% of the applied radioactivity. The pattern of metabolites was slightly different between buffer solution and pond water. The major radioactive fractions formed in the buffered system were M3, M7 and M8, whereas in the pond system M7, M8 and M10 were detected in amounts over 10%. M8 was identified by HPLC and LC/MS as N-(N-octyl) acetamide. M3 was identified as N-(N-octyl) ethyl amine. The very polar radioactive fractions, M7 and M10, were repeatedly re-generated in high dose experiments in buffer solution, natural and bi-distilled water. Several work-up procedures and mass spectrometric analysis were performed. Due to repeated clogging of the MS system after injection of purified M7/M10, structure elucidation for these fractions was not possible using LC/MS. However, chromatography and MS results indicate that the fractions must consist of several components, most probably polymers or adducts with differently sized fragments of the N-(N-octyl) side-chain.*

In the buffer system, the metabolites, N-(N-octyl) ethyl amine (M3) and M7 increased continuously and accounted for a maximum mean amount of 16.2% and 55.1%, respectively after 15 days of irradiation. The metabolite M8, N-(N-octyl) acetamide reached its maximum of 23.3% on day 4 and declined to 1.3% after 15 days of irradiation. All other metabolites were individually ≤ 4.0% (mean values).

Virtually no degradation of 2-(n-octyl)-4-[4,5-^{14}*C]isothiazolin-3-one (*^{14}*C-OIT) was observed in the dark control samples of the buffer test system. After 15 days of incubation, the test item still represented 95.4% of the applied radioactivity. The amount of carbon dioxide was ≤ 0.3%.*

In the pond water, a different pattern was observed. The major metabolite was M10 reaching the maximum of 58.7% of the initial amount after 15 days of irradiation. N-(N-octyl) acetamide (M8) reached its highest concentration of 25.1% on day 4 and degraded to 4.5% at day 15. M7 reached its maximum of 11.3% on day 4 and degraded thereafter to 5.0% on day 15. M3 metabolite represented only 5.2% at the end of the irradiation (day 15). All other metabolites were individually ≤ 6.2% (mean values).

Contrary to the buffer system, the test item degraded in the dark controls of the pond system from 97.4% initially to 42.6% within 15 days. Formation of carbon dioxide reached a maximum value of 7.9% at study end (mean value). Degradation observed in the pond system was due to microbial activity, since the samples were shown to be non-sterile on day 15. The dark metabolic pattern, was completely different from the photodegradation pattern observed for the irradiated systems being comparable with the one observed in the biodegradation study on 2-(n-octyl)-4-[4,5-^{14}*C]isothiazolin-3-one (*^{14}*C-OIT) [2]. However, the irradiated samples were shown to be sterile during the whole irradiation time.*

The rate of photodegradation of 2-(n-octyl)-4-[4,5-^{14}*C]isothiazolin-3-one (*^{14}*C-OIT) was described using first order kinetics. The following*

		half-lives and DT$_{90}$-values of the test item and its major metabolites are summarised in paragraph 4.2.3.	
5.3	**Conclusion**	*2-(n-octyl)-4-[4,5-^{14}C]isothiazolin-3-one (^{14}C-OIT) was rapidly photo-degraded in a buffered solution at pH 7 and in natural pond water with a photolytic Suntest half-life of about 2 days, equivalent to about 4-5 days summer sunlight at latitudes 50°N and 30 to 40°N, respectively. Photodegradation involved cleavage of the isothiazolone ring (cf table*	
5.3.1	Reliability	*1*	X
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	27 Nov 09
	2.3: The OECD Guideline states that the control samples should remain sterile throughout; this was not the case for the dark pond control in this study. The dark buffer solution control did not loose sterility within the study.
Materials and Methods	Applicant's version is considered acceptable noting the following:
	3.2: No reference substance was used.
	3.4.7: The robust study summary states that single samples were prepared for dark controls, which is contrary to page 22 of Adam (2007), where it is implied that the dark controls were prepared in duplicate. However, the study plan implies that single samples were taken for hydrolytically stable compounds and duplicate aliquots for irradiated samples if not hydrolytically stable.

Section A7.1.1.1.2 Phototransformation in water including identity of transformation products

