Section A7.2.2.1
Annex Point IIIA XII.1.1, XII.1.4

Rate and route of degradation including identification of the processes involved and identification of any metabolites and degrdation products in at least three soil types under appropriate conditions

			Official use only
		1 REFERENCE	
1.1	**Reference**	*Völkel, W., 2007b, 2-(n-octyl)-4-[4,5-^{14}C]- isothiazolin-3-one (^{14}C-OIT): Degradation and Metabolism in Three Soils Incubated under Aerobic Conditions,* [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 Guidelines for testing chemicals 307: Aerobic - Anaerobic Transformation in Soil (April 2002).* *Under consideration of:* *EPA Pesticide Assessment Guidelines, Subdivision N, Chemistry: Environmental Fate, Section 162-1, Aerobic Soil Metabolism Studies (October 1982).* *Guidelines for registration of pesticides in Canada: Environmental Chemistry and Fate, Section 6.2 C1 Soil-Degradation Pathways and Persistence (July 14, 1987). (If yes, give guidelines; if no, give justification, e.g. "no guidelines available" or "methods used comparable to guidelines xy")*	
2.2	**GLP**	*Yes*	
2.3	**Deviations**	*No*	
		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]	X
3.1.3	Radiolabelling	[redacted]	
3.1.4	Specific Radioactivity	[redacted]	
3.2	**Reference substance**	*No*	
3.3	**Test system**	*See table A7_2_2_1 _1-1*	
3.3.1	Soils	*Soil I (Senozan, France; silt loam), soil II (Speyer 2.2, Germany; sandy loam) and soil III (Speyer 6S, Germany; clay).*	X
3.3.2	Soil preparation	*After transportation to RCC Ltd, the soils were sieved through a 2-mm screen and stored at 4°C. Prior to use in the study, all soils were*	

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		conditioned to room temperature for about two weeks. The soils were finger-crumbled and turned over frequently to avoid excessive surface drying. The soils were watered if needed. One aliquot of soil I (sterile part of the study) was autoclaved at 120°C for 20 minutes before treatment.
3.3.3	Test conditions	*The treated soil samples were incubated at 20 ± 2°C and/or 6 ± 2°C in the dark under continuous ventilation with moistened air.*
3.3.4	Preparation of test solution and application	*An aliquot of 1.3 mL of the 2-(n-octyl)-4-[4,5-^{14}C]isothiazolin-3-one (^{14}C-OIT) stock solution was diluted with 10 mL acetone, mixed in an ultrasonic bath and then filled up with water to obtain a final volume of 100 mL. This application solution was submitted again to ultrasonic treatment. The amount of OIT in the application solution was determined by measuring five sub-samples of 10 µL by LSC. Based on the radioactivity measured and its specific activity, the amount of 2-(n-octyl)-4-[4,5-^{14}C]isothiazolin-3-one (^{14}C-OIT) was calculated to be 10.11 mg/100 mL.* *Soil samples (100 g) were separately dosed with the ^{14}C-test item as follows:* *An aliquot of 800 µL (or 500 µL for the sterile samples), calculated to contain about 0.07 mg of 2-(n-octyl)-4-[4,5-^{14}C]isothiazolin-3-one (^{14}C-OIT) was applied drop-wise to the soil surface of the respective samples using a 1000 µL Hamilton syringe. The treated soil was then mixed thoroughly. The total amount of organic solvent added to the samples was below 0.1% v/w.*
3.3.5	Rate of application	*The test item was applied to 100 g soil samples at a concentration of 0.7 mg/kg dry soil which is equivalent to an expected environmental concentration of 700 g OIT per hectare, assuming an even distribution of the test item in the top 10 cm of soil and a soil bulk density of 1 g/cm^3.*
3.3.6	Duration of the test	*100 days and 12 days (sterile part)*
3.3.7	Biomass determination	*Prior to treatment and after 100 days of incubation*
3.3.8	Sampling	*Individual samples from all soils (I to III) incubated at 20°C were taken for extraction and analysis immediately after treatment (day 0) and after 0.17 (4 hours), 0.33 (8 hours) 1, 2, 7, 14, 30, and 100 days. Samples from soil I incubated at 6°C were taken after 0.5 (12 hours), 2, 7, 14, 30, and 100 days. Sterile samples from soil I were worked-up after 2, 5 and 12 days of incubation.*
3.3.9	Extraction and identification	*Initially soils were extracted at room temperature using about 100 mL (1 mL/g soil) of [redacted] for 30 minutes on a shaker (200 - 250 strokes/min). Soils were extracted up to 4 times. The soils were then Soxhlet extracted using [redacted] The individual extracts were quantified by LSC then combined. The combined [redacted] extract was then concentrated under reduced pressure in a rotary evaporator at 30 to 35°C, and the radioactivity was re-measured by LSC. All extracts were chromatographed using HPLC and/or 1D- and 2D-TLC analysis.*
3.3.10	Determination of non-extractable radioactivity	*The residual radioactivity remaining in soil after the extraction procedure was quantified by LSC after combustion of aliquots of the air-dried and homogenised soil.*

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After the above extraction steps, the residual soil samples from day 100 were subsequently extracted with [redacted] by reflux for about four hours. The radioactivity in the individual extracts was quantified by LSC.

3.3.11 Analytical methods

Radioactivity: Liquid scintillation counting (LSC);

HPLC: (3 gradients)
Pre-column: [redacted]
Column: [redacted]
Column Temperature: [redacted]
Mobile Phase:
Solvent A: [redacted]
Solvent B: [redacted]
Gradient (one out of three):

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]
TLC:
Radio 1D- and 2D-TLC as secondary method on selected samples.

4 RESULTS

4.1 Recovery

Mean recoveries of radioactivity were 95.6 ± 3.0%, 95.5 ± 3.5% and 97.6 ± 3.0% AR for soils I to III, respectively, incubated at 20°C. For soil I incubated at 6°C and from soil I incubated under sterile conditions at 20°C, the mean recoveries were 96.7 ± 3.8% and 100.9 ± 1.5% AR, respectively (tables A7_2_2_1-2 through A7_2_2_1-6).

