

Section 7.1.2.2.1

Aerobic aquatic degradation study (freshwater)

Annex Point IIIA XII.2.1

		Official use only
		1 REFERENCE
1.1 Reference	<i>Mamouni, A., 2007a, 2-(n-octyl)-4-[4,5-¹⁴C]isothiazolin-3-one (¹⁴C-OIT): Aerobic Mineralisation in Surface Water – Simulation Biodegradation Test, RCC Ltd, [REDACTED]</i>	
1.2 Data protection	<i>Yes</i>	
1.2.1 Data owner	<i>THOR GmbH, [REDACTED]</i>	
1.2.2 Company with letter of access	<i>None</i>	
1.2.3 Criteria for data protection	<i>Data submitted on existing a.s. for the purpose of its entry into Annex I.</i>	
		2 GUIDELINES AND QUALITY ASSURANCE
2.1 Guideline study	<i>Yes</i> <i>OECD Guidelines for the Testing of Chemicals, Guideline 309 - Aerobic Mineralisation in Surface Water – Simulation Biodegradation Test, April 13, 2004.</i>	
2.2 GLP	<i>Yes</i>	
2.3 Deviations	<i>No</i>	
		3 METHOD
3.1 Test material	<i>2-(n-octyl)-4-[4,5-¹⁴C]isothiazolin-3-one (¹⁴C-OIT)</i>	
3.1.1 Lot/Batch number	[REDACTED]	
3.1.2 Radiochemical Purity	[REDACTED]	
3.1.3 Radiolabelling	[REDACTED]	
3.1.4 Specific Radioactivity	[REDACTED]	
3.1.5 Specific chemical analysis	<i>The following HPLC conditions were used:</i> <i>Instruments</i> <i>Pump: [REDACTED]</i> <i>Autosampler: [REDACTED]</i> <i>UV-detector: [REDACTED]</i> <i>¹⁴C-detector: [REDACTED]</i> <i>Mobile Phase:</i> <i>Solvent A: [REDACTED]</i> <i>Solvent B: [REDACTED]</i>	
3.2 Reference substance	<i>Aniline hydrochloride [¹⁴C(U)]</i>	
3.3 Test ing procedure		
3.3.1 Test system	<i>See table A7_1_2_2_1-1</i>	
3.3.2 Test conditions	<i>See table A7_1_2_2_1-2</i>	
3.3.3 Method of preparation of test	<i>In order to obtain target concentrations of 2-(n-octyl)-4-[4,5-¹⁴C]isothiazolin-3-one (¹⁴C-OIT) of 10 and 100 µg/L, aliquots of 45 and</i>	X

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solution	<i>455 µL of a stock solution were taken and spiked separately into 295 mL of sterilized surface water. Additional sterilized surface water was added to obtain a final volume of 300 mL (application solutions). Thereafter, the spiked water was submitted to ultrasonic treatment. LSC measurements of the application solutions resulted in 322 620 dpm (low dose) and 3 213 400 dpm per 10 mL (high dose). From these application solutions, 10 mL were added to 90 mL of natural surface water corresponding to initial 2-(n-octyl)-4-isothiazolin-3-one (OIT) concentrations of [REDACTED] (low dose) and [REDACTED] (high dose).</i>	
3.3.4 Duration of test	<i>29 days</i>	
3.3.5 Analytical parameter	<i>Parent substance, CO₂, metabolites, volatile substances</i>	
3.3.6 Sampling	<i>Duplicate water samples of both dosage groups were taken for analysis after 0, 0.25 (6 hours), 1, 3, 5, 7, 10, 20 and 29 days of incubation.</i>	
3.3.7 Intermediates/ degradation products	<i>Identified. The verification of the nature of the radioactivity in the sodium hydroxide traps was performed on the NaOH trapping solution samples after 7, 15 and 29 days of incubation for both doses. (quantitative precipitation by Ba(OH)₂).</i>	X

4 RESULTS**4.1 Degradation of test substance**

4.1.1 Recovery	<i>The total mean recoveries were 91.8% ± 4.6% and 93.0% ± 5.6% of the applied radioactivity at doses of 10 µg/L and 100 µg/L, respectively. See table A7_1_2_2_1-3.</i>	
4.1.2 Distribution water/ sediment	<i>No sediment was used. The level of radioactivity in the surface water decreased from 96.7% and 102.0% of the applied radioactivity on day 0 to 41.8% and 52.5% on day 29 of incubation for the low and high doses, respectively.</i>	
4.1.3 Mineralisation	<i>Significant amounts of ¹⁴CO₂ were formed during the incubation period. Radioactive carbon dioxide accounted for 47.9% at the lower application dose (10 µg/L) and 36.4% at the higher dose of 100 µg/L at study termination (29 days). See tables A7_1_2_2_1-3 through A7_1_2_2_1-5 and figure A7_1_2_2_1-1.</i> <i>The rate of mineralization of ¹⁴C-OIT in this system was greater than that of the ready biodegradable reference compound, ¹⁴C-aniline. These results provide additional confirmation of the rapid biodegradation of OIT and its subsequent mineralization to CO₂.</i>	
4.1.4 Degradation products	<i>Numerous transient metabolites fractions were formed which were subsequently mineralized to ¹⁴CO₂. Rapid metabolism involved cleavage of the isothiazolone ring and oxidation of the resulting alkyl metabolites which were further oxidized to CO₂. See tables A7_1_2_2_1-4 and tables A7_1_2_2_1-5 and figures A7_1_2_2_1-1 and A7_1_2_2_1-3.</i>	X
4.1.5 Non-extractable residues	<i>A significant part of radioactivity was bound to the dissolved organic matter. This radioactivity was not extractable from the solid after concentration of the samples.</i>	