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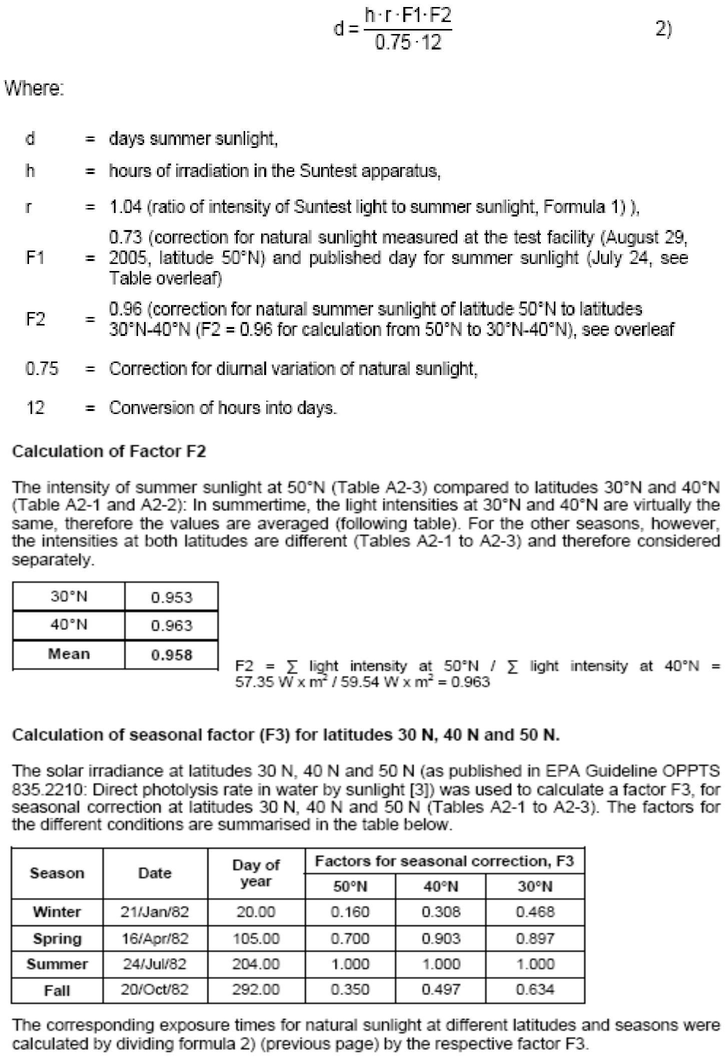
Results and discussion

Applicant’s version considered acceptable, noting the following:

A range of latitudes, such as 30-40°, should not be used. However the summer values are in good agreement and can be allowed, the values for spring, fall and winter are as follows. The applicant’s data was converted into the corresponding values for 30/40/50 ˚N using the conversion equations used by the applicant and described in Appendix II of the report. These and the resulting values are as follows:



$$d = \frac{h \cdot r \cdot F1 \cdot F2}{0.75 \cdot 12} \qquad 2)$$

Where:

d = days summer sunlight,
h = hours of irradiation in the Suntest apparatus,
r = 1.04 (ratio of intensity of Suntest light to summer sunlight, Formula 1)),
F1 = 0.73 (correction for natural sunlight measured at the test facility (August 29, 2005, latitude 50°N) and published day for summer sunlight (July 24, see Table overleaf)
F2 = 0.96 (correction for natural summer sunlight of latitude 50°N to latitudes 30°N-40°N (F2 = 0.96 for calculation from 50°N to 30°N-40°N), see overleaf
0.75 = Correction for diurnal variation of natural sunlight,
12 = Conversion of hours into days.

Calculation of Factor F2

The intensity of summer sunlight at 50°N (Table A2-3) compared to latitudes 30°N and 40°N (Table A2-1 and A2-2): In summertime, the light intensities at 30°N and 40°N are virtually the same, therefore the values are averaged (following table). For the other seasons, however, the intensities at both latitudes are different (Tables A2-1 to A2-3) and therefore considered separately.

30°N	0.953
40°N	0.963
Mean	**0.958**

F2 = ∑ light intensity at 50°N / ∑ light intensity at 40°N = 57.35 W x m^2 / 59.54 W x m^2 = 0.963

Calculation of seasonal factor (F3) for latitudes 30 N, 40 N and 50 N.

The solar irradiance at latitudes 30 N, 40 N and 50 N (as published in EPA Guideline OPPTS 835.2210: Direct photolysis rate in water by sunlight [3]) was used to calculate a factor F3, for seasonal correction at latitudes 30 N, 40 N and 50 N (Tables A2-1 to A2-3). The factors for the different conditions are summarised in the table below.

Season	Date	Day of year	Factors for seasonal correction, F3		
			50°N	40°N	30°N
Winter	21/Jan/82	20.00	0.160	0.308	0.468
Spring	16/Apr/82	105.00	0.700	0.903	0.897
Summer	24/Jul/82	204.00	1.000	1.000	1.000
Fall	20/Oct/82	292.00	0.350	0.497	0.634

The corresponding exposure times for natural sunlight at different latitudes and seasons were calculated by dividing formula 2) (previous page) by the respective factor F3.

