European Commission

Combined Draft Renewal Assessment Report prepared according to Regulation (EC) N° 1107/2009
and
Proposal for Harmonised Classification and Labelling (CLH Report) according to Regulation (EC) N° 1272/2008

THYMOL;
5-methyl-2-(propan-2-yl) phenol

Volume 3 – B.8 (AS)

Rapporteur Member State: Spain
Co-Rapporteur Member State: Greece

Version History

When	What
July 2022	Initial RAR-RMS Spain
November 2022	DRAR after CoRMS and Applicant comments
February 2023	RAR updated after EFSA CoCh received on 24/01/2023

The RMS is the author of the Assessment Report. The Assessment Report is based on the validation by the RMS, and the verification during the EFSA peer-review process, of the information submitted by the Applicant in the dossier, including the Applicant's assessments provided in the summary dossier. As a consequence, data and information including assessments and conclusions, validated and verified by the RMS experts, may be taken from the applicant's (summary) dossier and included as such or adapted/modified by the RMS in the Assessment Report. For reasons of efficiency, the Assessment Report should include the information validated/verified by the RMS, without detailing which elements have been taken or modified from the Applicant's assessment. As the Applicant's summary dossier is published, the experts, interested parties, and the public may compare both documents for getting details on which elements of the Applicant's dossier have been validated/verified and which ones have been modified by the RMS. Nevertheless, the views and conclusions of the RMS should always be clearly and transparently reported; the conclusions from the applicant should be included as an Applicant's statement for every single study reported at study level; and the RMS should justify the final assessment for each endpoint in all cases, indicating in a clear way the Applicant's assessment and the RMS reasons for supporting or not the view of the Applicant.

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ENVIRONMENTAL FATE AND BEHAVIOUR

B. 8.1 FATE AND BEHAVIOUR IN SOIL

B.8.1.1 Route and rate of degradation in soil

B. 8.1.1.1 Aerobic degradation

1. Information on the study

Data point	CA 7.1.1.1/01 (also relevant under CA 7.1.2.1.1/01)
Report author	A. Jones
Report year	2015a
Report title	Thymol: Aerobic soil metabolism
Report No.	Envigo Study No. PIF0008
Document No.	
Guidelines followed in study	OECD: Guideline 307, Aerobic and Anaerobic Transformation in Soil, 2002
Deviations from current test guideline	None Unclear if extraction methods were sufficiently robust.
Previous evaluation	Yes, Confirmatory Data 2016
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Yes

2. Full summary

The route and rate of degradation of thymol were studied in four soils in the laboratory under aerobic conditions. The soils were selected to represent a range of textural characteristics, pH (5.6 - 7.8) and organic carbon contents (1.8 – 4.4 %). Soil samples were set up and allowed to acclimatise before being treated with [^{14}C]-thymol at a nominal concentration of 1.04 mg/kg, approximately equivalent to a use rate of 1040 g ai/ha. The samples were incubated under aerobic conditions in the dark at about 20°C at a moisture content equivalent to pF 2 for periods of up to 120 days after application. Combined soil extracts were analysed by HPLC with fraction collection.

The overall recoveries of applied radioactivity (AR) were in the range 90 – 110% AR. The variable recoveries of radioactivity may be due to the inhomogeneous nature of the non-extractable residue, and the difficulties in accurately quantifying this fraction.

The proportion of radioactivity extracted from soil decreased with time with a corresponding increase in the levels of non-extractable radioactivity and $^{14}CO_2$ evolution. Extractable radioactivity declined with time from mean values of 80.8 – 91.8% AR in each soil at zero-time to mean values of 11.4 – 25.5% AR after 3 days incubation and then remained at a similar level throughout the incubation period. Non-extractable radioactivity accounted for mean values of 72.4 – 78.2% AR after 3 days and accounted for 62.0 – 78.2% AR after 120 days. Volatile radioactivity, characterised as $^{14}CO_2$, represented 11.9 – 28.2% AR after 120 days. Thymol declined rapidly in each soil type. The DT_{50} and DT_{90} values were 0.6-0.8 days and 1.8-2.6 days respectively.

Thymol degraded to five minor unidentified degradates (none of which individually accounted for >10% AR), bound residues and carbon dioxide.

I. MATERIALS AND METHODS

A MATERIALS

Test material 1 (used in standard rate tests)

Name:	[isopropyl-methyl-^{14}C]-Thymol
Description:	In acetonitrile solution
Lot/Batch no.:	370-031-0507-A-20140728-DRE
Radiochemical Purity:	Radiochemical purity 99.4%
Specific activity:	12.1 MBq/mg.
Storage :	-20°C

Non-labelled material (used in all tests)

Name:	Thymol, 2-isopropyl-5-methylphenol
Description:	Solid
Lot/Batch no.:	121700
Purity:	99.4%
CAS #:	
Storage/stability:	4°C in the dark

Four UK soil were obtained and used within 3 months of collection. Calke (sandy loam), Brierlow (clay loam/silty clay loam), Empingham (clay) and Ingleby (loamy sand) were supplied by LRA Labsoil, UK. Prior to use the soils were passed through a 2 mm sieve.

Table 8.1.1.1-1: Characterisation details of the test soils used to investigate the route of aerobic degradation of thymol

Soil parameter	**Value**			
Source	Brierlow, UK	Ingleby, UK	Empingham, UK	Calke, UK
HLS batch number	220914D	220914B	220914A	220914C
Particle size distribution, UK[1] % Sand (63 µm to 2 mm) % Silt (2 µm to 63 µm) % Clay (< 0.002µm)	 31 52 17	 89 5 6	 22 32 46	 71 16 13
Textural classification, UK	Silt loam	Sand	Clay	Sandy loam
USDA classification	Silt loam	Loamy sand	Silt clay loam	Sandy loam
Soil pH (water)	6.0	5.6	7.8	6.1
Soil pH (0.01M $CaCl_2$)	5.3	4.7	7.1	5.1
Organic carbon content, (%)	3.5	1.8	4.4	3.4
Cation exchange capacity, (meq/100 g soil)	18.6	6.5	35.5	15.1
Water content (% dry weight)	25.8	5.5	19.4	13.8
Water content at pF 2 (% dry weight)	46.0	15.0	44.5	31.0
Microbial biomass (mg C/kg soil)				
Start of incubation	445.97	1677.97	3019.17	1134.37
End of incubation	203.97	217.97	3654.77	530.37
Solvent treated	38.77	56.77	2432.37	211.57

B STUDY DESIGN

Experimental conditions

Glass test vessels were filled with 50 g (dry weight equivalent) of soil and distilled water was added to adjust the soil to a water content equal to that at pF2. Eighteen vessels were established for each soil type. Samples established for treatment with [^{14}C]-thymol were incorporated into separate flow-through systems arranged in a series. At intervals, the vessels were weighed and water added as necessary to maintain the water content equal to that at pF 2. All test systems were maintained in darkness at 20 ± 2°C in a temperature-controlled room.

The [^{14}C]-thymol was radiodiluted for application. The application solution was prepared by combining a portion of non-radiolabelled thymol (3.55 mg) with a portion (1.51 mg, 18.3 MBq) of [^{14}C]-thymol and diluting to volume (20 mL) with acetonitrile. The concentration of the application solution was 0.253 mg/mL and the radiodiluted specific activity was 217000dpm/µg.

Aliquots (0.206 mL) of this application solution were applied to the surface of the soil samples. The amount of thymol applied per sample was 52.2 µg. This equated to an application rate of 1.03 mg/kg. Following application each soil vessel was weighed and distilled water added as necessary to maintain the moisture content at that at pF 2. Each vessel (with the exception of those taken for zero-time analysis) was then reincorporated into its respective flow-through system. For the

main experiment, duplicate samples of each soil type were taken for analysis immediately after application, and after 3, 7, 14, 59 and 120 days of incubation. Trapping solutions were taken for analysis and replaced with fresh solutions at each sampling interval and additionally at 28, 42, 74, 88 and 105 days of incubation.

Analysis

The soil samples were extracted twice with acetonitrile and once with acetonitrile:0.1M Hydrochloric acid (8:2 v/v) (approximately 150 mL) by sonication for 5 minutes followed by shaking for 30 minutes. The extract solution and soil solids were separated by centrifugation and duplicate aliquots (1 mL) of each extract solution were taken for liquid scintillation counting (LSC).

The total weight of the soil debris remaining after extraction was measured and three weighed portions (approximately 0.05 – 0.1 g) were taken for combustion and radioassay. Extraction of the soil began on the day of sampling. Soil extracts were stored at <-15°C prior to analysis.

The volumes of trapping solutions were measured and duplicate 1 mL aliquots were taken for analysis using LSC.

The non-extractable radioactivity in one representative samples of each soil type (where this represented greater than 10% of the applied radioactivity) was characterised using an acid/base fractionation procedure.