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5 APPLICANT'S SUMMARY AND CONCLUSION		
5.1 Materials and methods	<p>The rate of biodegradation of ^{14}C-OIT, i.e. 2-(n-octyl)-4-[4,5-^{14}C]isothiazolin-3-one, was investigated in natural river water. The test item was applied to the system at application rates of 10 and 100 $\mu\text{g/L}$.</p> <p>Following application, the samples were incubated in the dark at $20 \pm 2^\circ\text{C}$. During the incubation period, a stream of air was allowed to pass through the samples. Organic volatiles and ^{14}C-carbon dioxide were collected in ethylene glycol and sodium hydroxide traps, respectively.</p> <p>Duplicate water samples of both dosage groups were taken for analysis after 0, 0.25 (6 hours), 1, 3, 5, 7, 10, 20 and 29 days of incubation. The radioactivity in the water phase was determined by LSC. Depending on the amount of radioactivity present in the water, samples were submitted to HPLC analysis directly after sampling or after concentration at about 35°C under reduced pressure in a rotary evaporator. Selected samples were additionally analyzed by TLC to confirm the HPLC results. A total radioactivity balance for each sample was established at each interval.</p>	
5.2 Results and discussion	<p>The total mean recoveries for the water samples treated with 2-(n-octyl)-4-[4,5-^{14}C]isothiazolin-3-one (^{14}C-OIT) were $91.8\% \pm 4.6\%$ and $93.0\% \pm 5.6\%$ of the applied radioactivity at doses of 10 $\mu\text{g/L}$ and 100 $\mu\text{g/L}$, respectively.</p> <p>The level of radioactivity in the surface water decreased from 96.7% and 102.0% of the applied radioactivity on day 0 to 41.8% and 52.5% on day 29 of incubation for the low and high doses, respectively. This decrease of the total radioactivity in water was directly correlated to the biodegradation of 2-(n-octyl)-4-isothiazolin-3-one (OIT) and its metabolites to $^{14}\text{CO}_2$. Additionally, a significant portion of the applied radioactivity was bound to the dissolved organic matter.</p> <p>The parent compound biodegraded rapidly in natural surface water with DT_{50} values of 0.6 and 1.4 days at application rates of 10 and 100 $\mu\text{g/L}$. See figure A7_1_2_2_1-2</p> <p>The major metabolite was $^{14}\text{CO}_2$. The mineralization rate was higher at the lower application rate of 10 $\mu\text{g/L}$ (47.9%) compared to the higher rate, 100 $\mu\text{g/L}$ (36.4%). No other type of volatile radioactivity was detected. Besides CO_2, numerous transient metabolite fractions were formed, none of which corresponded to the available reference standards. The main transient metabolite fractions were designated M1, M5 and M6, which were shown to contain multiple-components. The half-lives of sum of all components in these three radioactive fractions, determined by computational software, ranged from 3 to 36 days.</p> <p>The multi components radioactive fractions M1, M5, and M6 reached maximum amounts of 28.9%, 20.7%, and 16.4%, respectively. These transient fractions were mineralized to CO_2.</p>	X
5.3 Conclusion	<p>In conclusion, 2-(n-octyl)-4-isothiazolin-3-one (OIT) rapidly biodegrades in natural surface water with DT_{50} values of between 0.6 and 1.4 days at application rates of 10 and 100 $\mu\text{g/L}$. CO_2 is the major metabolite being present at 36% to 49% of the applied activity after 29 days of incubation. Metabolism involves cleavage of the isothiazolone ring and subsequent oxidation of the alkyl metabolites, ultimately to CO_2.</p>	

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Biodegradation rate of 2-(n-octyl)-4-isothiazolin-3-one (OIT)	Surface Water			
	Dose: 10 µg/L		Dose: 100 µg/L	
DT ₅₀ (days)	0.56	0.57	1.34	1.41
r ² (correlation coefficient)	0.9569	0.9815	0.9168	0.9798
Kinetic Model used	ModelMaker	Origin	ModelMaker	Origin

- 5.3.1 Reliability *1*
- 5.3.2 Deficiencies *No*

X

Evaluation by Competent Authorities

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

EVALUATION BY RAPPORTEUR MEMBER STATE

Date

18 Nov 09

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The applicant's version is considered acceptable, noting the following:

3.1: The expiry date of the test substance is not stated in the report. However, as the purity was determined before use, this is acceptable.

3.3: The analytical testing procedure is poorly described within the RSS, the procedure was as follows:

Aliquots of fresh water were taken for radioassaying and additional aliquots were acidified to quantitate dissolved $^{14}\text{CO}_2$. The remaining duplicate fresh water samples were concentrated using a rotary evaporator. Concentrated samples were chromatographed by HPLC with selected samples also analyzed by confirmatory TLC. A portion of the applied radioactivity remained adsorbed in the solid matter after concentration and therefore remained non-extractable.