Fall	Calculated Half-life (DT50) and DT90 in Natural Fall Sunlight days					
Compound	Sunlight 50° N		Sunlight 40° N		Sunlight 30° N	
	DT50	DT90	DT50	DT90	DT50	DT90
Parent	10.4	35.4	7.3	24.9	5.5	18.7
M8	11.6	38.8	8.2	27.3	6.1	20.6
Parent pond	14.5	47.5	10.2	33.5	7.7	26.2
M7	38.3	128.1	26.9	90.2	20.3	70.7
M8	19.1	62.6	13.5	44.1	10.1	34.6

Winter	Calculated Half-life (DT50) and DT90 in Natural Winter Sunlight days					
Compound	Sunlight 50° N		Sunlight 40° N		Sunlight 30° N	
	DT50	DT90	DT50	DT90	DT50	DT90
Parent	22.8	77.3	11.9	40.2	7.5	25.4
M8	25.4	84.9	13.2	44.1	8.3	27.9
Parent pond	31.7	104	16.5	54	10.4	35.5
M7	83.7	280.2	43.5	145.5	27.5	95.8
M8	41.8	136.9	21.7	71.1	13.7	46.8

4.2.4: The OECD Guideline astates that non linear kinetics should be used; the applicant has re-evaluated the data using first order kinetics to calculate t_{12} , rate constant and r^2 (data shown below within figure A7_1_1_2-4)

Conclusion

Applicant's version is considered acceptable noting the following:

5.2 : Metabolites M7 and M10 were not identified, despite the fact that these were present at >50 % in the samples It should be noted that Attempts were made to identify the very polar unknown major photodegradates, M7 and M10

Reliability

2

Study conducted in accordance with generally accepted scientific principles, possibly with incomplete reporting or methodological deficiencies, which do not affect the quality of relevant results.

Acceptability

Acceptable

Remarks

All endpoints and data presented in the summary and tables have been checked against the original study.The DT50 values can be considered acceptable, however the study fails to identify metabolite M7 and M10, while this is a requirement, the non-identification of the metabolites has no effect upon the resulting DT50 values.

As discussed within Doc IIA the photometabolites are not relevant to the final risk assessment.

All endpoints and data presented in the summary and tables have been checked against the original study.

COMMENTS FROM ...

Date

Section A7.1.1.1.2 Phototransformation in water including identity of transformation products

Annex Point IIA VII.7.6.2.2

Materials and Methods

Results and discussion

Conclusion

Reliability

Acceptability

Remarks

Table A7_1_1_2-1: Description of test solution and controls

Criteria	Details
Purity of water	*Purified water and natural pond water sterilised by gamma irradiation up to a maximal dose of 60 kGy.*
Preparation of test chemical solution	*Stock solution: 0.202 mg 2-(n-octyl)-4-[4,5-^{14}C]isothiazolin-3-one (^{14}C-OIT)/mL acetonitrile.*
Test concentrations (mg a.s./L)	*Initial concentrations:* [redacted] *(buffer) and* [redacted] *(natural pond water); The concentration of the co-solvent acetonitrile was 0.2%.*
Temperature (°C)	*About 25 °C*
Preparation of a.s. solution	*Not applicable*
Controls	*Dark controls*
Identity and concentration of co-solvent	*See above*

Table A7_1_1_2-2: Description of test system

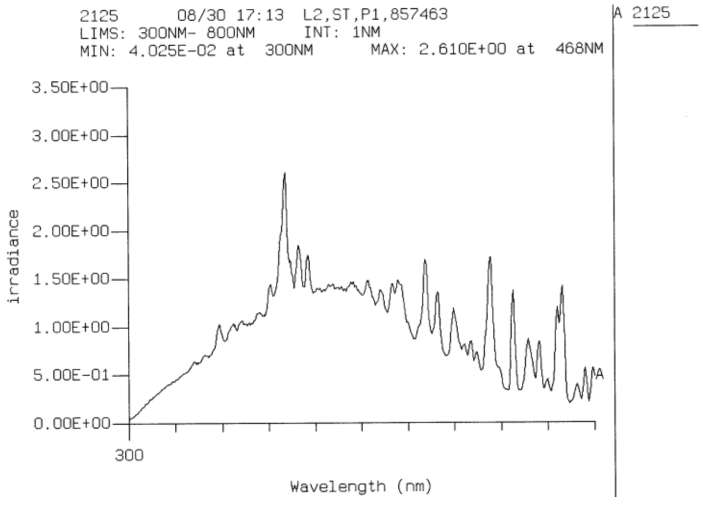
Criteria	Details
Laboratory equipment	*Photolysis was performed using cylindrical vessels constructed entirely of Pyrex glass covered with quartz glass plates (to cut off radiation below 290 nm similarly to the natural sunlight cut-off by ozone), which were screwed on top of the vessel. Exposed area 28.26 cm^2; 100 mL solution*
Test apparatus	[redacted]
Radiometer	*LI-1800 spectrophotometer* [redacted]
Properties of artificial light source:	
Nature of light source	[redacted] *Max. 765 W/m^2 at max. UV filtering (lambda < 800 nm) with irradiance between 400 W/m^2 and 765 W/m^2 to a pre-set value.)*
Emission wavelenght spectrum	
Light intensity	*400 W/m^2 to 765 W/m^2*
Filters	*UV filter with a 290 nm cut-off to simulate natural sunlight*

Table A7_1_1_2-3: Degradation of ^{14}C-OIT in the irradiated buffer samples at pH 7. Results shown in % of the applied radioactivity.