Characterisation of volatile radioactivity was performed by pooling the potassium hydroxide trapping solutions for selected samples. $^{14}CO_2$ was confirmed using sodium carbonate and barium chloride added to the above pooled trapping solutions. An absence of radioactivity in solution was indicative of precipitation of $^{14}CO_2$ as barium carbonate.

High Performance Liquid Chromatography (HPLC)
HPLC was carried out with UV and radioactivity detection. For each sample, the extract solutions were combined in proportion to their total weights. The samples were analysed directly by HPLC with fraction collection.

Table 8.1.1.1-2: HPLC method for soil samples

HPLC System			
Column	Prodigy ODS (25 cm x 4.6 mm)		
Mobile phases	Eluent A: 0.1% formic acid (aqueous) Eluent B: 0.1% formic acid in acetonitrile		
	Time (min)	% Eluent A	% Eluent B
	0	95	5
	10	95	5
	30	0	100
	40	0	100
	41	95	5
	50	95	5
Flow rate (mL/min)	1		

Liquid Chromatography – mass spectrometry (LC-MS)
LC-MS was used for the identification of unknown component B. however, due to the very low level of this unknown component is was not possible to elucidate the structure using LC-MS.

II. RESULTS

A MASS BALANCE

Total recoveries of radioactivity lay in the range 90.9 – 109.6% applied radioactivity (AR), except on four occasions where recoveries were outside this range (85.1-113.0%). The data suggests that the variable recoveries may be due to difficulties accurately quantifying the high levels of bound radioactivity. Due to the volatile nature of the test substance the solid residues were not dried prior to combustion. This made it difficult (after several attempts) to obtain a homogenous sample for analysis which led to high variance in the non-extractable residue combustions.
Extractable radioactivity declined with time from mean values of 80.8 – 91.8% AR in each soil at zero-time to mean values of 11.4 – 25.5% AR after 3 days, and then remained at a similar level throughout the incubation period. Non-extractable radioactivity increased to mean values of 72.4 – 78.8% AR after 3 days and accounted for 62.0 – 78.2% AR after 120 days incubation. Non extractable residues were mainly associated with the humin fraction (33.3 – 46.1% AR). Volatile radioactivity increased rapidly to means of 12.7 – 28.1% AR after 120 days.

Table 8.1.1.1-3: Distribution and recovery of radioactivity in Calke soil treated with [^{14}C]-thymol

Days	Ext 1	Ext 2	Ext 3	Total Extractable	NER	Ethyl Digol trap	KOH traps	Total
0	76.1	2.2	2.3	80.6	18.0	NS	NS	98.6
0	75.3	2.8	2.9	81.0	22.0	NS	NS	103.0
3	9.5	2.5	3.7	15.7	88.6	<0.1	0.7	105.0
3	8.9	2.1	2.9	13.9	67.2	ND	13.7	94.8
7	11.4	2.9	4.5	18.8	72.8	<0.1	3.7	95.3
7	10.7	2.9	4.1	17.7	71.0	ND	3.5	92.2
14	11.9	2.2	4.9	19.0	76.1	<0.1	5.4	100.5
14	11.6	2.2	4.7	18.5	71.2	ND	7.6	97.3
59	8.4	4.4	6.8	19.6	74.8	ND	12.0	106.4
59	8.5	5.2	6.4	20.1	64.8	ND	11.5	96.4
120	10.4	3.1	5.1	18.6	72.2	ND	16.9	107.7
120	9.7	3.1	6.0	18.8	73.6	ND	15.2	107.6

NS not sampled; NER non-extractable residues

Table 8.1.1.1-4: Distribution and recovery of radioactivity in Ingleby soil treated with [^{14}C]-thymol

Days	Ext 1	Ext 2	Ext 3	Total Extractable	NER	Ethyl Digol trap	KOH traps	Total
0	85.2	4.6	3.0	92.8	9.5	NS	NS	102.3
0	83.0	4.8	3.0	90.8	11.8	NS	NS	102.6
3	15.2	3.2	7.1	25.5	79.9	<0.1	2.4	107.8
3	15.3	2.8	7.3	25.4	76.4	ND	2.9	104.7
7	14.7	3.4	8.8	26.9	76.1	<0.1	3.5	106.5
7	15.0	2.9	8.1	26.0	71.1	<0.1	3.7	100.8
14	15.6	3.5	6.4	25.5	78.6	ND	4.7	108.8
14	13.8	2.1	8.4	24.3	77.6	ND	5.0	106.9
59	13.3	4.2	9.4	26.9	67.3	ND	11.8	106.0
59	14.5	3.4	9.8	27.7	54.5	ND	11.1	93.3
120	14.0	2.5	8.5	24.7	57.7	ND	13.5	95.9
120	12.2	2.4	10.2	24.8	71.0	ND	11.9	107.7

NS not sampled; NER non-extractable residues

Table 8.1.1.1-5: Distribution and recovery of radioactivity in Brierlow soil treated with [^{14}C]-thymol

Days	Ext 1	Ext 2	Ext 3	Total Extractable	NER	Ethyl Digol trap	KOH traps	Total
0	71.0	10.9	1.9	83.8	17.8	NS	NS	101.6
0	70.8	11.7	2.1	84.6	17.9	NS	NS	102.5
3	8.3	4.2	2.8	15.3	79.0	ND	15.3	109.6
3	7.8	3.2	2.4	13.4	65.8	ND	20.2	99.4
7	8.0	3.5	3.0	14.5	61.4	ND	15.0	90.9
7	8.0	3.8	2.7	14.5	65.5	ND	18.1	98.1
14	7.2	2.3	2.7	12.2	71.7	ND	23.9	107.8
14	8.6	1.8	2.6	13.0	68.4	ND	19.7	101.1
59	4.6	3.7	3.7	12.0	53.0	ND	28.7	93.7
59	4.9	3.3	3.9	12.1	72.9	ND	28.0	113.0
120	6.8	2.3	2.9	12.0	54.8	ND	27.9	94.7
120	6.3	2.0	3.0	11.3	69.2	ND	28.2	108.7

NS not sampled; NER non-extractable residues

Table 8.1.1.1-6: Distribution and recovery of radioactivity in Empingham soil treated with [^{14}C]-thymol

Days	Ext 1	Ext 2	Ext 3	Total Extractable	NER	Ethyl Digol trap	KOH traps	Total
0	82.4	6.2	1.5	90.1	12.5	NS	NS	102.6

0	83.9	5.2	1.4	90.5	11.7	NS	NS	102.2
3	7.5	2.3	2.1	11.9	73.3	ND	9.0	94.2
3	7.0	1.9	1.9	10.8	83.1	ND	12.1	106.0
7	7.4	2.7	3.3	13.4	62.6	ND	9.1	85.1
7	6.8	2.2	2.9	11.9	74.7	ND	10.4	97.0
14	8.5	2.2	2.6	13.3	66.9	ND	11.6	91.8
14	7.1	1.8	2.3	11.2	64.3	ND	11.5	87.0
59	4.1	2.0	2.6	8.7	83.9	ND	17.8	110.4
59	4.5	1.9	2.5	8.9	73.5	ND	19.6	102.0
120	4.5	1.2	2.1	7.8	75.9	ND	17.3	101.1
120	4.4	1.3	2.1	7.8	80.4	ND	17.1	105.3

NS not sampled; NER non-extractable residues

B CHARACTERISATION OF RADIOACTIVITY

The non-extractable radioactivity was further characterised in selected samples where this accounted for more than 10% applied radioactivity. Non-extractable radioactivity was shown to be distributed throughout the humic acid, fulvic acid and humin fractions.

Table 8.1.1.1-7: Characterisation of non-extractable radioactivity

Days	Vessel number	Soil	Total NER	NER associated with:		
				Fulvic acid	Humic acid	Humin
14	CA07	Calke	76.1	11.4	19.7	45.0
14	IN07	Ingleby	78.6	13.4	31.8	33.3
14	BR07	Brierlow	71.7	12.2	17.4	42.1
14	EM07	Empingham	66.9	8.7	12.1	46.1

NER non-extractable residues

Volatile radioactivity increased to means of 12.7 – 28.1% AR after 120 days. Radioactivity recovered in the potassium hydroxide trapping solution was confirmed as $^{14}CO_2$.

Thymol declined rapidly from means of 74.4 – 85.7% AR at Day 0 to 2.2 – 6.1% AR after 3 days. In addition to thymol, up to five minor unidentified degradates were detected. In the Ingleby soil unknown component B accounted for >5.0% at two consecutive time points (Day 3 and 7) and accounted for a maximum of 5.6% AR after 3 days incubation. Unknown component B was further investigated by LC-MS however due to the low concentrations of the degradate present in the samples structural identification was not possible. All other unknown components accounted for ≤ 4.8 % AR at all timepoints.