4.2 Extractable residues

For the soils incubated at 20°C, the amount of total extractable radioactivity decreased very rapidly from 94.8%, 97.7% and 95.9% AR immediately after treatment to 25.7%, 29.1% and 43.7% after just two days of incubation for soils I, II and III, respectively. At the end of incubation (day 100), only 8.5%, 10.9% and 11.5% AR was extractable from the corresponding soils. Soxhlet extraction released a maximum individual amount of 14.5% AR (8 hours, soil III). For soil I incubated at 6°C, the amount of extractables was higher, representing 59.4% and 18.1% AR on days 2 and 100, respectively. The amount of extractable radioactivity remained at the same level between days 2 and 12 for soil I incubated under sterile conditions (99.1-100.1% AR). For details see tables A7_2_2_1-2 through A7_2_2_1-6

4.2.1 Degradation products

Numerous radioactive fractions (up to 32) were formed. Only one accounted for more than 5% AR (M32), but never exceeded the mean value of 6% AR. Additionally M32 may contain more than one component as it corresponded to the unretained radioactivity by HPLC. Forming once the parent had significantly degraded, M32 steadily increased to reach maximum levels of 4.7%, 5.4%, 4.8% and 4.8% AR in soils I (20°C), II, III and I (6°C), respectively, between days 7 and 30. On day 100, it had decreased to represent between 1.9-4.1% AR. One of the very minor metabolites (M9) was characterised using the available

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		reference item as N-(n-octyl)oxamic acid. (tables A7_2_2_1-7 through A7_2_2_1-11).	
4.2.2	Unknowns	*See above.* *Metabolism involved cleavage of the isothiazolone ring and subsequent oxidation of the alkyl metabolites to CO_2. A significant portion of the ^{14}C-activity was incorporated into the bound residues as alkyl metabolites or CO_2.*	
4.3	**Non-extractable residues**	*The amount of non-extractable radioactivity for non-sterile soils reached peak values of 51.2% (day 14), 48.8% (day 7), 47.6% (day 14) and 49.6% (day 14) AR for soils I (20°C), II, III and I (6°C), respectively. Thereafter, the amount of non-extractables decreased by 8 to 15% AR in all soils until the end of incubation (day 100). Under sterile conditions in soil I, non-extractables did not account for more than 2% AR during the 12-day incubation period. For details see tables A7_2_2_1-2 through A7_2_2_1-6*	*X*
4.4	**Mineralisation**	*47.6%, 43.8%, 42.4% and 33.7% AR for soils I (20°C), II, III and I (6°C), respectively. There was no formation of $^{14}CO_2$ or other volatiles from the sterile soil. For details see tables A7_2_2_1-2 through A7_2_2_1-6.*	
4.5	**Degradation rates**	– DT_{50} soil I, 20°C: 0.3 days (or 7 hours) – DT_{50} soil II, 20°C: 0.3 days (or 7 hours) – DT_{50} soil III, 20°C: 0.5 days (or 12 hours) – DT_{50} soil I, 6°C: 1.4 days	*X*
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	*In the present study, the route and rate of biodegradation of 2-(n-octyl)-4-[4,5-^{14}C]isothiazolin-3-one (^{14}C-OIT) were investigated in three soils incubated under aerobic conditions for a period of up to 100 days.* *In order to investigate the influence of temperature and microbial degradation on the rate of disappearance of ^{14}C-OIT, one of the selected soils (I) was additionally incubated at 6°C and under sterile conditions.*	
5.2	**Results and discussion**	*Mineralisation to $^{14}CO_2$ was very high for all three soils and both incubation temperatures, reaching maximum levels of 47.6%, 43.8%, 42.4% and 33.7% AR for soils I (20°C), II, III and I (6°C), respectively.* *There was no formation of $^{14}CO_2$ or other volatiles from the sterile soil.* *The test item, 2-(n-octyl)-4-[4,5-^{14}C]isothiazolin-3-one (^{14}C-OIT), disappeared extremely rapidly from the non-sterile soils incubated at 20°C*	
5.2.1	Degradation pattern	*Besides the test item, numerous radioactive fractions (up to 32) were formed. Only one accounted for more than 5% AR (M32), but never exceeded the mean value of 6% AR. Additionally M32 may contain more than one component as it corresponded to the unretained radioactivity by HPLC. Forming once the parent had significantly degraded, M32 steadily increased to reach maximum levels of 4.7%, 5.4%, 4.8% and 4.8% AR in soils I (20°C), II, III and I (6°C), respectively, between days 7 and 30. On day 100, it had decreased to represent between 1.9-4.1% AR. One of the very minor metabolites (M9) was characterised using the*	

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available reference item as N-(n-octyl)oxamic acid. See also figure A7_2_2_1-3.

5.2.2 DT_{50}/ DT_{90}

Soil	Temperature (°C)	2-(n-octyl)-4-[4,5-^{14}C]isothiazolin-3-one (^{14}C-OIT	
		DT_{50} (d)	DT_{90} (d)
Soil I	20	0.3 (7 hours)	1.0 (24 hours)
Soil II	20	0.3 (7 hours)	0.9 (23 hours)
Soil III	20	0.5 (12 hours)	1.6 (38 hours)
Soil I	6	1.4 days	4.7 days

X

See figures A7_2_2_1-1 and A7_2_2_1-2.

5.3 Conclusion

2-(n-octyl)-4-[4,5-^{14}C]isothiazolin-3-one (^{14}C-OIT) biodegrades very rapidly in the soil environment. Metabolism involves cleavage of the isothiazolone ring and subsequent oxidation of the alkyl metabolites to CO_2. A significant portion of the ^{14}C-activity is also incorporated into the bound residues as alkyl metabolites or CO_2.

Only 2% AR remained bound to the sterile soil with the 12-day incubation period ***showing that the degradation and adsorption of residues to soil are only due to microbial degradation.***

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 Nov 2009
Materials and Methods	Applicant's version is considered acceptable noting the following: **Section 3.1.2:** The radiochemical purity of the test substance should be [redacted] **Section 3.3.1:** The pH of the chosen soils are similar, with no alkaline soils selected, however the UK CA note that there is no evidence from any of the other studies contained within the dossier which indicate that the behaviour of OIT is pH dependant. **Section 3.3.5:** A soil density of 1 g/cm^3 has been used. It is more usual to use a soil density of 1.5 g/cm3; however this would not have had any effect on the quality of the study.

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Results and discussion

Applicant's version is considered acceptable noting the following:

Section 4.3: Very large amounts of NER formed rapidly (*c*. 20% within few hours), peaking at *c*.48 to51 % after 7 to14 days and then declining slightly to c. 36 to 39 % by 100 days. However, only 2 % NER was seen in sterile soil after 12 days, suggesting that the majority of NER seen in non-sterile soil was likely to be minor metabolites formed following rapid microbial degradation of the parent compound and then released slowly over time, rather than unchanged parent compound itself. This supports the applicant's conclusion. Also further characterisation of the NER residue showed 75 % of the radioactivity was associated with humin and humic acid fractions rather than fulvic acid. The result of the sterile control also confirms that the most important route of degradation is microbial degradation.