Pattern		**IRRADIATION TIME IN DAYS**								
Irradiated Buffer (pH 7)	[Suntest]	**0**	**0.08**	**0.17**	**0.63**	**1**	**2**	**4**	**8**	**15**
(%applied)	[Sunlight][1]	**0**	**0.2**	**0.3**	**1.3**	**2.0**	**4.1**	**8.1**	**16.2**	**30.5**
Parent	A	98.1	93.9	88.6	76.0	65.5	46.1	21.2	5.0	0.6
	B	97.7	94.4	91.0	76.9	67.3	45.5	20.2	5.8	1.6
	mean	**97.9**	**94.2**	**89.8**	**76.5**	**66.4**	**45.8**	**20.7**	**5.4**	**1.1**
M1 (unknown)	A	*	*	*	*	*	*	0.7	*	*
	B	*	*	*	*	*	*	*	*	*
	mean	*****	*****	*****	*****	*****	*****	**0.4**	*****	*****
M2 (unknown)	A	*	0.4	*	*	*	0.9	2.3	4.7	3.0
	B	*	*	*	*	0.7	0.3	*	3.3	3.0
	mean	*****	**0.2**	*****	*****	**0.3**	**0.6**	**1.1**	**4.0**	**3.0**
M3	A	1.2	0.8	0.7	1.3	2.0	2.9	8.3	14.9	15.6
	B	0.6	1.1	0.8	1.5	1.7	4.4	7.2	14.3	16.8
	mean	**0.9**	**1.0**	**0.7**	**1.4**	**1.8**	**3.6**	**7.8**	**14.6**	**16.2**
M4 (unknown)	A	*	*	*	*	*	0.5	1.1	2.0	*
	B	*	*	*	*	*	0.5	2.6	*	3 5
	mean	*****	*****	*****	*****	*****	**0.5**	**1.8**	**1.0**	**1.7**
M5 (unknown)	A	0.4	0.4	0.4	*	*	*	*	*	*
	B	0.5	0 5	0.7	*	*	*	0.4	*	*
	mean	**0.4**	**0.5**	**0.6**	*****	*****	*****	**0.2**	*****	*****
M6 (unknown)	A	*	*	*	*	*	*	1 8	*	3 2
	B	*	*	*	*	*	*	1.6	1.8	3.2
	mean	*****	*****	*****	*****	*****	*****	**1.7**	**0.9**	**3.2**
M7 (unknown)	A	*	0 9	1 8	5 5	12.4	18 9	33 3	52 9	56 2
	B	1 2	1 0	2.1	5.7	9.9	19.4	30 0	51 8	54 0
	mean	**0.6**	**0.9**	**2.0**	**5.6**	**11.1**	**19.2**	**31.7**	**52.3**	**55.1**
M8	A	0.3	1.9	3.2	10 0	14.7	21 8	20 0	6.9	*
	B	*	1.8	3.4	10 3	15 2	22.7	26 5	14.4	2.5
	mean	**0.2**	**1.8**	**3.3**	**10.2**	**15.0**	**22.3**	**23.3**	**10.6**	**1.3**
M9	A	*	*	*	*	*	1.6	1.4	2.4	2.4
	B	*	*	0.3	*	0.6	1.3	1.6	2.1	2.2
	mean	*****	*****	**0.2**	*****	**0.3**	**1.5**	**1.5**	**2.2**	**2.3**
M10 (unknown)	A	*	*	0.3	1.3	*	*	*	1.8	3.0
	B	*	*	*	0.8	0.8	0.4	1.5	1.7	*
	mean	*****	*****	**0.1**	**1.1**	**0.4**	**0.2**	**0.8**	**1.7**	**1.5**
$^{14}CO_2$	A	n.p.	<0.1	<0.1	0.4	0.8	1.3	3.1	7.1	9.9
	B	n.p.	<0.1	<0.1	0.4	0.8	1.8	3.5	3.7	7.6
Volatiles in ethylene glycol	A	n.p.	<0.1	<0.1	<0.1	0.2	0.3	0.4	0.5	0.6
	B	n.p.	<0.1	<0.1	<0.1	0.2	0.4	0.5	0.6	0.7
TOTAL	**A**	**100.0**	**98.3**	**95.0**	**94.6**	**95.6**	**94.1**	**93.5**	**98.1**	**94.4**
	B	**100.0**	**98.8**	**98.4**	**96.6**	**97.9**	**97.3**	**97.1**	**101.3**	**95.0**

A/B:	Replicates
*:	not detected or lower than detection limit
[1]	natural summer sunlight at latitude 50°N
M3:	N-(n-octyl) ethyl-amine, contains also low amounts of N-(n-octyl)malonamic acid
M8:	N-(n-octyl)acetamide
M9:	N-(n-octyl)oxamic acid
M7/M10:	radioactive fractions conaining multiple components

Table A7_1_1_2-4: Distribution of ^{14}C-OIT in the dark control buffer samples at pH 7. Results shown in % of the applied radioactivity.

