

Section 7.1.2.2.1

Aerobic aquatic degradation study (sea water)

Annex Point IIIA XII.2.1

		Official use only	
		1 REFERENCE	
1.1	Reference	<i>Mamouni, A., 2007b, 2-(n-octyl)-4-[4,5-¹⁴C]isothiazolin-3-one (¹⁴C-OIT): Aerobic Mineralisation in Sea Water – Simulation Biodegradation Test</i>	
		[REDACTED]	
1.2	Data protection	<i>Yes</i>	
1.2.1	Data owner	<i>THOR GmbH</i> [REDACTED]	
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:</i> [REDACTED]	
		<i>Autosampler:</i> [REDACTED]	
		<i>UV-detector:</i> [REDACTED]	
		<i>¹⁴C-detector:</i> [REDACTED]	
		<i>Mobile Phase:</i>	
		<i>Solvent A:</i> [REDACTED]	
		<i>Solvent B:</i> [REDACTED]	
3.2	Reference substance	<i>Aniline hydrochloride [¹⁴C(U)]</i>	
3.3	Test ing procedure		

X

Section 7.1.2.2.1 Aerobic aquatic degradation study (sea water)**Annex Point IIIA XII.2.1**

3.3.1	Test system	See table A7_1_2_2_1-1	
3.3.2	Test conditions	See table A7_1_2_2_1-2	
3.3.3	Method of preparation of test solution	In order to obtain target concentrations of 2-(n-octyl)-4-[4,5- ¹⁴ C]isothiazolin-3-one (¹⁴ C-OIT) of 10 and 100 µg/L, aliquots of 29 and 310 µL of the stock solution were taken and spiked separately into approximately 196 ml of sea water. Additional sea water was added to obtain final volumes of 200 ml (low dose) and 215 ml (high dose), which served as application solutions. Thereafter, the application solutions were submitted to ultrasonic treatment and LSC measurement. Their radioactivity content was determined to be 315 350 dpm (low dose) and 3 118 100 dpm per 10 ml (high dose). From these application solutions, 10 ml were added to 90 ml of natural sea water corresponding to initial 2-(n-octyl)-4-isothiazolin-3-one (OIT) concentrations of [REDACTED] µg/L (low dose) and [REDACTED] (high dose).	X
3.3.4	Duration of test	17 days	
3.3.5	Analytical parameter	Parent substance, CO ₂ , metabolites, volatile substances	
3.3.6	Sampling	Duplicate water samples of both dosage groups were taken for analysis after 0, 1, 3, 5, 7, 11 and 17 days of incubation.	X
3.3.7	Intermediates/ degradation products	Identified. The verification of the nature of the radioactivity in the sodium hydroxide traps was performed on the NaOH trapping solution samples after 13 days of incubation for both doses. (quantitative precipitation by Ba(OH) ₂).	
4 RESULTS			
4.1	Degradation of test substance		
4.1.1	Recovery	The total mean recoveries for the sea water samples treated with 2-(n-octyl)-4-[4,5- ¹⁴ C]isothiazolin-3-one (¹⁴ C-OIT) were 91.4% ± 7.6% and 92.1 ± 8.5% of the applied radioactivity at doses of 10 µg/L and 100 µg/L, respectively. See table A7_1_2_2_1-3.	
4.1.2	Distribution water/ sediment	No sediment was used. The level of radioactivity in the sea water decreased from 100.7% and 102.6% of the applied radioactivity on day 0 to 40.4% and 47.0% on day 17 of incubation for the low and high doses, respectively.	
4.1.3	Mineralisation	The major metabolite was ¹⁴ CO ₂ . The mineralization rate was higher at the lower application rate of 10 µg/L (44.6%) when compared to the higher rate, 100 µg/L (36.8%). See tables A7_1_2_2_1-3 through A7_1_2_2_1-5 and figure A7_1_2_2_1-1. The rate of mineralization of ¹⁴ C-OIT in this system was greater than that of the ready biodegradable reference compound, ¹⁴ C-aniline. These results provide additional confirmation of the rapid biodegradation of OIT and its subsequent mineralization to CO ₂	
4.1.4	Degradation products	Numerous transient metabolites fractions were formed (all less than 10%) which were subsequently mineralized to ¹⁴ CO ₂ . Rapid metabolism involved cleavage of the isothiazolone ring and oxidation of the resulting alkyl metabolites which were further oxidized to CO ₂ . 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.	