Sodium hydroxide and ethylene glycol traps were radioassayed at each sampling interval. Verification and quantitation of $^{14}\text{CO}_2$ in the NaOH traps was accomplished by addition of saturated $\text{Ba}(\text{OH})_2$ forming BaCO_3 precipitate which was radioassayed.

Solutions were radioassayed using liquid scintillation counters.

Reversed phase HPLC was performed using either a C-18 column and a gradient consisting of water (adjusted to pH 2.5 with H_3PO_4) and acetonitrile or a C-8 column and gradients consisting of 4 mM H_3PO_4 in water (pH 2.5) and acetonitrile. UV and radioactivity monitors were employed for detection.

1-dimensional normal phase TLC was performed on silica gel plates. The following solvent systems were employed for samples treated with ^{14}C -OIT: 1) ethyl acetate:isopropanol:water formic acid (65:25:10:1); 2) n-butanol:water:acetic acid (80:20:20); and 3) chloroform:methanol:ammonia:water (20:70:5:5). The following solvent systems were employed for the analysis of aniline: 1) hexane:ethyl acetate (90:10); acetone; 2) acetonitrile: 5mM KH_2PO_4 (pH 7.3) (40:60); and 3) acetonitrile: 5mM KH_2PO_4 (pH 7.3) (40:60:1). Unlabeled substances were visualized by UV at 254 nm and 366 nm. Radioactive zones were detected using a phosphorimager.

3.3.7: This states that the metabolites were identified, which is not the case. Only CO_2 and volatile components were identified, while metabolites M1, M5 and M6 were observed but not identified. These reached maximum (mean) amounts of 22.8 %, 15.0 % and 10.5 % respectively. The applicant attempted to identify similar metabolites in other matrices using LC-MS, but stated that they were unsuccessful due to the low dosing concentration, the low concentration of the respective metabolic fractions and the fact that the fractions contain multiple components. The ionization of the metabolites, which is necessary for mass spectral detection, was also probably reduced and this was in part due to the presence of the multiple components. The metabolites also did not correspond to available reference standards.

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The applicant's version is considered acceptable, noting the following:

4.1.4: Within the low dose studies, both M4 and M7 appeared at 2 consecutive sampling points at amounts >5%, as such these metabolites should be considered to be major metabolites, where both metabolites are declining at study termination.

The UK CA does not agree that metabolites M1, M4, M5, M6 and M7 are transient in nature. While the metabolites do appear to ultimately appear to mineralise to CO₂ and the decline of the metabolites do not appear to be linked to the formation of any minor metabolites; the metabolites do not appear to be present for a short enough period of time to be termed as transient

Metabolites M1, 4, 5,6 and 7 were not identified; while this is not ideal, the UK CA considers that in this instance, this can be considered acceptable as identification was attempted and will have no effect upon the calculated DT₅₀ values, where applicable.

It is stated within the study that identification was not possible in part due to the metabolites (M1, 5 and 6) containing multiple components, however the study report does not contain sufficient evidence to illustrate that the metabolites are composed of multiple metabolite fractions. It is unclear from the study report whether attempts were made to identify metabolites M4 and M7.

First order non linear kinetics were not provided, the UK CA carried out non linear kinetics following SFO kinetics for both OIT and the metabolilitesM1, M5 and M6.The resulting dissipation rates are shown below.

Due to the low concentrations observed for M4 and M7 and the limited data sets, no reliable DT₅₀ or DT₉₀ values could be calculated for the metabolites, even when the top down approach was considered.

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OIT	Low Dose	High Dose
K	1.2160	0.5590
DT ₅₀	0.6	1.2
DT ₉₀	1.9	4.1
r ²	0.998	0.977
M1		
K	0.074	0.0370
DT ₅₀	9.4	18.7
DT ₉₀	31.1	62.2
r ²	0.810	0.634
Fraction of parent transformed (%)	19.7	16.5
M5		
K	0.0425	0.0680
DT ₅₀	16.3	10.2
DT ₉₀	54.2	33.9
r ²	0.745	0.653
Fraction of parent transformed (%)	13.2	14.5
M6		
K	0.1575	0.0573
DT ₅₀	4.4	12.1
DT ₉₀	14.6	40.2
r ²	0.546	0.5729
Fraction of parent transformed (%)	10.2	8.0