Pattern (pH 7) Dark control (% applied)	INCUBATION TIME IN DAYS							
	0	**0.08**	**0.17**	**0.63**	**1**	**2**	**8**	**15**
Parent	**98.5**	**99.0**	**96.9**	**98.0**	**99.0**	**97.4**	**98.2**	**95.4**
M1 (unknown)	*	*	*	*	*	*	*	*
M2 (unknown)	0.3	0.3	*	*	*	0.3	3.2	4.4
M3	0.7	0.8	0.8	*	0.6	0.6	1.3	0.4
M4 (unknown)	*	*	*	*	*	*	*	*
M5 (unknown)	0.5	0.4	0.7	*	0.4	0.5	0.5	*
M6 (unknown)	*	*	*	*	*	*	*	1.2
M7 (unknown)	*	*	*	*	*	0.3	0.4	0.4
M8	*	*	0.3	*	*	*	*	*
M9	*	*	*	*	*	*	0.3	0.7
M10 (unknown)	*	*	*	*	*	*	*	*
$^{14}CO_2$	n.p.	0.0	<0.1	<0.1	<0.1	<0.1	<0.1	0.3
TOTAL	**100.0**	**100.6**	**98.7**	**98.1**	**100.0**	**99.2**	**104.1**	**102.7**

*: not detected or lower than detection limit
M3: N-(n-octyl) ethyl-amine, contains also low amounts of N-(n-octyl)malonamic acid
M8: N-(n-octyl)acetamide
M9: N-(n-octyl)oxamic acid

Table A7_1_1_2-5: Degradation of ^{14}C-OIT in the irradiated natural pond water samples. Results shown in % of the applied radioactivity.

Pattern natural water (% applied)		**IRRADIATION TIME IN DAYS**								
	[Suntest]	**0**	**0.08**	**0.17**	**0.63**	**1**	**2**	**4**	**8**	**15**
	[Sunlight][1]	**0**	**0.2**	**0.3**	**1.3**	**2.0**	**4.1**	**8.1**	**16.2**	**30.5**
Parent	A	97.9	95.0	96.2	85.6	76.2	56.8	31.0	9.6	2.2
	B	98.2	95.0	95.2	87.4	74.3	56.2	31.5	11.0	2.8
	mean	**98.0**	**95.0**	**95.7**	**86.5**	**75.2**	**56.5**	**31.2**	**10.3**	**2.5**
M1 (unknown)	A	0.4	0.4	*	*	*	*	*	*	*
	B	0.8	*	0.4	*	1.3	*	*	*	*
	mean	**0.6**	**0.2**	**0.2**	*	**0.6**	*	*	*	*
M2 (unknown)	A	0.7	*	*	*	*	0.8	*	3.6	4.2
	B	0.6	*	*	*	1.5	0.5	*	4.4	4.3
	mean	**0.6**	*	*	*	**0.7**	**0.7**	*	**4.0**	**4.2**
M3	A	0.7	1.2	0.7	*	0.8	1.7	3.1	3.5	5.4
	B	0.8	1.2	0.7	*	0.9	0.8	2.4	5.3	5.0
	mean	**0.8**	**1.2**	**0.7**	*	**0.8**	**1.3**	**2.8**	**4.4**	**5.2**
M4 (unknown)	A	*	*	*	*	1.6	3.0	6.6	0.3	5.6
	B	*	0.6	0.4	*	2.2	4.6	5.8	4.9	4.6
	mean	*	**0.3**	**0.2**	*	**1.9**	**3.8**	**6.2**	**2.6**	**5.1**
M5 (unknown)	A	*	0.4	0.4	*	*	1.4	*	*	*
	B	*	*	0.5	*	*	1.4	*	*	*
	mean	*	**0.2**	**0.5**	*	*	**1.4**	*	*	*
M6 (unknown)	A	*	0.4	*	*	*	1.1	1.8	1.2	2.4
	B	*	0.3	0.6	*	*	2.0	1.6	*	*
	mean	*	**0.3**	**0.3**	*	*	**1.6**	**1.7**	**0.6**	**1.2**
M7 (unknown)	A	*	0.5	1.5	4.8	6.3	9.5	12.9	7.0	6.2
	B	*	0.8	0.9	3.9	4.2	6.8	9.7	10.6	3.9
	mean	*	**0.7**	**1.2**	**4.3**	**5.2**	**8.1**	**11.3**	**8.8**	**5.0**
M8	A	*	1.3	2.7	7.5	12.3	19.0	25.5	21.1	5.3
	B	*	1.6	2.4	8.8	11.1	16.8	24.7	15.4	3.6
	mean	*	**1.4**	**2.5**	**8.2**	**11.7**	**17.9**	**25.1**	**18.2**	**4.5**
M9	A	*	*	*	*	*	*	2.0	3.5	5.4
	B	*	0.4	*	*	*	1.2	2.5	4.4	5.9
	mean	*	**0.2**	*	*	*	**0.6**	**2.3**	**3.9**	**5.7**
M10 (unknown)	A	*	*	0.4	1.8	4.3	7.4	14.6	41.7	54.3
	B	*	0.3	0.5	1.5	4.4	8.2	17.8	42.0	63.2
	mean	*	**0.1**	**0.5**	**1.6**	**4.3**	**7.8**	**16.2**	**41.9**	**58.7**
$^{14}CO_2$	A	n.p.	<0.1	<0.1	0.1	0.3	0.7	1.6	4.1	6.8
	B	n.p.	<0.1	<0.1	0.2	0.3	0.4	1.4	1.8	6.7
Volatiles in ethylene glycol	A	n.p.	<0.1	<0.1	<0.1	0.1	0.3	0.5	0.6	0.7
	B	n.p.	<0.1	<0.1	<0.1	0.2	0.5	0.8	0.9	1.0
TOTAL	**A**	**99.7**	**99.3**	**101.9**	**99.9**	**101.8**	**101.8**	**99.5**	**96.3**	**98.5**
	B	**100.3**	**100.2**	**101.7**	**101.9**	**100.3**	**99.4**	**98.1**	**100.8**	**101.0**