Section 7.1.2.2.1**Aerobic aquatic degradation study (sea water)****Annex Point IIIA XII.2.1**

metabolite being present at 37% to 45% of the applied activity after 17 days of incubation. Metabolism involves cleavage of the isothiazolone ring and subsequent oxidation of the alkyl metabolites, ultimately to CO₂.

Biodegradation rate of 2-(n-octyl)-4-isothiazolin-3-one (OIT)	Sea water	
	Dose: 10 µg/L	Dose: 100 µg/L
DT ₅₀ (days)	1 60	2 13
r ² (correlation coefficient)	0 9212	0 9427
Model used	First-order	First-order

- 5.3.1 Reliability *I*
- 5.3.2 Deficiencies *No*

X

Evaluation by Competent Authorities

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

Date

12 Nov 2009

Materials and Methods

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.

3.1.2: The radiochemical purity was determined by the study author by HPLC to be [REDACTED] before use.

3.3.3: The seawater was also filtered through a 0.2 mm sieve and sorted in the dark for 4 days at 4 °C with aeration, before use.

3.3.3 and 3.3.6: The control samples were treated with 10 µg ¹⁴C-aniline/l and samples after 0, 3 and 6 days incubation.

Section 7.1.2.2.1**Aerobic aquatic degradation study (sea water)****Annex Point IIIA XII.2.1****Results and discussion**

Applicant's version is considered acceptable noting the following:

4.1.4 The UK CA does not agree that the metabolites 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

Within the high dose study. M21 appears at two consecutive sampling points at amounts $\geq 5\%$, and should be classified as a major metabolite. The applicant attempted to identify metabolites 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.

First order non linear kinetics were not used, when the UK CA carried out an independent evaluation of the kinetics the following values were calculated:

	Low Dose	High Dose
k	0.44	0.31
DT ₅₀	1.6 days	2.3 days
DT ₉₀	5.3 days	7.5 days
r ²	0.940	0.920

It should be noted that while the r² is within the acceptable range, the points below 20 % do not have a good fit, this does not have an effect upon the DT50 value, but the reliability of the DT90 is reduced.

Utiling the top down approach the following kinetics were calculated for metabolite M21.

M21	Low Dose
k	0.180
DT ₅₀	3.9
DT ₉₀	12.8
r ²	0.973

The UK CA have concluded that the metabolites M21 is 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

Section 7.1.2.2.1**Aerobic aquatic degradation study (sea water)****Annex Point IIIA XII.2.1**

Conclusion	<p>Applicant's version is considered acceptable, noting the following:</p> <p>5.2: The transient metabolites were not identified. However, it should be noted that some metabolites are still increasing in concentration at the end of the study, where none are $\geq 5\%$.</p> <p>The UK CA note a large amounts (up to 68.5 %) of non extractable radioactivity were reported to be contained within the solid matter after concentration of the water samples, however no further extraction procedures were carried out upon the solid matter. Clearly it was not ensured that the radioactivity was non-extractable from the solid matter after concentration of the sample but it is stated that the bound radioactive residue continued to mineralise to CO₂ (section 3.6 of report). The UK CA agrees with this in principle as when the results are examined it can be seen that while all metabolites and OIT decrease to below a measurable concentration bound residues increase, after which bound residues decrease but CO₂ continue to increase, however it can not be classified as non-extractable as no attempts were made to extract the bound residues.</p> <p>The applicant states that they have been unable to identify metabolites (report section 3.6). 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. As stated in OECD guideline 309 P1, a higher dosing concentration could be used to help to mitigate this problem. However, as the metabolites are present at concentrations of <10 %, this is acceptable.</p>
Reliability	<p>2</p> <p>The study contained minor methodological deviations which do not affect the quality of results.</p>
Acceptability	Acceptable
Remarks	<p>The end points and data presented in the summary and tables 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	<p>COMMENTS FROM ... (specify)</p>
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