Conclusion

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

Section 5.2.2: As a result of the rapid degradation (70 % within 1 day,) the first 6 sampling points are all within 7 days. The remaining samples are spaced over 93 days. The visual fit for the decline curve with single first order kinetics is good over the first 5 points, which is equivalent to approximately 80 to 90 % of the degradation. Also, in all 3 soils, less than10 % remained by 2 to 7 days. The visual fit of curve was less good over the last 4 time points, but these are associated with only low concentrations (1.5 to 8.5%), and therefore the fit to these later time points are considered less significant in the opinion of the UK CA, as the fit is acceptable up to the DT90 value, the UK CA found the kinetics to be of an acceptable reliability. The data shows that 50 % had degraded by 0.33 d, supporting the suggested DT_{50} of -0.3-0.5 days. The r^2 value is also acceptable (figure 7_1_2_2-1 and 7_1_2_2_2-2)

Using first order non-linear kinetics the UK CA calculated the K, DT50/90 and R^2 values to be:

Parameter	Soil I 20°C	Soil II 20°C	Soil III 20°C	Soil I 6°C
k	2.31	2.43	1.22	0.44
DT_{50} (days)	0.30	0.28	0.57	1.59
DT_{90} (Days)	1.00	0.95	1.89	5.28
R^2	0.985	0.987	0.952	0.963

This is broad agreement with the study values.

Reliability

1

Study conducted in compliance with agreed protocols, with no or minor deviations from standard test guidelines and /or minor methodological deficiencies, which do not affect the quality of relevant results.

Acceptability

Acceptable.

Remarks

All endpoints and data presented have been checked against the original study and are correct.

Section A7.2.2.1 **Annex Point IIIA XII.1.1, XII.1.4**	**Rate and route of degradation including identification of the processes involved and identification of any metabolites and degrdation products in at least three soil types under appropriate conditions**

	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A7_2_2_1-1: Classification and physico-chemical properties

Parameters	Soil I	Soil II	Soil III
Origin	Senozan France	Speyer 2.2 Germany	Speyer 6S Germany
Batch No.	[redacted]	[redacted]	[redacted]
pH ($CaCl_2$)	6.3	5.7	6.9
Organic carbon (g/100 g soil)	0.95	2.29	1.9
Cation exchange capacity (meq/100 g soil)	12.3	11	18
Carbonate (% $CaCO_3$)	<0.1	<0.1	1.2
Total nitrogen (%)	0.13	0.21	0.21
Bulk density (g/1000 mL)	1250	1158	1247
Particle size analyses (USDA, mm):			
Soil type (USDA)	Silt loam	Sandy loam	Clay
< 0.002 (clay) %	23.3	7.9	42.0
0.002 - 0.05 (silt) %	62.2	14.6	36.1
> 0.05 - 2 (sand) %	14.5	77.5	22.0
Max. water holding capacity [MWC; (g/100 g dry soil)]	59.30	48.4	41.7
Biomass* (mg carbon/100 g dry soil)			
Start of incubation	31.4	22.3	42.3
End of incubation (20°C / 6°C)	16.8 / 16.5	11.7	26.5

Parameters of soils as determined by LUFA Speyer, Germany

* Determined by RCC Ltd during the study.

Table A7_2_2_1-2: Balance of the applied radioactivity in soil I (Senozan) treated with ^{14}C-OIT and incubated at 20°C. Values in % AR.

OIT Soil I (Senozan) (% AR)	Duplicate	INCUBATION TIME IN DAYS								
		0	0.17	0.33	1	2	7	14	30	100
Extractables*	A	94.2	68.8	56.2	29.9	19.7	11.9	8.4	7.5	4.9
	B	95.4	67.1	53.9	31.0	20.8	11.8	8.3	7.4	4.8
Soxhlet**	A	n.p.	6.2	7.5	5.0	5.0	3.6	4.4	3.6	3.5
	B	n.p.	6.2	11.0	5.8	6.0	3.7	5.2	3.6	3.7
Total Extractables	mean	94.8	74.2	64.4	35.8	25.7	15.5	13.1	11.0	8.5
Non-Extractables	A	5.7	20.1	28.6	45.2	48.8	50.7	49.9	43.3	36.7
	B	5.5	20.8	28.4	45.3	47.6	48.8	52.4	44.1	35.9
	mean	5.6	20.5	28.5	45.2	48.2	49.7	51.2	43.7	36.3
$^{14}CO_2$	A	n.p.	2.0	4.5	15.1	22.0	25.7	34.8	39.4	47.4
	B	n.p.	1.6	0.8	13.9	19.9	27.3	35.8	38.2	47.8
	mean	n.p.	1.8	2.6	14.5	20.9	26.5	35.3	38.8	47.6
Other Volatiles in ethylene glycol	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
T O T A L	**A**	**99.9**	**97.1**	**96.8**	**95.2**	**95.5**	**91.9**	**97.4**	**93.8**	**92.5**
	B	**100.9**	**95.8**	**94.1**	**96.1**	**94.3**	**91.6**	**101.7**	**93.3**	**92.3**
MEAN ± SD					**95.6**	±	**3.0**			

Table A7_2_2_1-3: Balance of the applied radioactivity in soil II (Speyer 2.2) treated with ^{14}C-OIT and incubated at 20°C. Values in % AR.