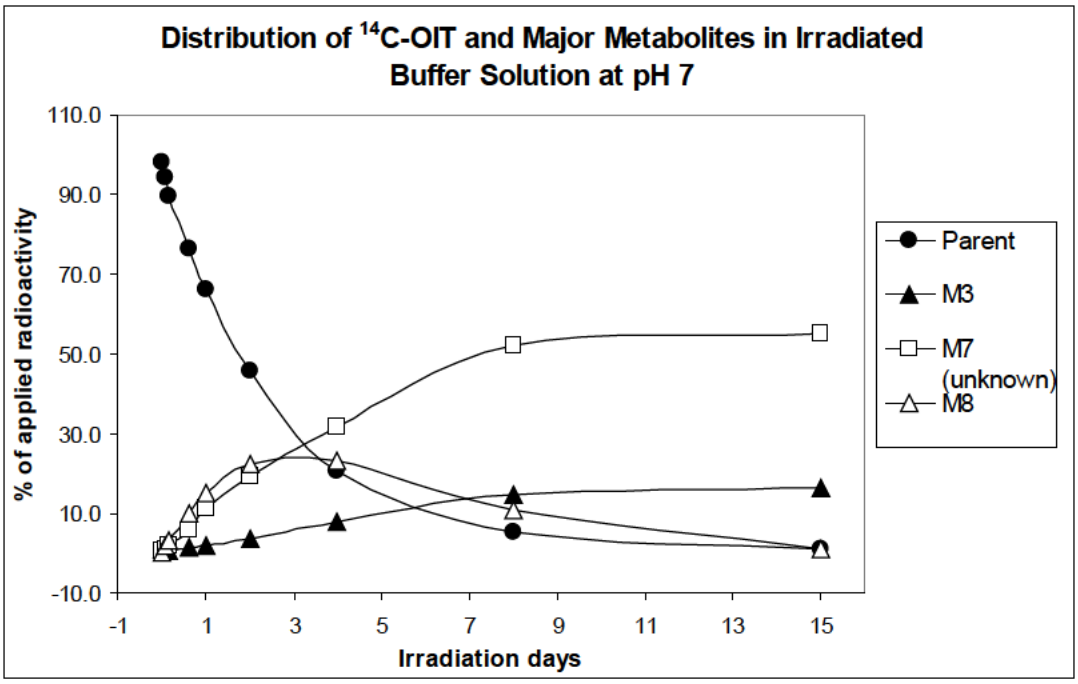
A/B:	Replicates
*:	not detected or lower than detection limit
[1]	natural summer sunlight at latitude 50°N
M3:	N-(n-octyl) ethyl-amine, contains also low amounts of N-(n-octyl)malonamic acid
M8:	N-(n-octyl)acetamide
M9:	N-(n-octyl)oxamic acid
M7/M10:	radioactive fractions conaining multiple components