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

Water	Sea water
Source	██████████
Sampling Date	██████████
Parameters measured at sampling:	
pH	8.07
Temperature	1°C
Parameters measured after arrival at RCC:	
Redox potential (mV)	204
pH	8.16
Oxygen content (mg/l)	8.61
Total organic carbon (TOC; ppm)	1.22
Dissolved organic carbon (DOC; ppm)	5.16
Total nitrogen (mg/l water)	1.86
Total phosphorus (mg/l water)	0.03
Nitrate (mg/l NO ₃ ⁻ -N)	1.69
Nitrite (mg/l NO ₂ ⁻ -N)	<0.50
Ammonium (mg/l NH ₄ ⁺ -N)	0.14

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

Natural Sea Water			
Incubation Time	Oxygen Conc.	pH	Room Temp.
(d)	(mg/l)		(°C)
0	8.11	7.88	20 ± 2
7	8.09	8.06	
14	8.56	7.97	
25	8.22	8.00	
Average	8.25	7.98	
± SD	0.22	0.08	

SD: Standard Deviation

Table A7_1_2_2_1-3: Balance of the applied radioactivity in the sea 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	Sample	Incubation Time (days)						
		0	1	3	5	7	11	17
Water phase	A	3.4	9.8	54.2	63.1	46.1	56.0	38.0
	B	3.4	9.3	58.3	58.6	54.4	58.2	32.7
	mean	3.4	9.5	56.3	60.9	50.2	57.1	35.4
Organic phase	A	97.6	88.1	26.4	10.3	10.6	16.2	5.7
	B	97.1	89.6	21.7	12.9	9.5	6.6	4.4
	mean	97.3	88.8	24.1	11.6	10.0	11.4	5.1
TOTAL in SOLUTION	A	101.0	97.8	80.6	73.4	56.6	72.3	43.7
	B	100.5	98.9	80.1	71.5	63.9	64.8	37.1
	mean	100.7	98.4	80.3	72.4	60.3	68.5	40.4
Dissolved CO ₂ in Water phase	A	---	---	---	6.6	7.3	6.7	4.5
	B	---	---	---	12.5	7.6	4.2	8.4
Evolved ¹⁴ CO ₂	A	n.p.	0.3	1.8	9.8	18.5	21.3	37.0
	B	n.p.	0.2	1.8	3.2	17.4	28.8	39.2
Total ¹⁴ CO ₂	A	n.p.	0.3	1.8	16.3	25.8	27.9	41.5
	B	n.p.	0.2	1.8	15.6	25.0	33.0	47.7
	mean	n.p.	0.3	1.8	16.0	25.4	30.5	44.6
other volatiles	A	n.p.	<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
	mean	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Total	A	101.0	98.1	82.4	89.7	82.5	100.3	85.3
	B	100.5	99.1	81.9	87.1	89.0	98.0	84.8
Mean +/- SD				91.4	±	7.6		

High dose	Sample	Incubation Time (days)						
		0	1	3	5	7	11	17
Water phase	A	3.7	6.0	24.1	44.8	48.8	45.5	32.2
	B	3.5	6.5	32.7	48.4	58.2	37.0	41.0
	mean	3.6	6.2	28.4	46.6	53.5	41.3	36.6
Organic phase	A	99.8	95.0	65.0	33.1	24.6	12.2	13.0
	B	98.1	96.0	59.4	33.4	14.2	19.7	7.9
	mean	99.0	95.5	62.2	33.3	19.4	15.9	10.4
TOTAL in SOLUTION	A	103.5	100.9	89.1	78.0	73.4	57.7	45.2
	B	101.7	102.5	92.1	81.8	72.5	56.7	48.8
	mean	102.6	101.7	90.6	79.9	72.9	57.2	47.0
Dissolved CO ₂ in Water phase	A	---	---	---	10.5	6.9	0.9	6.8
	B	---	---	---	11.2	14.4	---	---
Evolved ¹⁴ CO ₂	A	n.p.	<0.1	0.8	5.6	7.4	18.4	33.8
	B	n.p.	<0.1	0.7	4.7	5.6	25.0	33.1
Total ¹⁴ CO ₂	A	n.p.	<0.1	0.8	16.1	14.3	19.3	40.6
	B	n.p.	<0.1	0.7	15.9	20.0	25.0	33.1
	mean	n.p.	<0.1	0.7	16.0	17.2	22.1	36.8
other volatiles	A	n.p.	<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
	mean	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Total	A	103.5	101.0	89.9	94.1	87.7	77.0	85.8
	B	101.7	102.5	92.8	97.7	92.5	81.8	82.0
Mean +/- SD				92.1	±	8.5		