Table 8.1.1.1-8: Proportions of radioactivity in Calke soil treated with [^{14}C]-thymol

Days	Thymol	Unknown A	Unknown B	Unknown C	Unknown D	Unknown E	Others
0	74.6	0.7	2.1	1.0	0.5	0.6	1.0
0	74.2	1.3	2.3	1.4	0.6	0.5	0.7
3	3.2	1.0	3.2	3.0	2.2	1.9	1.2
3	3.5	0.9	3.0	2.5	2.0	1.2	0.9
7	4.2	1.4	3.7	3.5	2.4	2.4	1.1
7	3.9	1.3	3.5	3.2	2.3	2.2	1.3
14	3.7	1.7	2.7	4.1	2.6	2.5	1.6
14	3.5	1.6	3.2	3.6	2.7	2.5	1.5
59	4.2	2.2	3.4	3.7	2.4	2.2	1.5
59	4.1	2.3	3.6	3.7	2.5	2.1	1.7
120	4.2	2.2	3.3	3.3	2.3	2.0	1.2
120	4.5	2.3	3.3	3.3	2.1	1.8	1.4

Table 8.1.1.1-9: Proportions of radioactivity in Ingleby soil treated with [^{14}C]-thymol

Days	Thymol	Unknown A	Unknown B	Unknown C	Unknown D	Unknown E	Others
0	87.2	0.9	2.1	1.1	0.5	0.4	0.6
0	83.6	0.7	3.2	1.4	0.5	0.5	0.9
3	6.4	2.5	5.6	3.1	2.9	3.1	1.9
3	5.8	2.8	5.1	4.4	3.4	2.3	1.6
7	6.2	3.0	5.2	4.2	3.7	2.6	2.0
7	5.9	2.7	5.1	4.6	3.6	2.5	1.6
14	5.5	3.1	3.2	4.4	3.0	2.4	1.9
14	4.9	3.2	4.1	4.3	3.5	2.7	1.7
59	6.0	3.7	4.7	4.3	3.1	2.6	2.6
59	6.1	4.0	4.8	4.7	3.1	2.8	2.2
120	5.5	4.0	4.2	3.9	2.7	2.5	2.0
120	5.9	4.1	4.1	3.5	2.7	2.5	2.1

Table 8.1.1.1-10: Proportions of radioactivity in Brierlow soil treated with [^{14}C]-thymol

Days	Thymol	Unknown A	Unknown B	Unknown C	Unknown D	Unknown E	Others
0	76.8	0.8	2.7	1.1	0.6	0.5	1.3
0	78.1	0.8	2.1	1.2	0.7	0.5	1.2
3	4.0	0.7	3.9	2.6	2.0	1.3	0.8
3	3.4	0.9	3.2	1.2	1.7	2.3	0.7
7	3.6	0.8	3.1	1.4	2.1	2.5	1.0
7	3.4	0.8	3.1	2.6	2.1	1.5	1.1
14	2.5	0.8	2.1	2.3	1.8	1.6	1.2
14	2.7	0.9	2.5	2.3	2.0	1.6	1.0
59	2.4	1.1	2.1	2.0	1.8	1.7	0.9
59	2.5	1.2	2.2	2.1	1.5	1.8	0.9
120	2.2	1.4	2.0	1.9	1.7	1.6	1.1
120	2.2	1.1	1.7	2.0	1.7	1.6	0.9

Table 8.1.1.1-11: Proportions of radioactivity in Empingham soil treated with [^{14}C]-thymol

Days	Thymol	Unknown A	Unknown B	Unknown C	Unknown D	Unknown E	Others
0	85.1	0.5	2.0	1.0	0.6	0.5	0.5
0	86.3	0.4	2.3	1.0	0.4	0.2	0.0
3	2.3	0.5	2.7	2.4	0.9	1.9	1.1
3	2.1	0.4	2.4	2.3	1.8	1.2	0.7
7	2.9	0.7	2.9	1.4	2.2	2.2	1.1
7	2.1	0.5	2.3	2.3	2.1	1.6	1.0
14	2.5	0.8	2.8	2.7	2.0	1.7	0.9
14	2.1	0.5	2.2	2.3	1.7	1.6	0.8

C TRANSFORMATION PATHWAY

Thymol degraded rapidly to four minor unidentified degradates, bound residues and carbon dioxide.
The proposed degradation pathway is shown below in figure 8.1.1.1-1

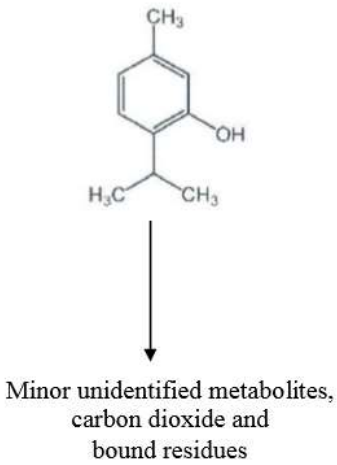


Figure 8.1.1.1-1: Degradation pathway

D DEGRADATION KINETICS

The decline of thymol in soil was modelled using the single first order model (SFO).
Modelled DT_{50} and DT_{90} values ranged from 0.6 – 0.8 days and 1.8 – 2.6 days, respectively.

Table 8.1.1.1-12: Kinetic data for the decline of thymol in aerobic soil

Soil	Kinetic model	DT_{50} (days)	DT_{90} (days)	Visual goodness-of-fit	Chi^2
Calke	SFO	0.6	2.1	Good[a]	13.5
Ingleby	SFO	0.8	2.6	Good[a]	17.7
Brierlow	SFO	0.6	2.1	Good[a]	9.5
Empingham	SFO	0.6	1.8	Good[a]	5.3

SFO Simple first order
[a] the visual fit was based on 2 sampling intervals only
NA insufficient data points to calculate Chi^2 value

III. CONCLUSION

Thymol degraded in aerobic soils at 20°C and pF2 with a DT_{50} of less than one day. Thymol degraded rapidly to five minor unidentified degradates (none of which individually accounted for >10% AR at a single timepoint or >5% AR at two consecutive timepoints), bound residues and carbon dioxide.

3. Assessment and Conclusion

Assessment and conclusion by applicant:

This study has been previously evaluated and accepted by the RMS. Nevertheless, the following comments were made:

- No samples were sterilised before treatment, which precludes characterisation by abiotic processes.
- It is unclear if extraction methods were sufficiently robust to yield higher extractable recoveries.

Given the very rapid degradation, it is considered that the deviations listed do not significantly alter the results of the study. The RMS stated that repeating the study may not yield significantly improved data. Calculated DT90s will be less than 2 days and there were no metabolites reported at >10%, >5% at 2 consecutive time points, or increasing at the study end.

Assessment and conclusion by RMS:

This study was previously evaluated and accepted in the thymol confirmatory data (January 2017).
Jones, 2015a was submitted to address the confirmatory data requirement on the groundwater exposure assessment for thymol since with default values, used in DAR 2011, the regulatory limit of 0.1 µg/L was exceeded in all the scenarios.
The route and rate of degradation of thymol were studied in four soils in the laboratory under aerobic conditions. Soil samples were treated with [^{14}C]-thymol at a nominal concentration of 1.04 mg/kg, approximately equivalent to a use rate of 1040 g ai/ha. The samples were incubated under aerobic conditions in the dark at about 20ºC at a moisture content equivalent to pF 2 for periods of up to 120 days after application.
Some deviations were found in the evaluation of the confirmatory data. The overall recoveries of applied radioactivity (AR) were in the range 85.1 – 113.0 % AR, which falls outside of the recommended range of 90 – 110 % for this kind of study. The explanations provided in the study report was that the variable recoveries of radioactivity were due to the inhomogeneous nature of the non-extractable residue and the difficulties in accurately quantifying this fraction.
Large amounts of non-extractable residues were found. RMS (UK) noted that the extraction method could not be sufficiently robust to yield higher extractable recoveries. Deviations were also observed regarding to the soil selection. The organic carbon content for Brierlow, Empingham, and Calke soils exceed the limit value of 2.5% (OCDE 307). In addition, the microbial biomass content of soils Brierlow, Empingham, and Calke soils was lower than the limit value of 1% OC. Furthermore, sterilized soil samples were not analysed to study the abiotic degradation.
All in all, these deviations were not considered to significantly alter the results of the study because the very rapid degradation of thymol.
Regarding to characterization of the degradation pathway, thymol degraded rapidly to five minors unidentified degradates, bound residues and carbon dioxide. Where the unextracted residue represented more than 10 % of the AR, radioactivity was further characterised using an acid/base fractionation procedure.
One of the degradates, the Unknown B in Ingleby soil, accounted for >5.0 % at two consecutive time points (Day 3 and 7) and accounted for a maximum of 5.6 % AR after 3 days incubation. Unknown component B was further investigated by LC-MS. However, due to the low metabolite concentrations, structural identification was not possible. This metabolite was found in lower proportions in the others three soils and was considered as transient in nature. One possible explanation given in the confirmatory data was that the low microbial content of this soil could explain the levels seen just over 5% at days 3 and 7.