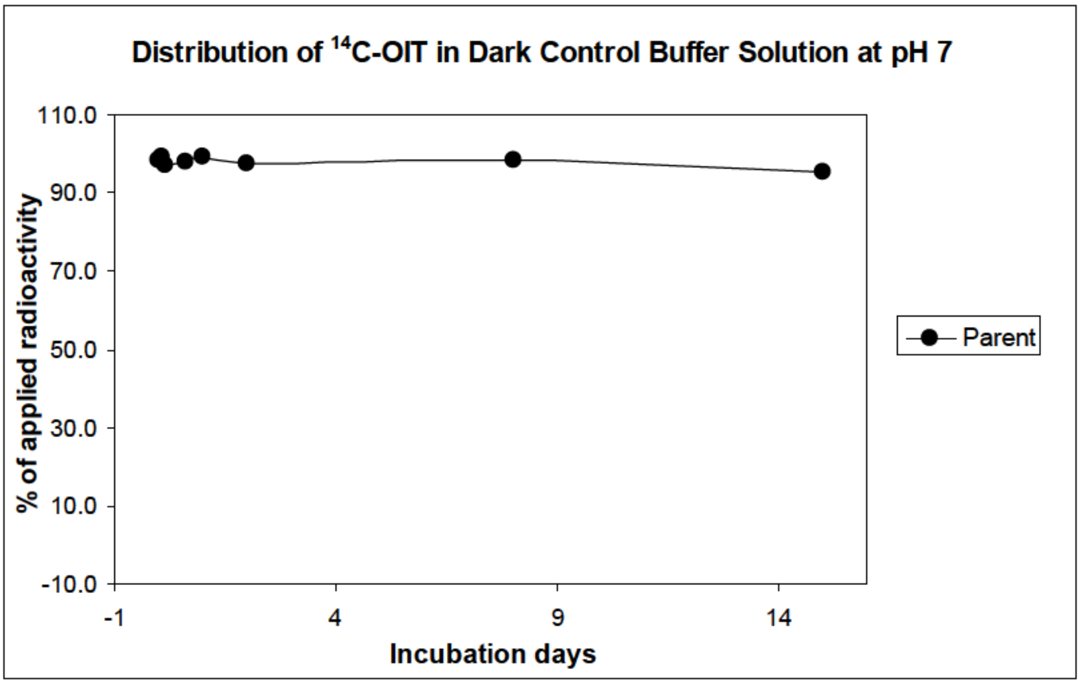
Table A7_1_1_2-6: Distribution of ^{14}C-OIT in the dark control natural pond water samples. Results shown in % of the applied radioactivity.

Pattern nat. water Dark control (% applied)	**INCUBATION TIME IN DAYS**								
	0	**0.08**	**0.17**	**0.63**	**1**	**2**	**4**	**8**	**15**
Parent	**97.4**	**95.4**	**97.6**	**97.4**	**93.9**	**93.3**	**86.9**	**82.0**	**42.6**
M1 (unknown)	0.4	*	0.4	1.5	1.8	1.2	*	*	9 5
M2 (unknown)	0.9	*	1.9	*	*	1.8	1.9	8.5	*
M3	0.5	1 2	0.7	*	0.7	0.6	1.2	1.7	*
M4 (unknown)	0.8	0.6	0.9	0.6	2.1	1.8	3.0	*	7.8
M5 (unknown)	*	*	*	*	*	*	2.5	*	*
M6 (unknown)	*	0 3	*	2.4	2.1	*	3.2	1.1	12 8
M7 (unknown)	*	0 8	*	*	*	*	*	*	*
M8	*	1.6	0 3	*	*	0.7	*	*	0.5
M9	*	0.4	*	*	*	0.5	*	0.9	0.8
M10 (unknown)	*	0.3	*	*	*	0.4	0.5	3.4	1.2
M11 (unknown)	*	*	*	*	*	*	*	*	1.1
M12 (unknown)	*	*	*	*	*	*	*	*	9.1
$^{14}CO_2$	n.p.	0 0	<0.1	<0.1	0.2	0.4	1.7	3.2	7 9
TOTAL	**100.0**	**100.6**	**101.9**	**102.0**	**100.9**	**100.6**	**100.9**	**100.9**	**93.3**

*: not detected or lower than detection limit
M3: N-(n-octyl) ethyl-amine, contains also low amounts of N-(n-octyl)malonamic acid
M8: N-(n-octyl)acetamide
M9: N-(n-octyl)oxamic acid