n.p.: Not performed

SD: Standard Deviation

Table A7_1_2_2_1-4: Pattern of biodegradation in the sea 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	Sample	Incubation Time (days)						
		0	1	3	5	7	11	17
OIT	A	95.3	88.1	22.0	1.9	3.9	n.a.	n.a.
	B	97.1	89.6	17.7	3.4	3.5	n.a.	n.a.
	Mean	96.2	88.8	19.9	2.7	3.7	n.a.	n.a.
M7	A	*	*	0.8	*	*	n.a.	n.a.
	B	*	*	*	*	*	n.a.	n.a.
	Mean	*	*	0.4	*	*	n.a.	n.a.
M5c	A	*	*	0.6	2.6	1.1	n.a.	n.a.
	B	*	*	1.2	*	1.0	n.a.	n.a.
	Mean	*	*	0.9	1.3	1.1	n.a.	n.a.
M1	A	*	*	*	1.0	1.4	n.a.	n.a.
	B	*	*	0.6	1.2	1.3	n.a.	n.a.
	Mean	*	*	0.3	1.1	1.4	n.a.	n.a.
M11	A	*	*	*	*	*	n.a.	n.a.
	B	*	*	*	1.4	*	n.a.	n.a.
	Mean	*	*	*	0.7	*	n.a.	n.a.
M12	A	*	*	*	1.5	*	n.a.	n.a.
	B	*	*	*	1.4	*	n.a.	n.a.
	Mean	*	*	*	1.4	*	n.a.	n.a.
M13	A	*	*	0.5	*	*	n.a.	n.a.
	B	*	*	*	*	*	n.a.	n.a.
	Mean	*	*	0.2	*	*	n.a.	n.a.
M15	A	*	*	*	*	*	n.a.	n.a.
	B	*	*	0.6	*	*	n.a.	n.a.
	Mean	*	*	0.3	*	*	n.a.	n.a.
M17	A	2.3	*	5.0	*	*	n.a.	n.a.
	B	*	*	4.0	*	*	n.a.	n.a.
	Mean	1.1	*	4.5	*	*	n.a.	n.a.
M20	A	*	*	*	*	*	n.a.	n.a.
	B	*	*	0.7	1.9	*	n.a.	n.a.
	Mean	*	*	0.3	1.0	*	n.a.	n.a.
M21	A	*	*	4.4	1.4	2.3	n.a.	n.a.
	B	*	*	3.3	2.2	2.1	n.a.	n.a.
	Mean	*	*	3.9	1.8	2.2	n.a.	n.a.
M22	A	*	*	*	1.8	1.8	n.a.	n.a.
	B	*	*	1.8	*	1.6	n.a.	n.a.
	Mean	*	*	0.9	0.9	1.7	n.a.	n.a.
M23	A	*	*	2.0	*	*	n.a.	n.a.
	B	*	*	1.6	*	*	n.a.	n.a.
	Mean	*	*	1.8	*	*	n.a.	n.a.
M24	A	*	*	1.2	*	*	n.a.	n.a.
	B	*	*	*	1.4	*	n.a.	n.a.
	Mean	*	*	0.6	0.7	*	n.a.	n.a.
adsorbed radioactivity **	A	3.4	9.8	44.2	63.1	46.1	72.3	43.7
	B	3.4	9.3	48.5	58.6	54.4	64.8	37.1
	Mean	3.4	9.5	46.3	60.9	50.2	68.5	40.4
¹⁴ CO ₂ ***	A	n.p.	0.3	1.8	16.3	25.8	27.9	41.5
	B	n.p.	0.2	1.8	15.6	25.0	33.0	47.7
	Mean	n.p.	0.3	1.8	16.0	25.4	30.5	44.6
EG	A	n.p.	<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
	Mean	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1