The decline of thymol in soil was modelled using the single first order model (SFO). DT50 and DT90 values ranged from 0.6 – 0.8 days and 1.8 – 2.6 days, respectively. The kinetic evaluation has been included under the point B.8.1.2.1.1. Aerobic degradation of the active substance.

Outcome and conclusion of the study:

Despite the reported deviations, the study is considered acceptable.
Thymol degraded in aerobic soils at 20ºC and pF2 with a DT50 of less than one day. Thymol degraded rapidly to five minors unidentified degradates, bound residues and carbon dioxide.

B.8.1.1.2 Anaerobic degradation

No data has been submitted. Based on the application timing and the fast degradation rate of thymol it is unlikely that thymol will be present when anaerobic conditions may occur.

B.8.1.1.3 Soil photolysis

No data has been submitted. The following justification has been submitted by applicant: based on the fast degradation rate of thymol it is unlikely that thymol will be present long enough for photolysis to occur.

However, according to section 7.1.1.3 of Regulation (EU) No. 283/2013 a soil photolysis study shall be submitted unless the applicant shows that deposition of the active substance on the soil surface is unlikely to occur or that photolysis is not expected to contribute significantly to the degradation of the active substance in soil for example due to low light absorbance of the active substance.

Therefore, RMS has identified a data gap for a soil photolysis study.

The following comment was added by applicant during the co-RMS-applicant´s initial revision:

Photolysis is not expected to contribute significantly to the degradation of thymol as Thymol degraded rapidly to five minor unidentified degradates, bound residues and carbon dioxide under aerobic conditions with a soil biodegradation DT50 of 1 day and the aqueous photolysis gave a DT50 of 33 days. Biotic degradation is the most significant route.

B.8.1.2 Rate of degradation in soil

B.8.1.2.1 Laboratory studies

B.8.1.2.1.1 Aerobic degradation of the active substance

1. **Information on the study**

Data point	CA 7.1.2.1.1/01 (also relevant under CA 7.1.1.1/01)
Report author	A. Jones
Report year	2015a
Report title	Thymol: Aerobic soil metabolism
Report No.	Envigo Study No. PIF0008
Document No.	
Guidelines followed in study	OECD: Guideline 307, Aerobic and Anaerobic Transformation in Soil, 2002
Deviations from current test guideline	None
Previous evaluation	Yes, Confirmatory Data 2016
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Yes

2. **Full summary**

Please refer to the summary provided under CA 7.1.1.1/01.

The declines of thymol in aerobic soils were modelled using the Single First Order (SFO) kinetic model. The software ModelMaker (version 4.0, ModelKinetix, Oxford, UK) was used, employing default Marquardt optimisation and Runge-Kutta integration settings. An assessment of goodness-of-fit was made by visual inspection and by Chi-squared statistical analysis. Both replicate values were used in the kinetic modelling. For thymol the radiochemical purity value was used in lieu of the measured zero-time values.

Table 8.1.2.1.1-1: Kinetic data for the decline of thymol in soil from the applicant (ModelMaker, version 4.0, ModelKinetix, Oxford, UK).

Soil	Parameter	Value	Standard error	Visual fit	Chi^2	DT50 (days)	DT90 (days)
Calke	K M0	1.10333 96.9959	0.24458 2.55538	Bad[a]	13.5	0.6	2.1
Ingleby	K M0	0.899528 96.9864	0.187649 3.62096	Bad[a]	17.7	0.8	2.6
Brierlow	K M0	1.07571 96.9983	0.152431 1.72196	Bad[a]	9.5	0.6	2.1
Empingham	K M0	1.25012 96.9995	0.210962 1.39566	Bad[a]	5.3	0.6	1.8

In the Student's t-test, the majority of values were significant at the 0.1% level

[a] the visual fit for DT50 and DT90 was based on 2 sampling intervals only

The visual analysis is shown below:

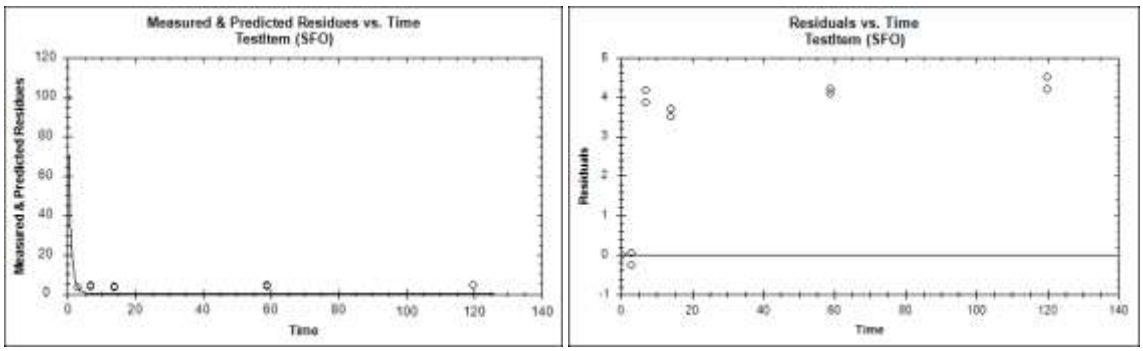


Figure 8.1.2.1.1-1: SFO curve for Calke soil.

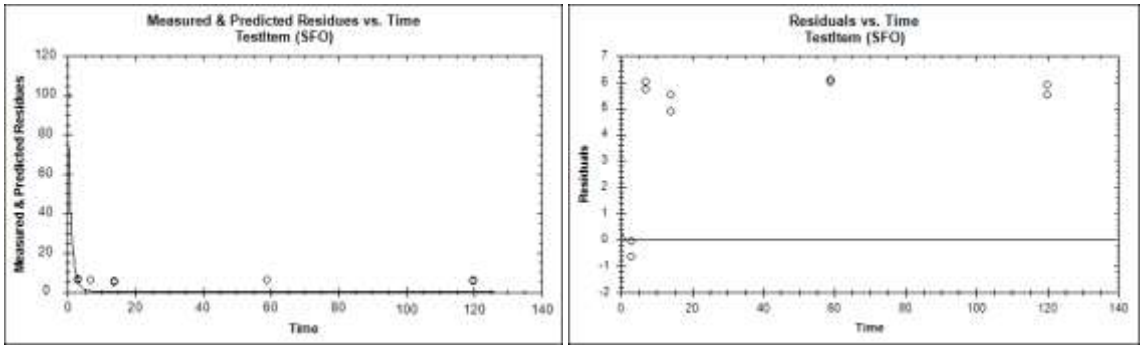


Figure 8.1.2.1.1-2: SFO curve for Ingleby soil.

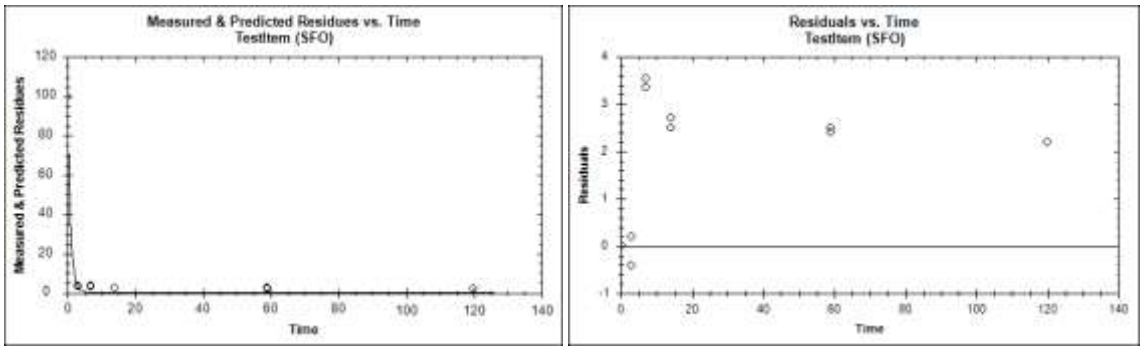


Figure 8.1.2.1.1-3: SFO curve for Brierlow soil.