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	<p>It should be noted that while the r^2 of OIT is within the acceptable range the final points do not have a good fit, this does not have an effect upon the DT_{50} value but the reliability of the DT_{90} value is reduced.</p> <p>Based upon the r^2 values calculated for the metabolite kinetics it would appear that there is poor correlation between the measured values and the modelled values, however upon inspection of visual fits (figure 1-6) it is clear to see that the kinetics provide a conservative estimation of the degradation to an acceptable quality for the DT_{50} to be relied upon, however the reliability of the DT_{90} is reduced.</p> <p>For all the kinetic evaluation the same problem is causing these poor fits to occur, which is that once the maximum occurrence of the metabolite is reached, the metabolite sharply decreases within the following sampling point. Looking at the data after the maximum occurrence all metabolites decrease to less than 50% of this occurrence in less than 4 days, except M1 within the low dose where only <5 days can be stated. This would mean that if the kinetics were improved to fit this observation the dissipation rates would decrease.</p> <p>However it should be noted that the applicant has stated that metabolites M1, 5 and 6 contain multiple components, therefore the poor correlation would not be unexpected as each of the individual components will follow a different degradation pathway, however as discussed above the UK CA are of the opinion that at the current time, there is not sufficient evidence to accept this statement.</p> <p>The UK CA have concluded that the metabolites M1, M4, M5, M6 and M7 are relevant for environmental risk assessment (see Doc IIA), and PEC_{sw} calculation will be required. While the metabolites have not been identified, this will not have an effect upon the resulting risk assessment as the PECs shall be reported in terms of g OIT/L</p> <p>Conclusion The applicant's version is considered acceptable, noting the following:</p> <p>5.2: The applicant reports that a significant portion of the applied radioactivity was bound to dissolved organic matter. It appears that this was calculated as the difference between the applied and the extracted radioactivity. Although bound residues contained within the solid matter post concentration account for up to 30 % of applied radioactivity, the amount of dissolved organic matter contained within the solid matter does not appear to be reported. It is also noted that in section 3.2.1 of the study report, low recoveries are attributed to high adsorption of the test item to particulates; as such it is possible that the solid matter contained more than organic matter alone. The UK CA note that at time within the RSS the bound residues have been classified as non-extractable residues, this is not acceptable as no attempts were made to extract the residues from the solid matter</p> <p>Reliability 2</p> <p>The study contained minor methodological deviations which do not affect the quality of results.</p> <p>Acceptability Acceptable</p> <p>Remarks All endpoints and data presented in the summary have been checked against the original study.</p> <p>The reliability of the study has been decreased as no attempts were made to extract the residues from the solid matter post concentration.</p>
Date	COMMENTS FROM ... (specify)



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Reliability

Acceptability

Remarks

Table A7_1_2_2_1-1: Properties of the river water used in the study.

Water	River water
Source	
Sampling Date	October 12, 2005
Parameters measured at sampling:	
pH	8.04
Redox potential (mV)	235
Oxygen content (mg/L)	9.65
Temperature	15.2°C
Hardness (°dH)	11.5
Parameters measured after arrival at RCC	
pH	7.93
Redox potential (mV)	131
Oxygen content (mg/L)	8.21
Total organic carbon (TOC; ppm)	2.07
Hardness (°dH)	10.0

Table A7_1_2_2_1-2: Parameters (pH and oxygen concentration) measured in a control river water sample at various incubation intervals.

River natural water			
Incubation Time (d)	Oxygen Conc. Water (mg/l)	pH Water	Room Temp. (°C)
	0	8.40	
3	8.55	7.98	
5	8.43	7.88	
7	8.46	7.92	
10	8.42	8.23	
12	8.26	8.13	
14	8.32	8.14	
18	8.25	8.21	
26	8.32	8.14	
Average	8.38	8.10	
± SD	0.10	0.14	

SD: Standard Deviation

Table A7_1_2_2_1-3: Balance of the applied radioactivity in the river surface water treated with 2-(n-octyl)-4-[4,5-¹⁴C]isothiazolin-3-one (14C-OIT) at doses of 10 µg/L (top) and 100 µg/L (bottom). Values are given in percent of the applied radioactivity.

Low Dose % applied	Sample	Incubation Time (days)								
		0	0.25	1	3	5	7	10	20	29
Radioactivity in Solution	A	95.4	94.3	92.3	81.9	84.0	72.0	49.8	38.7	43.1
	B	97.9	92.9	97.5	91.4	81.2	67.3	48.5	32.1	40.4
	mean	96.7	93.6	94.9	86.7	82.6	69.6	49.1	35.4	41.8
dissolved ¹⁴ CO ₂	A	n.p.	<0.1	<0.1	4.9	5.1	3.0	11.4	7.1	2.9
	B	n.p.	<0.1	<0.1	2.3	0.3	7.5	15.3	5.3	2.6
	mean	n.p.	<0.1	<0.1	3.6	2.7	5.3	13.4	6.2	2.8
Evolved ¹⁴ CO ₂	A	n.p.	0.2	0.5	3.3	6.4	14.9	21.2	51.4	44.4
	B	n.p.	0.1	0.6	2.9	6.0	15.2	22.5	47.8	46.0
	mean	n.p.	0.1	0.6	3.1	6.2	15.1	21.9	49.6	45.2
Total ¹⁴ CO ₂	A	n.p.	0.2	0.5	8.3	11.5	18.0	32.6	58.5	47.3
	B	n.p.	0.1	0.6	5.2	6.3	22.7	37.8	53.1	48.6
	mean	n.p.	0.1	0.6	6.8	8.9	20.3	35.2	55.8	47.9
Other volatiles	A	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
	B	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
	mean	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Total	A	95.4	94.5	92.8	90.2	95.5	89.9	82.4	97.1	90.3
	B	97.9	93.0	98.1	96.7	87.6	90.0	86.3	85.2	89.0
Mean +/- SD		91.8 +/- 4.6								