* Not detected

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

*** Total ¹⁴CO₂

n.p. Not performed

n.a.: Not analysed due to insufficient radioactivity after concentration

Table A7_1_2_2_1-5: Pattern of biodegradation in the sea 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	1	3	5	7	11	17
OIT	A	99.8	95.0	37.3	11.2	3.7	1.4	0.8
	B	98.1	96.0	45.5	19.0	2.6	3.0	0.7
	Mean	99.0	95.5	41.4	15.1	3.1	2.2	0.7
M7	A	*	*	*	0.1	*	*	0.6
	B	*	*	*	*	*	*	0.5
	Mean	*	*	*	0.1	*	*	0.5
M10	A	*	*	*	*	*	*	0.1
	B	*	*	4.7	0.9	*	*	*
	Mean	*	*	2.4	0.4	*	*	0.1
M5b	A	*	*	*	*	*	1.0	0.1
	B	*	*	*	*	1.0	*	0.1
	Mean	*	*	*	*	0.5	0.5	0.1
M5c	A	*	*	0.4	0.4	*	*	*
	B	*	*	0.3	0.9	1.7	*	*
	Mean	*	*	0.4	0.6	0.9	*	*
M1	A	*	*	0.3	0.8	1.4	1.7	2.3
	B	*	*	0.3	0.8	0.9	1.8	1.9
	Mean	*	*	0.3	0.8	1.1	1.8	2.1
M11	A	*	*	*	*	*	1.6	0.1
	B	*	*	*	0.8	1.8	*	*
	Mean	*	*	*	0.4	0.9	0.8	0.1
M12	A	*	*	3.3	3.9	3.0	*	*
	B	*	*	*	0.6	*	*	*
	Mean	*	*	1.6	2.3	1.5	*	*
M13	A	*	*	0.6	0.1	*	*	*
	B	*	*	0.6	0.5	*	*	*
	Mean	*	*	0.6	0.3	*	*	*
M14	A	*	*	*	*	*	*	*
	B	*	*	0.1	0.4	*	*	*
	Mean	*	*	0.0	0.2	*	*	*
M15	A	*	*	*	*	*	*	*
	B	*	*	*	0.2	*	*	*
	Mean	*	*	*	0.1	*	*	*
M16	A	*	*	2.4	*	*	*	*
	B	*	*	1.8	1.1	*	*	*
	Mean	*	*	2.1	0.5	*	*	*
M17	A	*	*	*	2.5	*	*	*
	B	*	*	*	0.9	*	*	*
	Mean	*	*	*	1.7	*	*	*
M18	A	*	*	*	*	*	*	*
	B	*	*	*	0.5	*	*	*
	Mean	*	*	*	0.3	*	*	*
M19	A	*	*	5.1	*	*	*	*
	B	*	*	1.4	0.8	*	*	*
	Mean	*	*	3.3	0.4	*	*	*
M20	A	*	*	*	*	*	0.3	*
	B	*	*	*	0.9	*	*	*
	Mean	*	*	*	0.5	*	0.1	*
M21	A	*	*	7.6	6.4	6.3	2.3	0.8
	B	*	*	10.8	4.4	2.9	2.9	*
	Mean	*	*	9.2	5.4	4.6	2.6	0.4

* Not detected

Table A7_1_2_2_1-5 continued:

OIT Biodegradation High dose	Duplicate	Incubation Time (days)						
		0	1	3	5	7	11	17
M22	A	*	*	3.5	*	5.4	*	*
	B	*	*	1.5	0.7	2.6	0.7	1.5
	Mean	*	*	2.5	0.3	4.0	0.3	0.8
M23	A	*	*	6.7	4.7	4.6	4.6	4.4
	B	*	*	0.5	2.0	0.7	8.2	2.3
	Mean	*	*	3.6	3.3	2.6	6.4	3.4
M24	A	*	*	7.5	3.7	3.5	1.0	3.8
	B	*	*	3.3	2.4	2.0	3.6	1.7
	Mean	*	*	5.4	3.1	2.7	2.3	2.8
M25	A	*	*	*	5.9	*	1.4	1.5
	B	*	*	1.8	0.4	2.1	3.1	1.4
	Mean	*	*	0.9	3.2	1.1	2.2	1.4
M26	A	*	*	*	*	*	*	1.4
	B	*	*	*	*	*	0.9	*
	Mean	*	*	*	*	0.4	*	0.7
M27	A	*	*	*	*	*	*	*
	B	*	*	*	*	*	*	*
	Mean	*	*	*	*	*	*	*
adsorbed radioactivity**	A	3.7	6.0	14.5	38.2	45.6	42.6	29.2
	B	3.5	6.5	19.5	43.4	53.2	33.5	38.7
	Mean	3.6	6.2	17.0	40.8	49.4	38.0	34.0
¹⁴ CO ₂ ***	A	n.p.	<0.1	0.8	16.1	14.3	19.3	40.6
	B	n.p.	<0.1	0.7	15.9	20.0	25.0	33.1
	Mean	n.p.	<0.1	0.7	16.0	17.2	22.1	36.8
EG	A	n.p.	<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
	Mean	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1

* Not detected

** Radioactivity adsorbed to dissolved organic matter in the sea 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 sea 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.