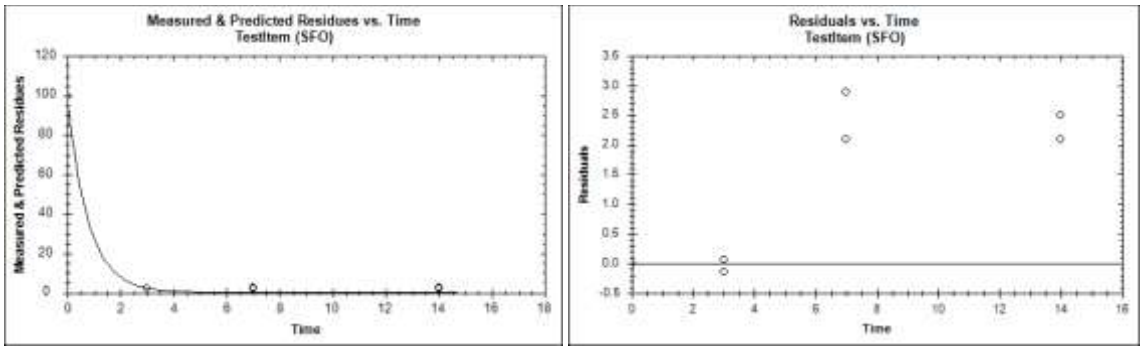


Figure 8.1.2.1.1-4: SFO curve for Empingham soil.

3. Assessment and conclusions

Further kinetic analysis on the Aerobic degradation was not performed due to the rapid degradation of the test substance and the worst-case DT_{50} value of 1 day is used for the risk assessment.

Assessment and conclusion by RMS:
The decline of thymol in soil was modelled using the single first order model (SFO). Since a significant decrease of thymol was observed at the first sampling points, the radiochemical purity value was used by applicant instead of the measured zero-time values. This is in line with the recommendations of Focus Kinetic guideline which states that for kinetic fitting, metabolite levels can be set to zero and their values summed as parent for the time zero sample.

DT50 and DT90 values ranged from 0.6 – 0.8 days and 1.8 – 2.6 days, respectively.

Outcome and conclusion of the study:
The study is considered acceptable.
This study confirms the rapid degradation of thymol. For modelling purposes, a conservative DT 50 of 1 day has been proposed to replace the previous default value of 30 days used in Annex I inclusion.

1. Information on the study

Data point	CA 7.1.2.1.1/02 (and CA 7.2.2.3/01)
Report author	D. Hu and J. Coats
Report year	2008
Report title	Evaluation of the environmental fate of thymol and phenethyl propionate in the laboratory
Report No.	NA
Document No.	Pest Management Science 64:775-779 (2008)
Guidelines followed in study	Not stated
Deviations from current test guideline	^{3}H used, only one soil, no microbial biomass stated, no trapping of CO_2, open water system
Previous evaluation	No
GLP/Officially recognised testing facilities	No
Acceptability/Reliability	2, supporting data

2. Full summary

ABSTRACT (copied from original literature)
BACKGROUND: The natural monoterpenoid pesticides thymol and phenethyl propionate (PEP) are used indoors and outdoors, but their fate in the environment has not been reported. In order better to understand their impact on the environment, water metabolism and soil metabolism studies were conducted with thymol and PEP at a concentration of 10 µg g^{-1} in water and in soil under laboratory conditions.

RESULTS: Dissipation half-lives of thymol and PEP were 16 and 5 days in water and 5 and 4 days in soil. 2-Phenylethanol and 2-(4-hydroxyphenyl)ethanol were detected as primary degradation products of PEP. Over time, a considerable volatilization loss of thymol, but not of phenethyl propionate, was found in the 1 month study under the experimental conditions used. Less than 6% of thymol and PEP were detected as bound residues, and less than 3% were mineralized during the 30 day study.

CONCLUSION: In order to maximize the pesticidal effect, more attention should be paid to the temperature for thymol than for PEP when they are being applied, owing to the high volatility of the former. Thymol and PEP pose low risks to the ecosystem because of their rapid dissipation and low bound residues in the environment.

I. MATERIALS AND METHODS
A MATERIALS
Test material

Name: (^{3}H)-Thymol

CAS number:	NS
Purity:	NS
Specific Activity:	27.7 mCi $mmol^{-1}$

Synthesized by PerkinElmer Life Sciences and Analytical Sciences (Boston, MA)

Test Conditions
Soil was collected at the Iowa State University Agronomy and Agricultural Engineering Research Farm near Ames, Iowa. The soil was classified as a sandy loam, a Nicollet–Webster complex with 1.6% organic matter, 60% sand, 22% silt and 18% clay, and pH 7.0. Soil (50 g) was kept in a 250 mL French square bottle, and soil moisture content was adjusted to 75% of −33 J kg^{-1} moisture. The temperature was maintained at 25 ± 2 ◦°C. Soil samples were treated with ^{3}H-thymol in 300 µL of acetone carrier at 10 µg g^{-1}. Thymol had a total radioactivity of 1.8 µCi applied in this study. There were four replications for each group. Bottles were sealed with perforated caps to maintain an aerobic condition. Polyurethane foam was suspended above the treated soils inside each bottle to capture volatile compounds. Polyurethane foam was changed biweekly. Anhydrous calcium sulfate (Drierite®; 1.5 g) was used to trap ^3H_2O in the air in the container. Drierite was changed when it turned pink. Soil samples were taken at pretreatment and 1, 3, 7, 14 and 30 days post-treatment, and were stored in the −20 °C freezer prior to analysis
The dissipation half-lives (DT_{50}) were calculated using a first-order dissipation model.

II. RESULTS

Thymol and related degradation products were quantified by a HPLC method coupled with LSC. Recoveries of ^{3}H-thymol from soil were more than 90%, as determined by spike-recovery experiments at the initial time point. All measured concentrations of thymol were corrected for their recovery in the study.
The dissipation half-live (DT_{50}) of thymol in soil were calculated using the first-order dissipation model
In soil, the DT_{50} of thymol was 5 days, and cumulative volatilization in 1 month reached 26%. The lower volatilization from soil compared with that from water is probably due to adsorption to soil colloids. The testing system of the soil metabolism study was not totally open as for the water metabolism study, which might also have reduced the evaporation of thymol from the soil. The overall mass balance ranged from 86 to 100% of the applied radioactivity. The evolved ^3H_2O remained consistently less than 1% throughout the study period, indicating that little mineralization occurred. The average radioactivity as bound residues remained below 3% in 1 month.

3. Conclusion

In conclusion, thymol and phenethyl propionate dissipate rapidly in water and in soil under aerobic conditions. The primary degradation products of phenethyl propionate are 2-phenyl ethanol and 2-(4- hydroxyphenyl)ethanol. Bound 3H residues formed in soil were not considerable. Rapid dissipation and low residues indicate that thymol and PEP pose little risk to the ecosystem. Thymol is much more volatile than PEP when applied to soil and water in the experimental period. When they are used as pesticides, volatilization may be a major route of dissipation for thymol.
In order to maximize the pesticidal effect, more attention should be paid to the temperature for thymol than for PEP when they are being applied, owing to the high volatility of the former. Thymol and PEP pose low risks to the ecosystem because of their rapid dissipation and low bound residues in the environment.

Assessment and conclusion
Reliability assessment
For full details and justification, please refer to Document K.
This paper confirms that thymol degrades rapidly in soil with moderate mineralisation, as shown in the study by Jones (2015).
Proposed category: 2 supporting data

Assessment and conclusion by RMS:
The fate of thymol and PEP in pond water and agronomic soil was investigated to determine the dissipation half-life and the identity and quantity of degradation products that form in water or soil. As a part of the environmental fate studies, volatilization, mineralization and bound residues in soil were also determined.
The study was carried out applying 10 m/kg of ^{3}H-Thymol in soil. The temperature was maintained at 25 ± 2 ◦C and the soil moisture was adjusted to 75% of -33 Jkg^{-1}. No information about microbial activity was included in the report. In this study little mineralization occurred and the average radioactivity as bound residues remained below 3% in 1 month which contrast with the results given by Jones, 2015a. Not degradation products were determined.
The DT50 of thymol was 5 days in soil, and cumulative volatilization in 1 month reached 26%.
Lower volatilization from soil was found compared what that from water. This was attributed to the adsorption of thymol to soil colloids but also to the study design (testing system of the soil metabolism study was not totally open as for the water metabolism study).

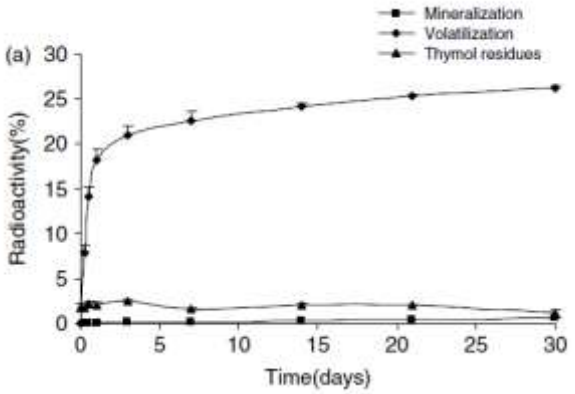


Figure 8.1.2.1.1-4: Dissipation profiles of thymol in soil (*n* = 4). Volatilization and mineralization values are cumulative.