High Dose % applied	Sample	Incubation Time (days)								
		0	0.25	1	3	5	7	10	20	29
Radioactivity in Solution	A	101.2	98.8	97.8	79.8	83.9	70.4	34.4	52.2	48.1
	B	102.8	99.2	97.0	90.8	78.3	80.6	42.9	44.6	56.9
	mean	102.0	99.0	97.4	85.3	81.1	75.5	38.7	48.4	52.5
dissolved ¹⁴ CO ₂	A	n.p.	<0.1	<0.1	5.2	0.6	10.6	13.6	9.4	8.9
	B	n.p.	<0.1	<0.1	2.1	<0.1	5.0	0.8	10.8	0.2
	mean	n.p.	<0.1	<0.1	3.6	0.3	7.8	7.2	10.1	4.6
Evolved ¹⁴ CO ₂	A	n.p.	<0.1	<0.1	1.8	3.9	9.0	40.4	30.6	32.3
	B	n.p.	<0.1	<0.1	4.6	13.1	3.1	40.1	36.4	31.3
	mean	n.p.	<0.1	<0.1	3.2	8.5	6.1	40.2	33.5	31.8
Total ¹⁴ CO ₂	A	n.p.	<0.1	<0.1	6.9	4.5	19.7	54.0	40.0	41.2
	B	n.p.	<0.1	<0.1	6.6	13.1	8.1	40.9	47.2	31.5
	mean	n.p.	<0.1	<0.1	6.8	8.8	13.9	47.4	43.6	36.4
Other volatiles	A	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
	B	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
	mean	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Total	A	101.2	98.8	97.9	86.8	88.4	90.0	88.4	92.2	89.3
	B	102.8	99.2	97.0	97.4	91.5	88.7	83.9	91.8	88.4
Mean +/- SD		93.0 +/- 5.6								

n.p.: Not performed

SD: Standard Deviation

Table A7_1_2_2_1-4: Pattern of biodegradation in the river surface water treated with 2-(n-octyl)-4-[4,5-¹⁴C]isothiazolin-3-one (¹⁴C-OIT) at a dose of 10 µg/L (low dose). Values are given in percent of the applied radioactivity.

OIT Biodegradation Low Dose	Duplicate	Incubation Time (days)								
		0	0.25	1	3	5	7	10	20	29
OIT	A	95.4	77.2	14.9	3.1	*	*	*	*	*
	B	97.9	73.9	38.2	8.0	1.8	*	*	*	*
	Mean	96.7	75.5	26.6	5.6	0.9	*	*	*	*
M1	A	*	*	9.1	14.7	19.0	12.4	7.6	2.4	2.0
	B	*	*	10.2	17.9	19.1	18.0	5.9	2.8	2.6
	Mean	*	*	9.6	16.3	19.0	15.2	6.7	2.6	2.3
M2	A	*	*	*	1.5	3.9	1.5	1.2	1.7	1.9
	B	*	*	*	3.4	2.5	1.8	1.2	1.6	1.7
	Mean	*	*	*	2.5	3.2	1.6	1.2	1.6	1.8
M4	A	*	*	*	4.9	5.1	5.4	3.9	1.6	1.4
	B	*	*	2.1	4.8	5.5	5.2	1.3	1.2	0.7
	Mean	*	*	1.0	4.9	5.3	5.3	2.6	1.4	1.0
M5	A	*	*	8.4	13.7	15.4	6.8	6.6	6.7	4.8
	B	*	*	6.9	13.9	14.7	6.8	5.9	5.3	5.6
	Mean	*	*	7.6	13.8	15.0	6.8	6.2	6.0	5.2
M5a	A	*	*	*	*	3.3	1.7	1.3	1.1	1.5
	B	*	*	*	*	2.0	1.3	1.3	1.4	1.5
	Mean	*	*	*	*	2.7	1.5	1.3	1.3	1.5
M5b	A	*	*	*	1.3	1.9	1.5	1.9	*	*
	B	*	*	*	*	2.5	1.5	0.6	0.9	*
	Mean	*	*	*	0.7	2.2	1.5	1.2	0.4	*
M5c	A	*	*	*	*	1.5	2.6	1.5	*	*
	B	*	*	*	*	1.2	1.1	*	*	*
	Mean	*	*	*	*	1.3	1.9	0.8	*	*
M6	A	*	*	9.3	4.3	2.1	3.6	3.2	3.1	2.4
	B	*	*	8.7	8.9	3.9	4.4	2.4	2.0	3.4
	Mean	*	*	9.0	6.6	3.0	4.0	2.8	2.5	2.9
M7	A	*	*	1.5	4.3	3.7	4.6	4.9	2.4	1.1
	B	*	*	2.4	5.7	9.0	10.0	2.1	1.9	1.7
	Mean	*	*	1.9	5.0	6.4	7.3	3.5	2.1	1.4
M10	A	*	*	3.3	1.8	5.0	6.4	1.8	5.1	4.4
	B	*	*	4.8	3.1	2.3	2.8	7.5	1.3	1.0
	Mean	*	*	4.1	2.5	3.6	4.6	4.7	3.2	2.7
M11	A	*	*	*	*	*	1.5	0.6	*	*
	B	*	*	*	*	0.9	1.0	*	*	*
	Mean	*	*	*	*	0.5	1.2	0.3	*	*
M12	A	*	*	*	0.9	*	1.1	*	*	*
	B	*	*	*	*	1.1	*	*	*	*
	Mean	*	*	*	0.4	0.5	0.6	*	*	*
M13	A	*	*	*	*	*	*	*	*	*
	B	*	*	*	*	1.1	*	*	*	*
	Mean	*	*	*	*	0.5	*	*	*	*