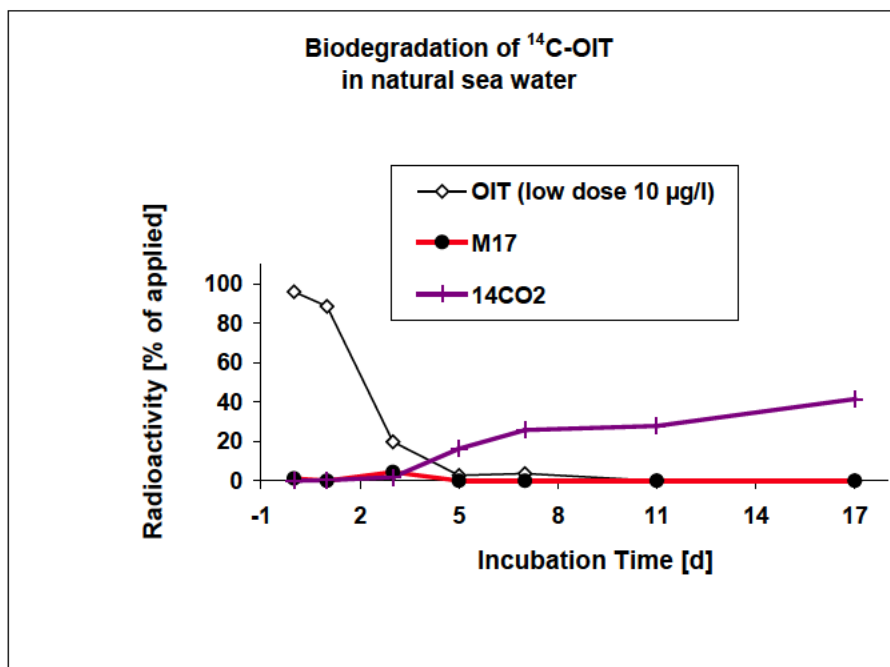
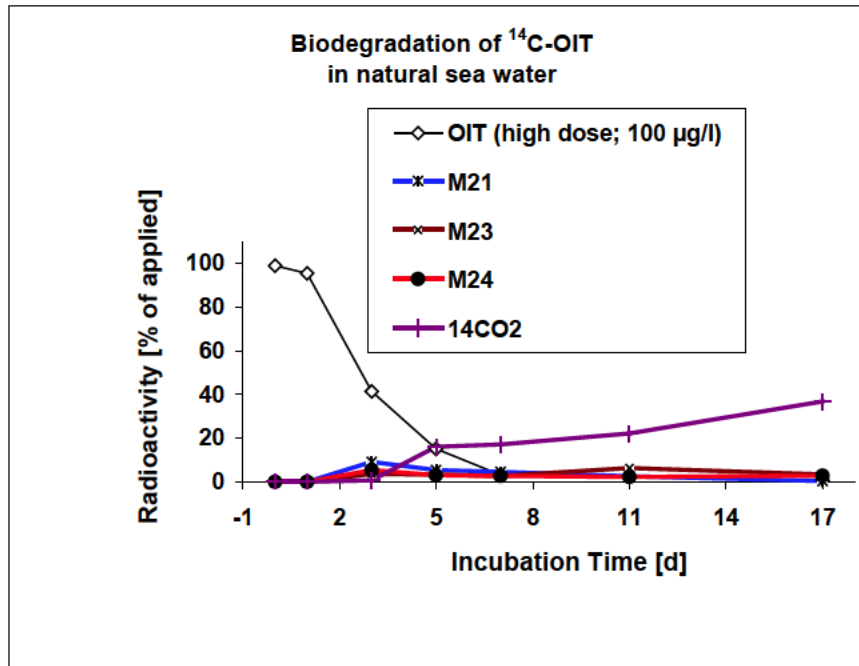