Outcome and conclusion of the study:
Not GLP study.
Supplementary information

B.8.1.2.1.2 Aerobic degradation of metabolites, breakdown and reaction products
From the data generated under CA 7.1.1.1, in studies designed to investigate the route and rate of aerobic soil degradation of thymol, no major soil metabolites or degradation products are observed at levels exceeding 10% of the applied amount, nor at above 5% of the applied amount for two sequential measurements, nor above 5% of the applied amount at the final measurement. Therefore, the rate of aerobic degradation for any metabolites is not required and no further work has been performed.

B.8.1.2.1.3 Anaerobic degradation of the active substance
No data submitted.

B.8.1.2.1.4 Anaerobic degradation of metabolites, breakdown and reaction products
No data submitted

B.8.1.2.2 Field studies
Based on the laboratory DT_{90} of > 2 days, field studies are not triggered for thymol and therefore no data is submitted.

B.8.1.2.2.1 Soil dissipation studies
Not required.

B.8.1.2.2.2 Soil accumulation studies
Not required.

B.8.1.3 Adsorption and desorption in soil

B.8.1.3.1.Adsorption and desorption of the active substance

1. Information on the study

Data point	CA 7.1.3.1.1/01
Report author	A. Jones
Report year	2015b
Report title	Thymol: Adsorption/Desorption in Five Soils
Report No.	Envigo Study No. PIF0009
Document No.	
Guidelines followed in study	OECD: Guideline 106, Adsorption-Desorption Using a Batch Equilibrium Method (January 2000)
Deviations from current test guideline	In the preliminary experiment the mass balance and stability investigations were conducted in a single replicate only.
Previous evaluation	Yes, Confirmatory Data 2016
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	No

2. Full summary

The sorption properties of thymol were studied in five soils using the batch equilibrium method. The test soils included a range of textural classes, with pH values between 3.9 and 7.3 and organic carbon contents between 1.1 and 4.4%. Thymol radiolabelled with carbon-14 was used.

Preliminary experiments were conducted to ascertain (1) an appropriate soil-to-solution ratio, (2) the time required to achieve equilibrium between thymol in solution and that adsorbed to soil and (3) the extent of any binding of thymol to the glass test vessels.

Nominal initial solution concentrations of thymol in the range 0.05 – 5 mg/L in aqueous 0.01 M calcium chloride were studied. Soil-to-solution ratios of 1:10 w/v (Bromsgrove and Warsop) or 1:20 w/v (Elmton, Evesham 3, and Calke) were used. For the adsorption phase all five soils were equilibrated for 24 hours. Soil samples were then desorbed once with fresh calcium chloride solution for either 24 hours. All equilibrations were carried out at 25°C in the dark.

Concentrations of radioactivity in solution were measured directly by LSC and, from these results, the concentration of radioactivity in the soil was determined. Both values were corrected for actual proportions of thymol present in solution and soil exacts, as determined by chromatographic analysis.

Freundlich adsorption and desorption coefficients, these coefficients corrected for organic carbon content, and Freundlich exponents (1/n) were calculated. The adsorption / desorption values are shown in the table below.

Soil	Texture class	Soil pH ($CaCl_2$)	OC (%)	Adsorption			Desorption		
				K_f	K_{foc}	1/n	K_f	K_{foc}	1/n
Bromsgrove	Sandy loam	5.6	1.5	2.67	178	0.65	33.5	2230	0.82
Evesham 3	Clay loam	7.3	4.0	7.59	190	0.89	34.8	870	0.86
Elmton	Sandy Clay Loam	7.0	4.4	8.14	185	0.68	169	3840	0.87
Warsop	Sand	3.9	1.1	2.38	216	0.82	4.23	385	0.71
Calke	Sandy loam	5.1	3.4	6.15	181	0.78	128	3760	1.03

There was no correlation between soil pH and the degree of adsorption/desorption. For each soil, the desorption coefficient was higher than the adsorption coefficient, indicating some degree of irreversibility to the adsorption of thymol. Using the McCall scale to assess a chemical's potential mobility, the coefficient values indicate a medium potential for mobility of thymol in the soils tested.

I. MATERIALS AND METHODS

A MATERIALS

Test material 1 (used in standard rate tests)

Name:	[isopropyl-methyl-^{14}C]-thymol
Description:	In acetonitrile solution
Lot/Batch no.:	370-031-0507-A-20140728-DRE
Radiochemical Purity:	Radiochemical purity 99.3%
Specific activity:	12.1 MBq/mg.
Storage :	<-20°C

Non-labelled material (used in all tests)

Name:	Thymol, 2-Isopropyl-5-methylphenol
Description:	Solid
Lot/Batch no.:	121700
Purity:	99.4%
CAS #:	
Storage/stability:	4°C in the dark

Five UK soil were chosen to cover a wide range of agriculture soil types. Elmton (sandy clay loam) and Bromsgrove (sandy loam) soils were supplied by LandLook (Midlands), UK. Warsop (sand) and Calke (sandy loam) soils were supplied by Land Research Associates. Evesham 3 (clay loam) soil was acquired from within the site at Envigo. Prior to use the soils were air dried and passed through a 2 mm sieve.

Table 8.1.3.1.1-1: Characterisation details for the soil used to investigate the adsorption desorption of thymol

Soil parameter	**Value**				
Source	Bromsgrove	Evesham	Elmton	Warsop	Calke
HLS batch number	101213C	030413A	181213A	190713C	220914C
Particle size distribution, UK[1] % Sand (63 µm-2 mm) % Silt (2 µm-63 µm) % Clay (< 0.002µm)	 72 16 12	 11 27 62	 51 22 27	 90 6 4	 71* 16* 13*
Textural classification, UK	Sandy loam	Clay loam	Sandy clay loam	Sand	Sandy loam*
Soil pH (water)	6.4	7.9	7.7	4.9	6.1
Soil pH (0.01M $CaCl_2$)	5.6	7.3	7.0	3.9	5.1
Organic carbon (%)	1.5	4.0	4.4	1.1	3.4
Organic matter (%)	2.6	6.9	7.6	1.9	5.8
Cation exchange capacity,(meq/100 g soil)	9.1	45.0	26.9	2.8	15.1
Nitrogen (%)	0.13	0.41	0.41	0.07	0.31
Water content (% dry weight)	6.5	11.5	26.6	2.4	13.7

* USDA classification

B STUDY DESIGN

Experimental conditions

Preliminary experiments were initially performed to determine the soil-to-solution ratio and the adsorption equilibration time for the main isotherm experiment. As a result of these preliminary experiments, a soil-to-solution ratio of 1:20 w/v was selected for Elmton, Evesham 3, and Calke soils and a soil-to-solution ratio of 1:10 w/v was selected for Bromsgrove and Warsop. An adsorption equilibration time of 24 hours was selected for all soils.
For the main study and for each soil type, eleven vessels were prepared. The appropriate amounts (1 g (Elmton, Evesham 3, and Calke) or 2 g (Bromsgrove and Warsop) dry weight equivalent) of the soils were weighed into each tube. An aliquot of 0.01 M calcium chloride solution was added to achieve soil-to-solution ratios of 1:10 or 1:20 w/v. The tubes were pre-equilibrated by shaking in the dark at approximately 25ºC for approximately 21 hours.
Following pre-equilibration, an aliquot (20 µL) of the appropriate thymol treatment solution was added to duplicate vessels of each soil type. This gave initial nominal thymol concentrations of 5.0, 1.5, 0.5, 0.15 and 0.05 mg/L. A third replicate vessel was prepared at the highest concentration and used to assess any degradation of thymol during the adsorption process. The quantity of radioactivity applied to the tubes was accurately determined from triplicate aliquots (20 µL), which were taken from the treatment solutions into 10 mL volumetric flasks. These aliquots were diluted to 10 mL using acetonitrile. Duplicate aliquots (20 µL) were taken from each dilution for analysis by LSC.
After shaking in the dark at approximately 25ºC for 24 hours the soil and solution were separated by centrifugation to remove particles larger than 0.2 µm diameter from solution (45 minutes at 2500 rpm) and the supernatant removed. The volume of each supernatant was measured and duplicate aliquots (0.5 mL) taken for LSC. One replicate sample for each soil at the highest concentration was not taken through the subsequent desorption phase.
Following the adsorption phase, the solution that was removed was replaced with the same volume of fresh 0.01 M calcium chloride solution. Duplicate samples for each test substance concentration and soil type were removed after shaking in the dark at approximately 25ºC for 24 hours. The soil and solution were separated by centrifugation to remove particles larger than 0.2 µm diameter from solution (45 minutes at 2500 rpm) and the supernatant removed. The volume of each supernatant was measured and duplicate aliquots (1.0 mL) taken for LSC.
Samples treated at the highest concentration (one replicate removed after 24 hours adsorption and both replicates removed after 24 hours desorption), were further processed in order to obtain a mass balance and assess the stability of thymol. Soil samples were extracted once with acetonitrile (2 x ca 5 mL) by sonicating for 5 minutes then shaking for 30 minutes. The extracts and soil were separated by centrifugation. The procedure was repeated using acetonitrile:0.1 M hydrochloric acid (8:2 v/v). The supernatant was removed, the volume measured and duplicate aliquots (0.25 mL) were taken for LSC. Aqueous supernatants and soil extracts were pooled where appropriate and analysed by HPLC to determine the proportion of thymol in each sample. Soil residues were analysed by combustion and LSC.