OIT Biodegradation Low Dose	Duplicate	Incubation Time (days)								
		0	0.25	1	3	5	7	10	20	29
M14	A	*	*	*	*	*	*	*	*	*
	B	*	*	*	*	1.4	*	*	*	*
	Mean	*	*	*	*	0.7	*	*	*	*
M15	A	*	*	2.9	*	*	*	*	*	*
	B	*	*	4.0	*	*	*	*	*	*
	Mean	*	*	3.4	*	*	*	*	*	*
adsorbed radioactivity**	A	---	17.1	43.0	31.4	23.1	22.9	15.4	14.6	23.4
	B	---	19.0	20.3	25.7	12.2	13.5	20.2	13.8	22.1
	Mean	---	18.1	31.6	28.5	17.6	18.2	17.8	14.2	22.7
¹⁴ CO ₂ ***	A	n.p.	0.2	0.5	8.3	11.5	18.0	32.6	58.5	47.3
	B	n.p.	0.1	0.6	5.2	6.3	22.7	37.8	53.1	48.6
	Mean	n.p.	0.1	0.6	6.8	8.9	20.3	35.2	55.8	47.9
Other volatiles	A	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
	B	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
	Mean	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1

* Not detected

** Radioactivity adsorbed to dissolved organic matter in the surface water (non-extractable)

*** Total ¹⁴CO₂

n.p. Not performed

Table A7_1_2_2_1-5: Pattern of biodegradation in the river surface water treated with 2-(n-octyl)-4-[4,5-¹⁴C]isothiazolin-3-one (¹⁴C-OIT) at a dose of 100 µg/L (high dose). Values are given in percent of the applied radioactivity.

OIT Biodegradation High dose	Duplicate	Incubation Time (days)								
		0	0.25	1	3	5	7	10	20	29
OIT	A	101.2	96.8	68.8	*	5.2	*	*	*	*
	B	102.8	90.0	81.5	16.1	0.9	3.4	*	*	*
	Mean	102.0	93.4	75.1	8.1	3.0	1.7	*	*	*
M1	A	*	*	*	19.8	14.1	15.9	8.1	8.0	7.8
	B	*	*	*	8.1	18.7	29.8	5.2	4.7	9.4
	Mean	*	*	*	13.9	16.4	22.8	6.6	6.4	8.6
M2	A	*	*	*	*	2.3	*	1.2	1.3	1.4
	B	*	*	*	2.0	1.6	2.1	1.1	0.7	1.8
	Mean	*	*	*	1.0	2.0	1.0	1.1	1.0	1.6
M4	A	*	*	*	9.3	1.3	2.9	1.7	1.5	2.7
	B	*	*	*	3.8	3.0	2.1	4.3	1.7	2.6
	Mean	*	*	*	6.5	2.2	2.5	3.0	1.6	2.7
M5	A	*	*	*	19.8	15.9	14.3	3.5	1.9	5.7
	B	*	*	*	7.2	10.7	15.1	4.7	2.8	4.6
	Mean	*	*	*	13.5	13.3	14.7	4.1	2.3	5.2
M5a	A	*	*	*	*	6.9	3.7	2.2	1.8	1.0
	B	*	*	*	*	3.0	2.6	1.3	1.7	1.3
	Mean	*	*	*	*	4.9	3.1	1.7	1.8	1.2
M5b	A	*	*	*	*	*	*	0.6	0.6	*
	B	*	*	*	*	3.0	1.8	0.8	0.7	*
	Mean	*	*	*	*	1.5	0.9	0.7	0.7	*
M5c	A	*	*	*	*	1.2	*	0.4	*	1.2
	B	*	*	*	*	1.4	2.5	0.3	1.1	0.9
	Mean	*	*	*	*	1.3	1.2	0.3	0.5	1.1
M6	A	*	*	4.1	7.2	11.7	5.5	1.2	2.0	2.1
	B	*	*	*	13.7	4.5	4.2	2.8	1.7	4.9
	Mean	*	*	2.1	10.5	8.1	4.9	2.0	1.8	3.5
M7	A	*	*	*	*	6.0	4.1	1.1	0.8	1.3
	B	*	*	*	2.4	1.6	7.2	2.6	0.8	2.1
	Mean	*	*	*	1.2	3.8	5.7	1.9	0.8	1.7
M10	A	*	*	*	*	2.3	2.2	0.8	0.6	1.7
	B	*	*	*	*	*	1.5	2.0	0.4	3.5
	Mean	*	*	*	*	1.1	1.8	1.4	0.5	2.6
M11	A	*	*	*	*	*	*	*	*	*
	B	*	*	*	1.8	*	*	1.2	*	*
	Mean	*	*	*	0.9	*	*	0.6	*	*
M12	A	*	*	*	*	*	*	*	*	*
	B	*	*	*	*	*	*	0.5	*	*
	Mean	*	*	*	*	*	*	0.2	*	*
M15	A	*	*	*	*	2.7	*	*	*	*
	B	*	*	*	*	*	*	*	*	*
	Mean	*	*	*	*	1.4	*	*	*	*
adsorbed radioactivity**	A	---	2.0	24.9	23.6	14.2	21.7	13.7	33.7	23.3
	B	---	9.2	15.5	35.8	29.7	8.4	16.4	28.4	25.6
	Mean	---	5.6	20.2	29.7	22.0	15.0	15.0	31.0	24.4
¹⁴ CO ₂ ***	A	n.p.	<0.1	<0.1	6.9	4.5	19.7	54.0	40.0	41.2
	B	n.p.	<0.1	<0.1	6.6	13.1	8.1	40.9	47.2	31.5
	Mean	n.p.	<0.1	<0.1	6.8	8.8	13.9	47.4	43.6	36.4
Other volatiles	A	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
	B	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
	Mean	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1