OIT Soil II (Speyer 2.2) (% AR)	Duplicate	INCUBATION TIME IN DAYS								
		0	0.17	0.33	1	2	7	14	30	100
Extractables*	A	96.4	67.2	56.1	31.6	21.9	11.9	8.9	8.1	5.3
	B	99.1	70.5	52.6	32.1	21.0	11.4	8.8	7.5	5.4
Soxhlet**	A	n.p.	7.9	11.7	7.9	8.0	6.2	7.5	5.7	5.6
	B	n.p.	7.2	13.1	8.8	7.4	6.9	7.5	6.4	5.6
Total Extractables	mean	97.7	76.4	66.8	40.2	29.1	18.2	16.4	13.8	10.9
Non-Extractables	A	4.8	17.1	25.8	40.4	45.0	48.7	46.7	41.0	36.9
	B	4.8	17.7	26.7	40.3	46.0	48.9	44.6	42.4	35.4
	mean	4.8	17.4	26.2	40.3	45.5	48.8	45.7	41.7	36.1
$^{14}CO_2$	A	n.p.	1.3	4.3	15.2	22.3	28.8	34.3	36.1	43.3
	B	n.p.	2.0	3.9	15.2	21.5	27.1	32.3	35.2	44.2
	mean	n.p.	1.7	4.1	15.2	21.9	27.9	33.3	35.7	43.8
Other Volatiles in ethylene glycol	A	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.2
	B	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
T O T A L	**A**	**101.2**	**93.4**	**98.0**	**95.2**	**97.2**	**95.6**	**97.4**	**90.9**	**91.3**
	B	**103.8**	**97.5**	**96.3**	**96.4**	**95.9**	**94.3**	**93.2**	**91.5**	**90.6**
MEAN ± SD					**95.5**	±	**3.5**			

Table A7_2_2_1-4: Balance of the applied radioactivity in soil III (Speyer 6S) treated with ^{14}C-OIT and incubated at 20°C. Values in % AR.

OIT Soil III (Speyer 6S) (% AR)	Duplicate	INCUBATION TIME IN DAYS								
		0	0.17	0.33	1	2	7	14	30	100
Extractables*	A	95.4	70.3	60.5	49.4	39.3	22.9	13.8	10.5	6.6
	B	96.4	72.1	60.6	49.8	36.4	21.2	14.5	10.7	7.0
Soxhlet**	A	n.p.	8.8	14.5	6.2	7.4	7.2	6.5	5.8	4.8
	B	n.p.	9.4	10.4	5.9	4.3	5.8	5.9	6.4	4.6
Total Extractables	mean	95.9	80.3	73.0	55.6	43.7	28.5	20.3	16.7	11.5
Non-Extractables	A	6.6	17.0	21.6	34.1	41.3	44.0	47.2	42.1	39.0
	B	5.2	16.3	24.9	35.1	38.9	46.0	47.9	43.7	40.2
	mean	5.9	16.7	23.3	34.6	40.1	45.0	47.6	42.9	39.6
$^{14}CO_2$	A	n.p.	1.3	2.8	6.1	14.5	23.7	31.3	34.4	42.5
	B	n.p.	1.1	2.7	6.2	14.0	25.2	31.1	35.1	42.3
	mean	n.p.	1.2	2.8	6.2	14.2	24.5	31.2	34.7	42.4
Other Volatiles in ethylene glycol	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
T O T A L	**A**	**102.0**	**97.4**	**99.5**	**95.8**	**102.5**	**97.9**	**98.9**	**92.8**	**92.9**
	B	**101.6**	**98.8**	**98.6**	**97.0**	**93.5**	**98.2**	**99.4**	**95.9**	**94.1**
MEAN ± SD					**97.6**	±	**3.0**			

Table A7_2_2_1-5: Balance of the applied radioactivity in soil I (Senozan) treated with ^{14}C-OIT and incubated at 6°C. Values in % AR.

OIT Soil I/6°C (Senozan) (% AR)	Duplicate	INCUBATION TIME IN DAYS						
		0	0.5	2	7	14	30	100
Extractables*	A	94.2	71.0	50.6	27.5	17.6	14.3	9.8
	B	95.4	72.5	57.0	26.0	17.5	14.7	12.1
Soxhlet**	A	n.p.	7.3	5.3	8.2	8.4	7.7	6.7
	B	n.p.	7.0	5.9	6.8	9.3	6.8	7.4
Total Extractables	mean	94.8	78.9	59.4	34.2	26.4	21.8	18.1
Non-Extractables	A	5.7	20.2	40.3	46.8	49.8	46.0	42.7
	B	5.5	18.2	33.7	47.8	49.5	46.3	40.2
	mean	5.6	19.2	37.0	47.3	49.6	46.1	41.4
$^{14}CO_2$	A	n.p.	<0.1	6.8	15.8	17.7	24.4	34.3
	B	n.p.	1.9	4.4	11.5	18.8	25.1	33.1
	mean	n.p.	1.0	5.6	13.7	18.2	24.8	33.7
Other Volatiles in ethylene glycol	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
T O T A L	**A**	**99.9**	**98.6**	**103.0**	**98.3**	**93.4**	**92.4**	**93.5**
	B	**100.9**	**99.6**	**101.0**	**92.1**	**95.1**	**92.9**	**92.9**
MEAN ± SD				**96.7**	±	**3.8**		

Table A7_2_2_1-6: Balance of the applied radioactivity in soil I (Senozan) treated with ^{14}C-OIT and incubated at 20°C under sterile conditions. Values in % AR.

OIT Soil I/sterile (Senozan) (% AR)	Duplicate	INCUBATION TIME IN DAYS		
		2	5	12
Extractables*	A	93.6	94.4	94.1
	B	97.7	96.3	93.3
Soxhlet**	A	3.8	4.8	5.5
	B	3.7	4.6	5.3
Total Extractables	mean	99.4	100.1	99.1
Non-Extractables	A	0.9	1.0	2.1
	B	0.9	1.0	1.9
	mean	0.9	1.0	2.0
$^{14}CO_2$	A	<0.1	<0.1	<0.1
	B	<0.1	<0.1	<0.1
	mean	<0.1	<0.1	<0.1
Other Volatiles	A	<0.1	0.2	<0.1
in ethylene glycol	B	<0.1	0.1	<0.1
T O T A L	**A**	**98.3**	**100.4**	**101.7**
	B	**102.4**	**102.1**	**100.6**
MEAN ± SD		**100.9**	±	**1.5**

Table A7_2_2_1-7: Pattern of degradation and formation of metabolites in soil I (Senozan) treated with ^{14}C-OIT and incubated at 20°C. Values in % AR.