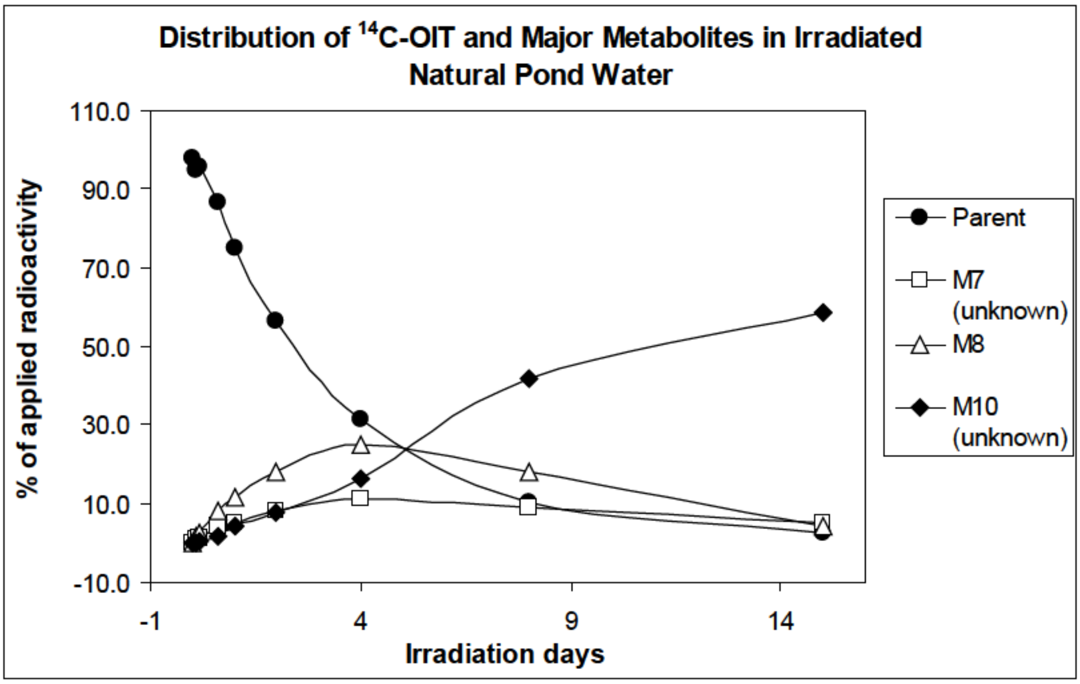
Figure A7_1_1_2-1: **Degradation of 14C-OIT and major metabolites in sterilised buffer solution at pH 7. Irradiated samples (top), dark controls (bottom).**

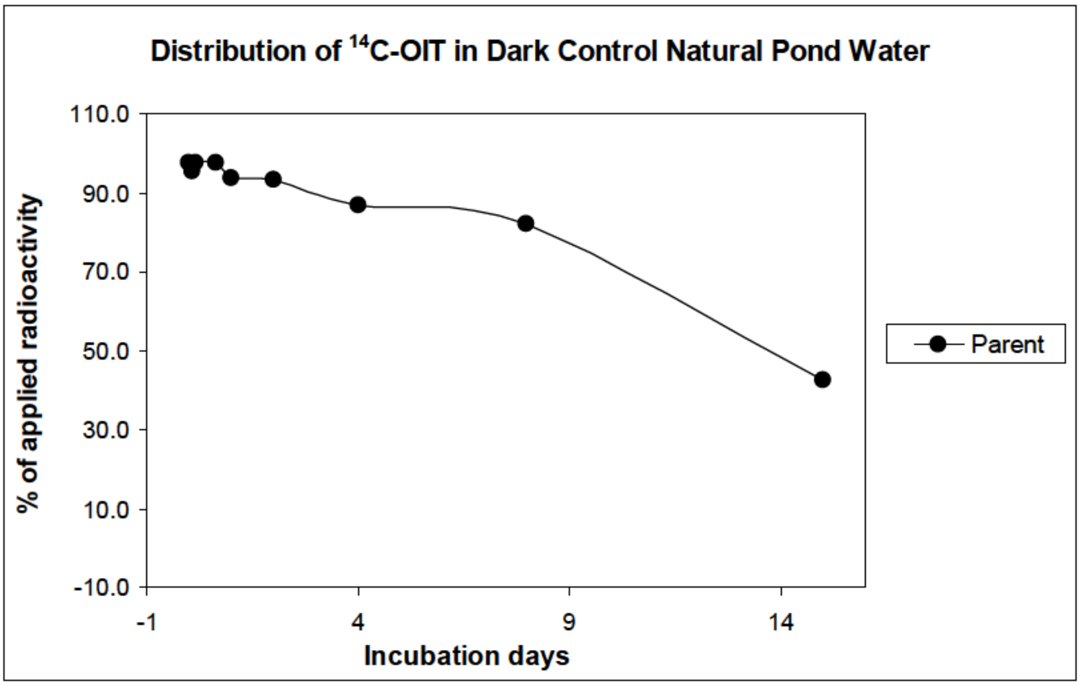




M3: N-(n-octyl) ethyl-amine, contains also low amounts of N-(n-octyl)malonamic acid
M8: N-(n-octyl)acetamide
M7: radioactive fraction containing multiple components

Figure A7_1_1_2-2: **Degradation of ^{14}C-OIT and major metabolites in sterile natural pond water.**





Note: Degradation observed in the dark control was due to microbial contamination. In the sterile dark controls of the buffered system ^{14}C-OIT was stable.

M8: N-(n-octyl)acetamide
M7/M10: radioactive fractions containing multiple components

Figure A7_1_1_2-3: Proposed degradation pathway of irradiated OIT in aqueous system