Figure A7_1_2_2_1-2: Rate of biodegradation of 2-(n-octyl)-4-[4,5-¹⁴C]isothiazolin-3-one (¹⁴C-OIT) in the sea water at treatment rates of 10 µg/L (low dose; top) and 100 µg/L (high dose; bottom). Calculation

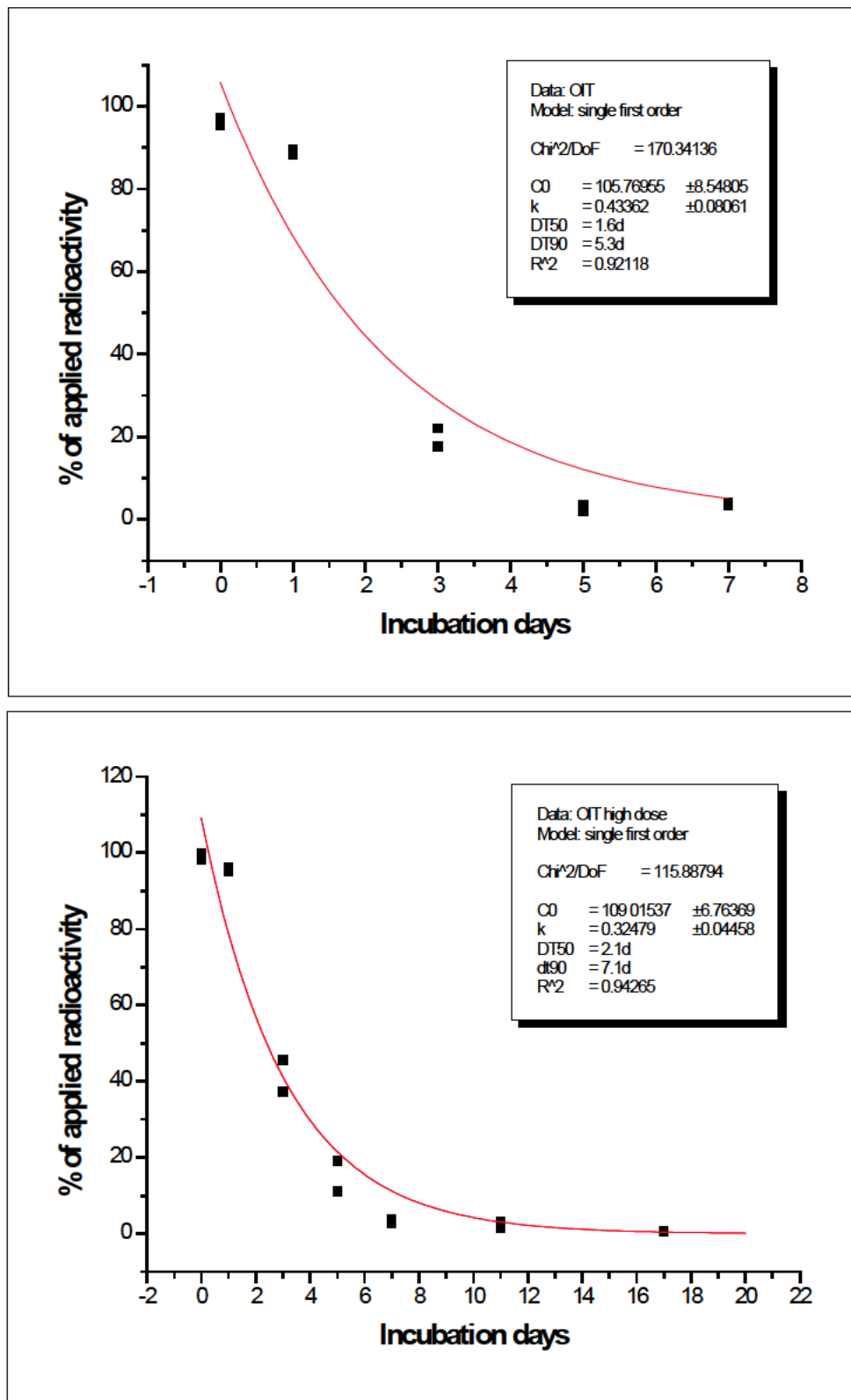




Figure A7_1_2_2_1-3: Proposed metabolic pathway of 2-(n-octyl)-4-[4,5- ^{14}C]isothiazolin-3-one (^{14}C -OIT) in natural sea water.