Soil I (Senozan) Pattern (% AR)	Duplicate	INCUBATION TIME IN DAYS								
		0	**0.17**	**0.33**	**1**	**2**	**7**	**14**	**30**	**100**
Parent	A	92.7	59.8	42.0	13.4	6.9	2.9	3.5	1.3	1.3
	B	95.4	56.8	40.0	15.3	8.5	4.2	4.1	2.0	1.6
	mean	**94.0**	**58.3**	**41.0**	**14.3**	**7.7**	**3.5**	**3.8**	**1.7**	**1.5**
M6	A	*	1.5	2.9	2.1	0.9	0.6	0.5	*	*
	B	*	1.4	3.3	3.1	0.9	0.5	0.6	*	*
	mean	*****	**1.4**	**3.1**	**2.6**	**0.9**	**0.5**	**0.5**	*****	*****
M7	A	*	0.4	*	2.4	3.9	1.1	0.9	*	0.4
	B	*	1.2	*	3.3	3.1	1.2	1.0	*	0.5
	mean	*****	**0.8**	*****	**2.8**	**3.5**	**1.2**	**0.9**	*****	**0.5**
M17	A	0.8	2.1	1.6	1.8	0.7	*	*	*	*
	B	*	2.4	1.6	1.7	0.8	*	*	*	*
	mean	**0.4**	**2.2**	**1.6**	**1.7**	**0.8**	*****	*****	*****	*****
M21	A	*	1.3	2.6	0.9	1.1	1.1	0.9	0.2	0.6
	B	*	1.0	4.2	1.2	1.6	0.7	0.9	2.3	2.1
	mean	*****	**1.1**	**3.4**	**1.1**	**1.3**	**0.9**	**0.9**	**1.3**	**1.4**
M22	A	*	0.9	1.0	0.7	0.6	1.1	1.0	1.9	0.8
	B	*	0.1	*	1.0	0.8	1.0	1.1	2.2	1.7
	mean	*****	**0.5**	**0.5**	**0.9**	**0.7**	**1.0**	**1.1**	**2.0**	**1.2**
M23	A	*	*	*	0.5	0.4	1.1	0.7	2.2	1.3
	B	*	*	*	0.3	1.6	1.7	0.8	0.4	0.4
	mean	*****	*****	*****	**0.4**	**1.0**	**1.4**	**0.7**	**1.3**	**0.9**
M25	A	*	1.1	3.1	*	1.5	1.0	*	1.0	0.4
	B	*	1.7	2.7	0.9	2.0	*	*	*	*
	mean	*****	**1.4**	**2.9**	**0.5**	**1.8**	**0.5**	*****	**0.5**	**0.2**
M32	A	*	*	*	1.2	3.1	5.0	4.6	1.8	2.1
	B	*	*	*	0.9	2.2	4.5	4.2	3.1	1.7
	mean	*****	*****	*****	**1.0**	**2.7**	**4.7**	**4.4**	**2.4**	**1.9**
Non-Extractables	**mean**	**5.6**	**20.5**	**28.5**	**45.2**	**48.2**	**49.7**	**51.2**	**43.7**	**36.3**
$^{14}CO_2$	**mean**	**n.p.**	**1.8**	**2.6**	**14.5**	**20.9**	**26.5**	**35.3**	**38.8**	**47.6**

* not detected or below limit of quantification n.p.: not performed

Minor metabolites (m) below 2% AR and their maximum amounts:

m	% AR	m	% AR	m	% AR	m	% AR	m	% AR
m2	0.5	m9*	0.6	m13	0.5	m24	0.8	m29	1.3
m3	1.3	m10	1.9	m19	0.5	m26	0.4	m31	1.2
m4	0.9	m11	1.0	m20	1.7	m27	0.6		
m5	1.2	m12	1.2	m23	1.4	m28	0.5		

* Characterised by HPLC and TLC using the available reference item to be N-(n-octyl)oxamic acid

Table A7_2_2_1-8: Pattern of degradation and formation of metabolites in soil II (Speyer 2.2) treated with ^{14}C-OIT and incubated at 20°C. Values in % AR.

Soil II (Speyer 2.2) Pattern (% AR)	Duplicate	INCUBATION TIME IN DAYS								
		0	0.17	0.33	1	2	7	14	30	100
Parent	A	96.4	58.8	43.9	14.0	6.2	2.6	3.6	3.4	1.6
	B	99.1	61.3	39.5	12.0	8.1	3.1	4.2	4.0	1.9
	mean	**97.7**	**60.1**	**41.7**	**13.0**	**7.2**	**2.9**	**3.9**	**3.7**	**1.7**
M4	A	*	1.0	1.6	1.4	0.6	*	0.2	*	*
	B	*	0.8	1.8	2.4	*	*	*	*	*
	mean	*****	**1.8**	**3.4**	**1.9**	**0.3**	*****	**0.1**	*	*
M6	A	*	1.6	2.8	2.9	1.2	0.5	0.3	0.3	0.4
	B	*	1.3	3.2	2.3	1.9	0.4	0.4	0.3	0.2
	mean	*****	**1.5**	**3.0**	**2.6**	**1.6**	**0.4**	**0.3**	**0.3**	**0.3**
M7	A	*	0.5	0.9	1.5	3.0	1.0	1.8	1.3	0.6
	B	*	0.7	0.9	1.6	4.5	1.8	1.6	1.3	1.0
	mean	*****	**0.6**	**0.9**	**1.6**	**3.8**	**1.4**	**1.7**	**1.3**	**0.8**
M10	A	*	1.3	1.6	4.5	*	*	*	*	*
	B	*	0.9	1.8	4.5	*	*	*	*	*
	mean	*****	**1.1**	**1.7**	**4.5**	*****	*****	*****	*****	*****
M12	A	*	2.2	3.2	1.0	0.6	0.4	0.3	*	0.3
	B	*	2.6	2.3	0.8	0.7	0.6	0.4	0.3	0.3
	mean	*****	**2.4**	**2.8**	**0.9**	**0.6**	**0.5**	**0.3**	**0.2**	**0.3**
M23	A	*	0.5	1.9	2.2	4.7	2.9	0.8	0.3	2.7
	B	*	1.2	2.6	3.5	2.3	2.6	0.6	0.8	0.6
	mean	*****	**0.8**	**2.3**	**2.8**	**3.5**	**2.7**	**0.7**	**0.6**	**1.6**
M32	A	*	0.2	*	0.9	2.0	2.2	4.9	4.8	1.5
	B	*	*	*	0.6	2.0	3.2	4.2	4.8	4.4
	mean	*****	**0.1**	*****	**0.8**	**2.0**	**2.7**	**4.6**	**4.8**	**2.9**
Non-Extractables	**mean**	**4.8**	**17.4**	**26.2**	**40.3**	**45.5**	**48.8**	**45.7**	**41.7**	**36.1**
$^{14}CO_2$	**mean**	**n.p.**	**1.7**	**4.1**	**15.2**	**21.9**	**27.9**	**33.3**	**35.7**	**43.8**

* not detected or below limit of quantification n.p.: not performed

Minor metabolites (m) below 2% AR and their maximum amounts:

m	% AR	m	% AR	m	% AR	m	% AR	m	% AR
m1	0.8	m9*	1.0	m17	1.9	m24	1.0	m28	1.1
m2	1.2	m11	0.9	m20	0.9	m25	0.9	m30	0.2
m3	1.0	m13	0.2	m21	1.9	m26	0.9	m31	0.6
m5	0.6	m14	0.3	m22	1.6	m27	0.7		

* Characterised by HPLC and TLC using the available reference item to be N-(n-octyl)oxamic acid

Table A7_2_2_1-9: Pattern of degradation and formation of metabolites in soil III (Speyer 6S) treated with ^{14}C-OIT and incubated at 20°C. Values in % AR.