Analysis

High performance Liquid chromatography (HPLC)
HPLC was carried out with UV and radioactivity detection. For each sample, the extract solutions were combined in proportion to their total weights. The samples were analysed directly by HPLC with fraction collection.

Table 8.1.3.1.1-2: HPLC method for soil samples

HPLC System			
Column	Prodigy ODS (25 cm x 4.6 mm)		
Mobile phases	Eluent A: 0.1% Formic acid (aqueous) Eluent B: 0.1% Formic acid in Acetonitrile		
	Time (min)	% Eluent A	% Eluent B
	0	95	5
	10	95	5
	30	0	100
	40	0	100
	41	95	5
	50	95	5
Flow rate (mL/min)	1		

II. RESULTS

A ADSORPTION

The mass balance of radioactivity was measured in one vessel of each soil type treated at the highest thymol concentration following the adsorption phase. Recoveries of radioactivity were in the range 88.8 – 95% of the amounts applied.

Table 8.1.3.1.1-3: Recovery of radioactivity after adsorption at an initial thymol concentration of 5.0 mg/L

Parameter/Soil	Bromsgrove	Evesham 3	Elmton	Warsop	Calke
Adsorption solution	60.6	57.9	56.4	72.3	40.9

Soil extracts	15.6	24.4	13.7	21.8	20.8
Non-extractable	14.2	8.4	18.7	0.9	32.7
Total Recovery	90.4	90.7	88.8	95.0	94.4

Thymol represented 91.1 – 99.9% sample radioactivity in the aqueous solution and 44.5 – 86.9% sample radioactivity in soil extracts, however significant amounts of applied radioactivity remained on the soil residues post extraction. This was probably due to the degradation of the Thymol to form degradates which bound to the soil. Thymol was readily extractable in the extraction solvents used therefore it is unlikely that the remaining radioactivity on the soil residues was thymol. The proportion of thymol in total soil (soil extracts and soil non-extractable residue) was calculated based on the amount of thymol extracted and quantified by HPLC. Thymol was therefore considered to represent 17.3 – 83.5% of total radioactivity in the soil.

Table 8.1.3.1.1-4: Proportions of thymol in aqueous and soil extracts after adsorption at an initial concentration of 5.0 mg/L

Parameter/Soil	Bromsgrove	Evesham 3	Elmton	Warsop	Calke
% Aqueous solution	98.9	99.3	91.1	99.9	98.2
%Soil extracts	58.5	83.2	65.2	86.9	44.5
%Total soil radioactivity	30.6	61.9	27.6	83.5	17.3

The concentrations of thymol (corrected for degradation) in solution and on soil after equilibration and the associated Kd values are shown in the following tables:

Table 8.1.3.1.1-5: Adsorption of thymol on Bromsgrove soil

Nominal initial concentration in solution (mg/L)	Concentration in solution (µg/mL) [a]	Concentration in soil (µg/g) [a]	Kd
5.000	2.973	5.675	1.99
1.500	0.8281	2.271	2.75
0.500	0.2652	1.029	3.90
0.150	0.04037	0.3785	9.38
0.050	0.007962	0.1095	13.8

[a] Values are corrected for thymol degradation and are the means of two replicates.

Table 8.1.3.1.1-6: Adsorption of thymol on Evesham 3 soil

Nominal initial concentration in solution (mg/L)	Concentration in solution (µg/mL) [a]	Concentration in soil (µg/g) [a]	Kd
5.000	3.319	18.78	5.66
1.500	0.9157	9.491	10.3
0.500	0.3678	2.878	7.83
0.150	0.09314	0.8678	9.43
0.050	0.02239	0.2629	11.9

[a] Values are corrected for thymol degradation and are the means of two replicates.

Table 8.1.3.1.1-7: Adsorption of thymol on Elmton soil

Nominal initial concentration in solution (mg/L)	Concentration in solution (µg/mL) [a]	Concentration in soil (µg/g) [a]	Kd
5.000	2.874	9.322	3.25
1.500	0.3435	11.25	34.2
0.500	0.1904	2.164	11.4
0.150	0.0269	0.738	29.1
0.050	0.006637	0.200	30.2

[a] Values are corrected for thymol degradation and are the means of two replicates.
[b] single replicate only

Table 8.1.3.1.1-8: Adsorption of thymol on Warsop soil

Nominal initial concentration in solution (mg/L)	Concentration in solution (µg/mL) [a]	Concentration in soil (µg/g) [a]	Kd
5.000	3.92	7.778	1.99
1.500	1.26	2.68	2.13
0.500	0.4545	1.241	2.74
0.150	0.1163	0.3983	3.43
0.050	0.02815	0.1298	4.61

[a] Values are corrected for thymol degradation and are the means of two replicates.

Table 8.1.3.1.1-9: Adsorption of thymol on Calke soil

Nominal initial concentration in solution (mg/L)	Concentration in solution (µg/mL) [a]	Concentration in soil (µg/g) [a]	Kd
5.000	2.033	9.628	4.86
1.500	0.4944	6.542	13.1
0.500	0.243	1.224	5.04
0.150	0.03704	0.4344	11.8
0.050	0.006068	0.1298	21.4

[a] Values are corrected for thymol degradation and are the means of two replicates.

The Freundlich adsorption coefficients (K_F^{ads}) were in the range 2.38 – 8.14 indicating some adsorption of thymol to the test soils. Adsorption coefficients corrected for organic carbon content (K_{Foc}^{ads}) were in the range 178-216. Values of the exponent 1/n were in the range 0.65 – 0.89. There was no correlation between soil pH and the degree of adsorption.

Table 8.1.3.1.1-10: Adsorption characteristics of thymol

Soil	Organic carbon content (%)	K_F	K_{foc}	1/n
Bromsgrove	1.5	2.67	178	0.65
Evesham 3	4.0	7.59	190	0.89
Elmton	4.4	8.14	185	0.68
Warsop	1.1	2.38	216	0.82
Calke	3.4	6.15	181	0.78

B DESORPTION

The mass balance of radioactivity was measured in the two remaining vessels of each soil type treated at the highest thymol concentration following the desorption phase. Recoveries of radioactivity were in the range 93.6 – 103.0% of the applied radioactivity.

Thymol represented 5.6 – 91.1% sample radioactivity in the aqueous solution and 4.6 – 57.0% of total radioactivity in soil (soil extracts and non-extractable residue).

Table 8.1.3.1.1-10: Adsorption characteristics of thymol. Proportions of thymol in aqueous and soil extracts after desorption at an initial concentration of 5.0 mg/L

Parameter/Soil	Bromsgrove	Evesham 3	Elmton	Warsop	Calke
Aqueous solution	28.8	67.9	5.6	91.1	29.0
Total radioactivity in soil	12.6	28.0	5.9	57.0	4.6

For each soil the Freundlich desorption coefficient (K_F^{des}, in the range 4.23 – 169) was higher than the adsorption coefficient, indicating some degree of irreversibility to the adsorption of thymol.

Table 8.1.3.1.1-12: Desorption characteristics of thymol

Soil	Organic carbon content (%)	K_F	K_{foc}	1/n
Bromsgrove	1.5	33.5	2230	0.82
Evesham 3	4.0	34.8	870	0.86
Elmton	4.4	169	3840	0.87
Warsop	1.1	4.23	385	0.71
Calke	3.4	128	3760	1.03

III. CONCLUSION

The Freundlich adsorption coefficients (K_F^{ads}) were in the range 2.38 – 8.14 indicating some adsorption of thymol to the test soils. Adsorption coefficients corrected for organic carbon content (K_{Foc}^{ads}) were in the range 178 – 216. Using the McCall scale to assess a chemical's potential mobility, these values indicate a medium potential for mobility of thymol in the soils tested. There was no correlation between soil pH and the degree of adsorption. For each soil the desorption coefficient was higher than the adsorption coefficient, indicating some degree of irreversibility to the adsorption of thymol.

3. Assessment and conclusions

Assessment and conclusion by applicant:

When the RMS reviewed this study, they did not consider that the data provided in this study were robust enough to be used to derive the sorption endpoint for thymol. The RMS suggested that due to the rapid degradation of thymol, it's stability during a batch equilibrium adsorption/desorption study cannot be relied upon.

Values from this study have been used in the risk assessment until the new study is available.