* Not detected

** Radioactivity adsorbed to dissolved organic matter in the surface water (non-extractable)

*** Total ¹⁴CO₂

n.p. Not performed

Figure A7_1_2_2_1-1: Biodegradation of 2-(n-octyl)-4-[4,5-¹⁴C]isothiazolin-3-one (¹⁴C-OIT) and pattern of metabolites in the river surface water treated with 2-(n-octyl)-4-[4,5-¹⁴C]isothiazolin-3-one (¹⁴C-OIT) at doses of 10 µg/L (low dose; top) and 100 µg/L (high dose; bottom). Values are given in percent of the applied radioactivity.

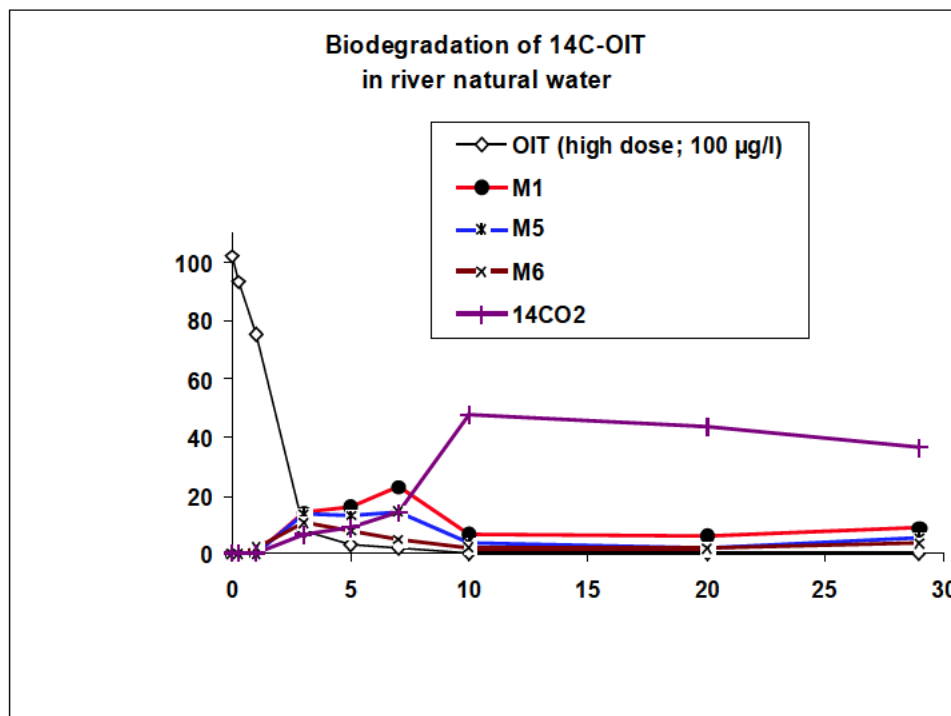
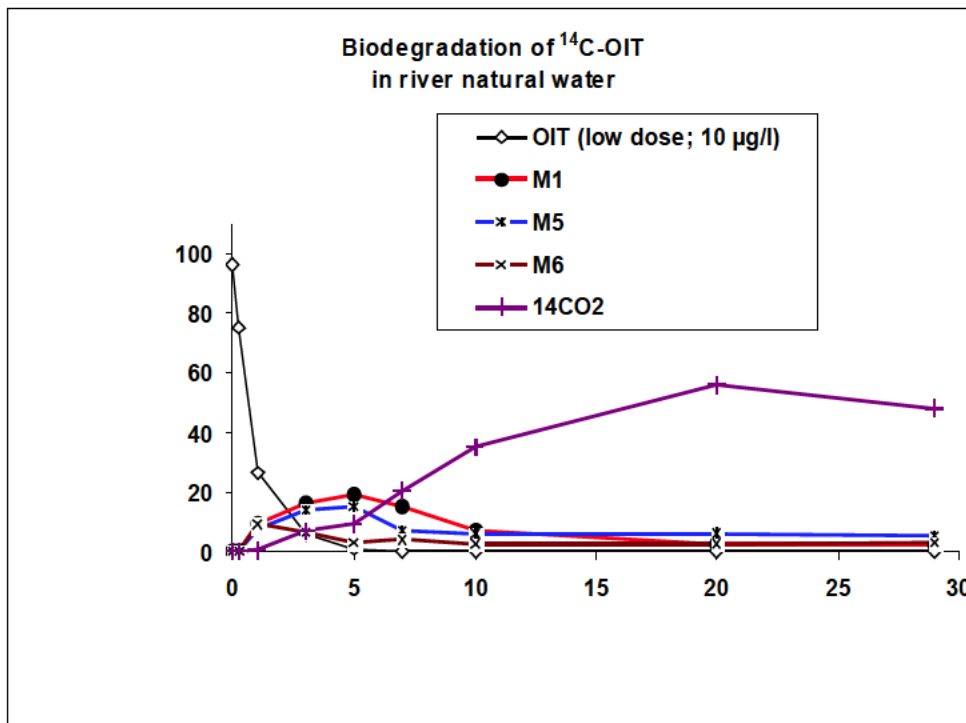