Soil III (Speyer 6S) Pattern (% AR)	Duplicate	INCUBATION TIME IN DAYS								
		0	**0.17**	**0.33**	**1**	**2**	**7**	**14**	**30**	**100**
Parent	A	94.8	66.4	54.4	25.9	18.8	9.2	5.9	6.1	2.0
	B	96.4	67.3	50.2	27.5	19.3	7.8	8.0	5.9	2.8
	mean	**95.6**	**66.8**	**52.3**	**26.7**	**19.0**	**8.5**	**6.9**	**6.0**	**2.4**
M6	A	0.6	1.2	0.8	3.6	2.4	0.4	0.6	*	0.2
	B	*	1.1	0.7	3.8	2.2	0.8	0.4	0.2	0.3
	mean	**0.3**	**1.2**	**0.8**	**3.7**	**2.3**	**0.6**	**0.5**	**0.1**	**0.3**
M7	A	*	0.8	0.6	2.4	3.6	3.1	1.8	0.9	0.6
	B	*	0.3	0.7	1.5	2.7	1.6	1.8	0.9	0.8
	mean	*****	**0.6**	**0.6**	**1.9**	**3.2**	**2.3**	**1.8**	**0.9**	**0.7**
M17	A	*	2.1	3.6	2.1	1.6	0.7	0.2	0.4	0.3
	B	*	1.4	2.9	1.8	1.5	0.5	0.8	0.4	0.1
	mean	*****	**1.7**	**3.2**	**2.0**	**1.5**	**0.6**	**0.5**	**0.4**	**0.2**
M21	A	*	1.0	2.4	2.5	2.5	1.3	0.6	1.1	0.6
	B	*	1.1	3.3	1.1	1.0	0.8	0.4	0.1	0.1
	mean	*****	**1.1**	**2.8**	**1.8**	**1.8**	**1.0**	**0.5**	**0.6**	**0.4**
M22	A	*	0.7	1.4	1.7	1.4	0.9	0.7	0.6	0.7
	B	*	1.3	2.0	2.8	0.7	0.6	0.9	0.4	0.1
	mean	*****	**1.0**	**1.7**	**2.3**	**1.1**	**0.8**	**0.8**	**0.5**	**0.4**
M24	A	*	0.1	0.9	1.5	2.1	3.2	0.7	*	0.8
	B	*	0.3	0.7	2.3	1.7	1.6	0.8	0.3	0.1
	mean	*****	**0.2**	**0.8**	**1.9**	**1.9**	**2.4**	**0.8**	**0.2**	**0.4**
M25	A	*	0.5	0.8	3.4	0.7	1.9	*	*	*
	B	*	0.5	*	2.4	0.8	1.1	*	*	0.2
	mean	*****	**0.5**	**0.4**	**2.9**	**0.8**	**1.5**	*****	*****	**0.1**
M28	A	*	0.8	1.5	4.4	2.5	1.6	*	*	*
	B	*	1.3	2.6	3.3	1.6	1.1	*	0.3	0.1
	mean	*****	**1.1**	**2.0**	**3.9**	**2.1**	**1.3**	*****	**0.1**	**<0.1**
M32	A	*	*	*	*	2.1	3.5	4.8	4.9	3.3
	B	*	*	0.2	0.7	1.3	4.9	4.5	5.9	5.0
	mean	*****	*****	**0.1**	**0.3**	**1.7**	**4.2**	**4.7**	**5.4**	**4.1**
Non-Extractables	**mean**	**5.9**	**16.7**	**23.3**	**34.6**	**40.1**	**45.0**	**47.6**	**42.9**	**39.6**
$^{14}CO_2$	**mean**	**n.p.**	**1.2**	**2.8**	**6.2**	**14.2**	**24.5**	**31.2**	**34.7**	**42.4**

* not detected or below limit of quantification n.p.: not performed

Minor metabolites (m) below 2% AR and their maximum amounts:

m	% AR	m	% AR	m	% AR	m	% AR	m	% AR
m1	0.2	m4	0.6	m10	0.6	m13	0.6	m31	1.5
m2	1.4	m5	0.4	m11	0.9	m20	1.6		
m3	0.8	m9*	1.2	m12	0.9	m23	1.3		

* Characterised by HPLC and TLC using the available reference item to be N-(n-octyl)oxamic acid

Table A7_2_2_1-10: Pattern of degradation and formation of metabolites in soil I (Senozan) treated with ^{14}C-OIT and incubated at 6°C. Values in % AR.

Soil I/6°C (Senozan) Pattern (% AR)	Duplicate	INCUBATION TIME IN DAYS						
		0	**0.5**	**2**	**7**	**14**	**30**	**100**
Parent	A	92.7	63.5	33.7	14.8	8.6	7.4	2.2
	B	95.4	65.7	40.0	10.2	9.0	6.5	4.0
	mean	**94.0**	**64.6**	**36.8**	**12.5**	**8.8**	**6.9**	**3.1**
M4	A	*	*	1.3	1.9	0.8	0.8	0.8
	B	*	*	0.9	1.3	2.3	0.6	0.6
	mean	*****	*****	**2.2**	**1.6**	**1.6**	**0.7**	**0.7**
M7	A	*	0.8	1.5	2.7	3.1	1.6	1.1
	B	*	1.0	1.1	1.7	3.2	2.0	2.5
	mean	*****	**0.9**	**1.3**	**2.2**	**3.2**	**1.8**	**1.8**
M10	A	*	0.8	1.6	5.1	*	*	*
	B	*	0.4	1.1	3.9	*	*	*
	mean	*****	**0.6**	**1.3**	**4.5**	*****	*****	*****
M21	A	*	3.0	3.7	0.7	*	1.8	0.9
	B	*	2.1	1.1	1.0	*	0.2	1.0
	mean	*****	**2.5**	**2.4**	**0.9**	*****	**1.0**	**1.0**
M22	A	*	*	3.0	2.7	*	2.2	1.0
	B	*	2.2	5.0	2.8	*	0.9	0.8
	mean	*****	**1.1**	**4.0**	**2.8**	*****	**1.5**	**0.9**
M23	A	*	*	*	*	*	*	3.0
	B	*	*	*	*	*	*	1.6
	mean	*****	*****	*****	*****	*****	*****	**2.3**
M24	A	*	*	*	1.3	1.1	*	0.6
	B	*	1.5	*	3.5	1.3	1.2	0.2
	mean	*****	**0.7**	*****	**2.4**	**1.2**	**0.6**	**0.4**
M28	A	*	1.7	2.6	*	2.0	*	0.4
	B	*	0.2	2.6	1.5	1.1	1.4	0.6
	mean	*****	**1.0**	**2.6**	**0.8**	**1.5**	**0.7**	**0.5**
M32	A	*	*	0.4	4.7	4.8	4.7	1.7
	B	*	*	0.9	4.7	4.8	4.8	3.5
	mean	*****	*****	**0.7**	**4.7**	**4.8**	**4.8**	**2.6**
Non-Extractables	**mean**	**5.6**	**19.2**	**37.0**	**47.3**	**49.6**	**46.1**	**41.4**
$^{14}CO_2$	**mean**	**n.p.**	**1.0**	**5.6**	**13.7**	**18.2**	**24.8**	**33.7**

* not detected or below limit of quantification n.p.: not performed

Minor metabolites (m) below 2% AR and their maximum amounts:

m	% AR	m	% AR	m	% AR	m	% AR
m1	0.7	m6	1.8	m13	0.3	m26	0.6
m2	0.7	m9*	1.3	m17	1.4	m27	0.2
m3	1.4	m11	0.4	m21	1.0	m30	0.1
m5	1.4	m12	1.4	m25	1.0	m31	0.3

* Characterised by HPLC and TLC using the available reference item to be N-(n-octyl)oxamic acid

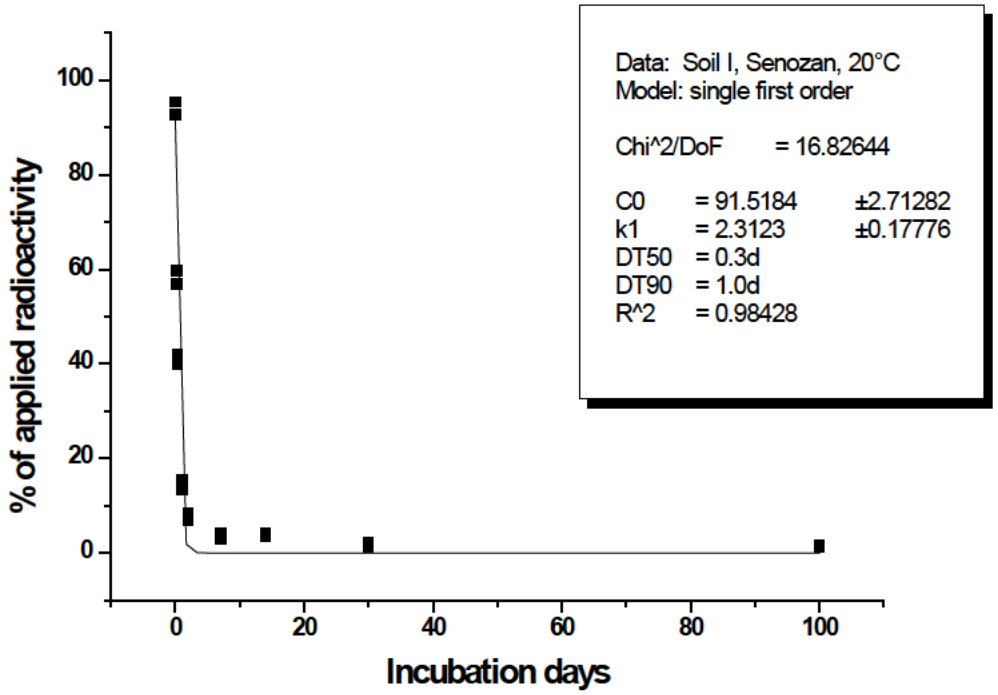
Table A7_2_2_1-11: Pattern of degradation and formation of metabolites in soil I (Senozan) treated with ^{14}C-OIT and incubated at 20°C under sterile conditions. Values in % AR (top) and mg OIT equivalents/kg dry soil (bottom).

Soil I/sterile (Senozan) Pattern (% AR)	Duplicate	INCUBATION TIME IN DAYS		
		2	5	12
Parent	A	97.4	99.2	99.6
	B	101.5	100.9	98.7
	mean	**99.4**	**100.1**	**99.1**
Non-Extractables	**mean**	**0.9**	**1.0**	**2.0**
$^{14}CO_2$	**mean**	**<0.1**	**<0.1**	**<0.1**

Soil I/sterile (Senozan) Pattern (mg/kg)	Duplicate	INCUBATION TIME IN DAYS		
		2	5	12
Parent	A	0.696	0.709	0.711
	B	0.725	0.721	0.705
	mean	**0.710**	**0.715**	**0.708**
Non-Extractables	**mean**	**0.006**	**0.007**	**0.014**
$^{14}CO_2$	**mean**	**<0.001**	**<0.001**	**<0.001**

Figure A7_1_2_2_1-1: Rate of degradation of ^{14}C-OIT from soils I and II incubated at 20°C.

Top: Soil I (Senozan) incubated at 20°C.
Bottom: Soil II (Speyer 2.2) incubated at 20°C.