Figure A7_1_2_2_1-2: Rate of biodegradation of 2-(n-octyl)-4-[4,5-¹⁴C]isothiazolin-3-one (¹⁴C-OIT) in the river surface water at treatment rates of 10 µg/L (low dose; top) and 100 µg/L (high dose; bottom). Calculation using [redacted]

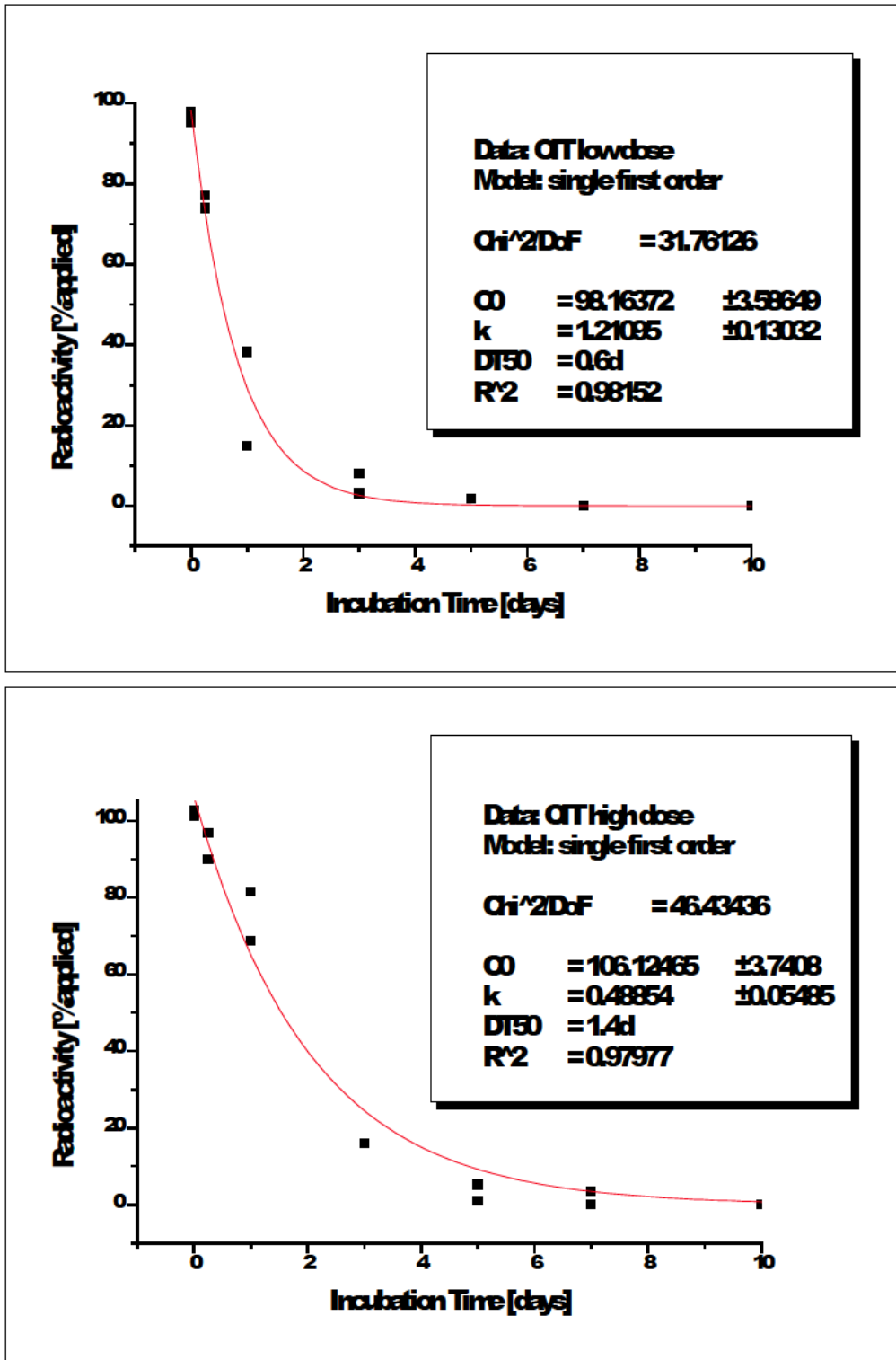


Figure A7_1_2_2_1-3: Proposed metabolic pathway of 2-(n-octyl)-4-[4,5-¹⁴C]isothiazolin-3-one (¹⁴C-OIT) in natural river water.