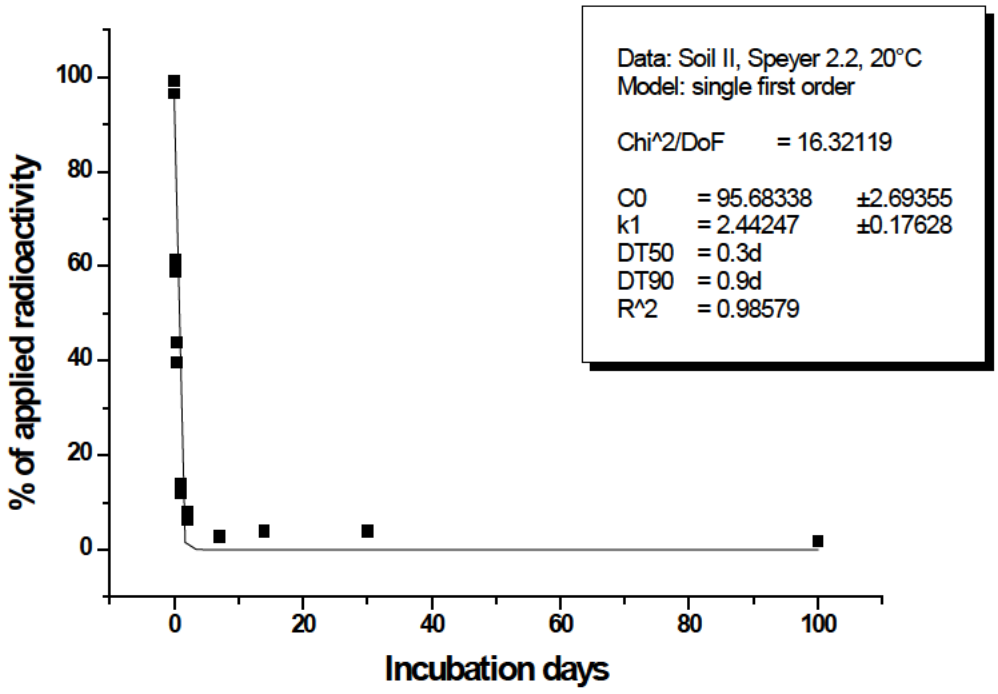
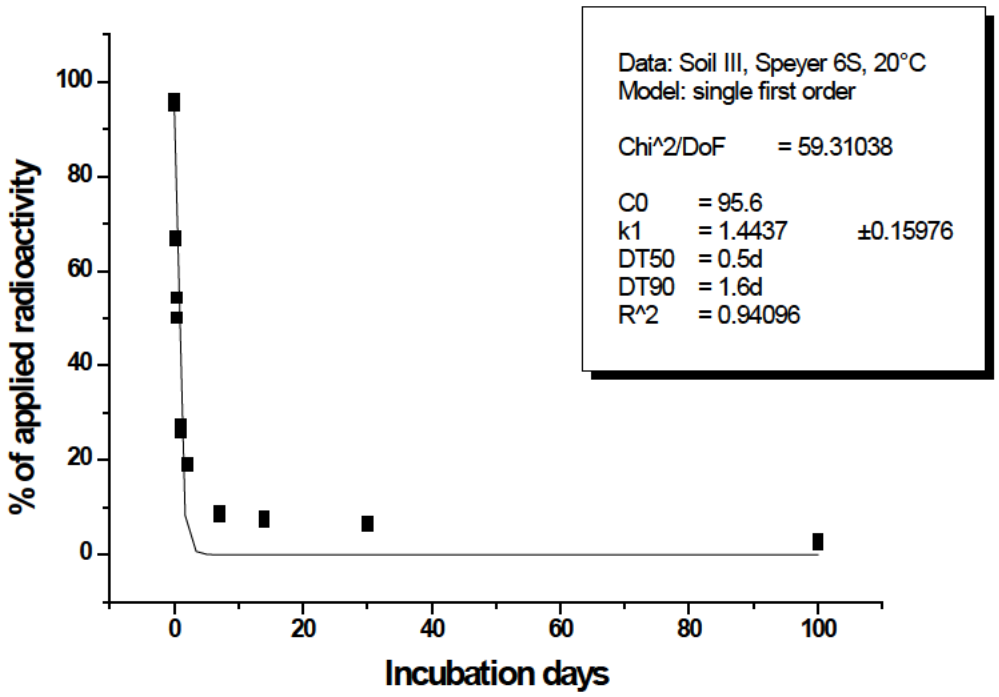


Figure A7_1_2_2_1-2: Rate of degradation of ^{14}C-OIT from soil III incubated at 20°C and soil I incubated at 6°C.

Top: Soil III (Speyer 6S) incubated at 20°C.
Bottom: Soil I (Senozan) incubated at 6°C.



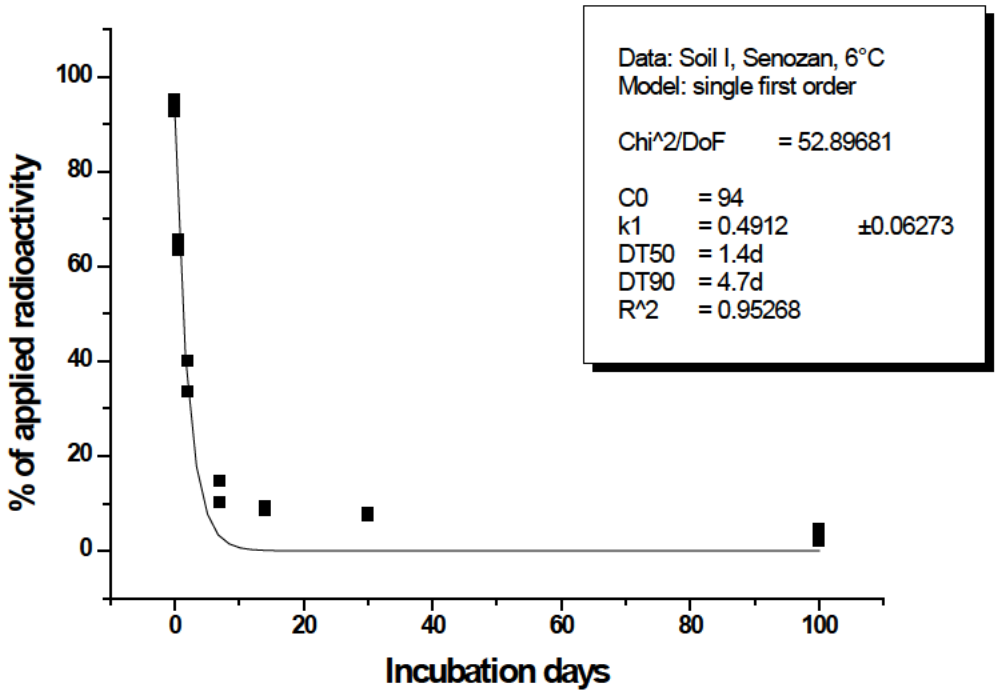


Figure A7_1_2_2_1-3: Proposed metabolic pathway of ^{14}C-OIT in soil incubated under aerobic non-sterile conditions.