A new OECD 106 has been conducted, and this study is reported here for reference only.

Assessment and conclusion by RMS:

A batch equilibrium adsorption/desorption study for thymol according to OECD 106 and to GLP was submitted and evaluated by United Kingdom in the context of confirmatory data (2016). The following deviations were found in the previous evaluation and has been replicated below:

- The test substance stability was assessed in two soils, using only single replicates. Thymol shown to be unstable in this preliminary experiment.
- The study stated that equilibrium had generally been reached at 24 hours. However, significant reductions in solution radioactivity were seen after 24 hours. The route and rate study showed rapid degradation of thymol (section B.8.1). Therefore, the RMS considered it likely that equilibrium was reached during the preliminary study, but was not captured because of the time point spacing (2, 4, 8, 24 and 48 hours). The RMS suggested that the decrease in the % radioactivity in the solution at 24 and 48 hours represents the adsorption of one or more metabolites, rather than of thymol.
- A soil-to-solution ratio of 1:20 w/v was selected for Elmton, Calke and Evesham soils and a ratio of 1:10 w/v was selected for Warsop and Bromsgrove soils. The selected soil-to-solution ratio for Warsop and Bromsgrove soil was not tested in the preliminary experiment. The highest ratio tested was 1:20 w/v which gave an adsorption of 4.5 and 14.9 % for Warsop and Bromsgrove, respectively. Based on these results, a ratio of 1:10 w/v was used, without further preliminary investigations.
- Details of the pesticide and fertiliser treatment for the 5 years prior to sampling as well as grid references or GPS/map coordinates to identify the specific sampling sites were not included.
- Soil characterisation was not conducted according to GLP.
- Lower temperature was not used (e.g. 20 ºC), given the high volatility of the test substance and the test solution pH was not measured during the main experiment.
- The study only reported the mass balance for samples with an initial thymol concentration of 5.0 mg/L in the main experiment. The total recoveries ranged from 88.8 – 95.0 % AR which is slightly outside the OECD106 guideline values. Significant proportion of the overall recovery was classed as unextracted radioactivity, particularly in Calke soil. The RMS suggested that stronger extraction techniques could have been employed to achieve a greater total extracted radioactivity.
- Radioactivity in the soil was determined indirectly by analysis of radioactivity in the supernatant (indirect method). The study also stated that these two values were corrected for degradation. An example calculation was included for one soil type (Warsop) at one initial thymol concentration (5 mg/L). However, radiochromatograms were not included for Warsop soil, so verification was not possible. Additionally, the study did not describe how the corrections are made for test solutions at lower concentrations.

In conclusion, RMS considered that the data provided in this study were not robust enough to be used to derive the sorption endpoint for thymol.

For completeness with respect to the previous evaluation, the OECD 106 checklist has been included below together with the graphical outputs:

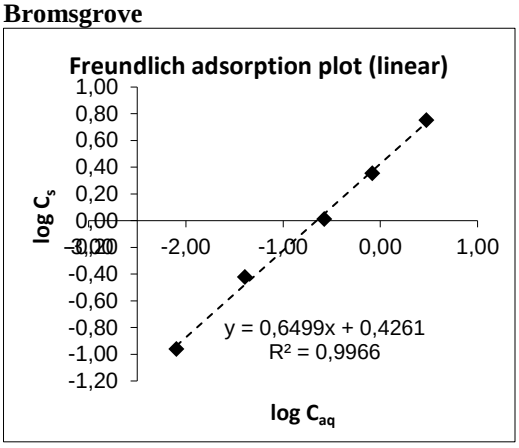
Soil	**Units**	Bromsgrove	Evesham 3	Elmton	Warsop	Calke
Adsorption method	-	Indirect method				
Soil:solution ratio	g dw/mL	1:10	1:20	1:20	1:10	20:1
Mass balance of (at highest tested conc.)*	%	n.r	n.r	n.r	n.r	n.r
Adsorbed percentage	%	40.54-84.08	26.44-55.22	42.52-86.73	9.10-43.7	51.4-87.86
K_d × (soil: solution ratio)	-	0.19-1.38	0.28-0.59	0.16-1.64	0.20-0.46	0.24-1.07
${}^{ads}K_F$ (95% confidence interval)	L/kg dw	2.668	7.594	8.142	2.379	6.155
${}^{ads}1/n$ (95% confidence interval)	L/kg dw	0.650	0.887	0.677	0.823	0.776
${}^{ads}R^2$	-	0.997	0.989	0.865	0.999	0.949
${}^{ads}K_{F,\,OC}$	L/kg OC	177.8	189.9	185.0	216.3	181.0

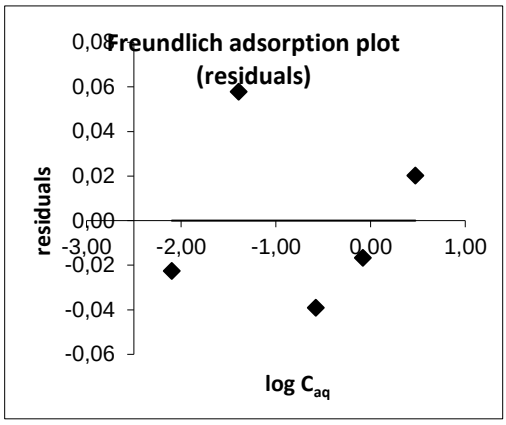
K_{fE} /K_f	-	-	-	-	-	-

The checklist has been completed using the concentration values in aqueous solution and soil extracts corrected for the degradation of thymol as it was provided in the original study report. It is noted that only an example of correction was provided at the highest application rate (Warsop soil at 5 mg/L).
Parental mass balance was not reported. HPLC was carried out for all soils at the highest concentration; however, the chromatograms were not included with the exception of the chromatogram for Bromsgrove soil. Theoretically, the

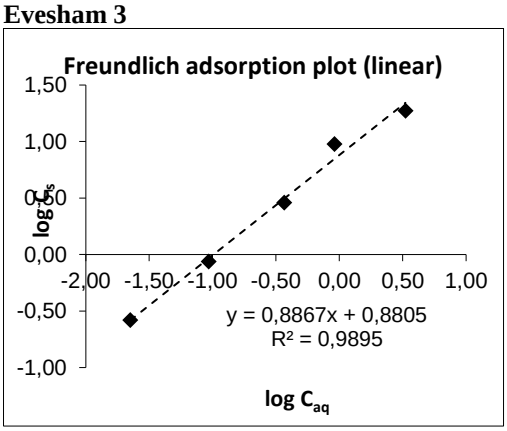
proportions of thymol in aqueous and soil extracts after adsorption found were used for the correction at all the tested concentration.

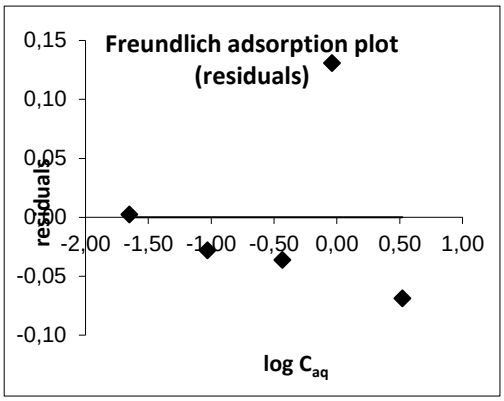
Bromsgrove



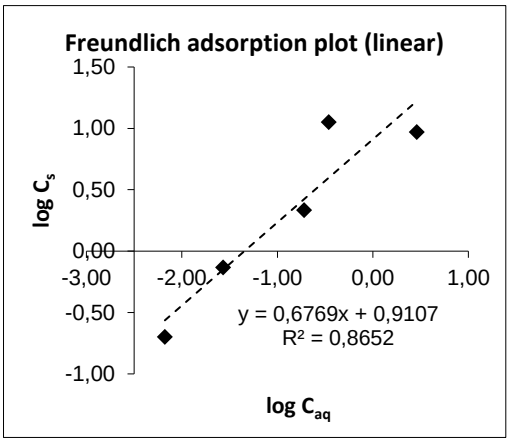


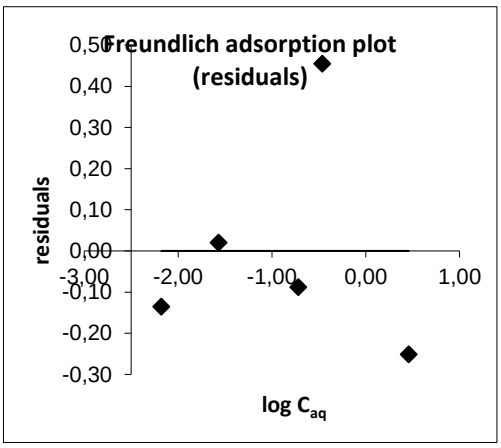
Evesham 3



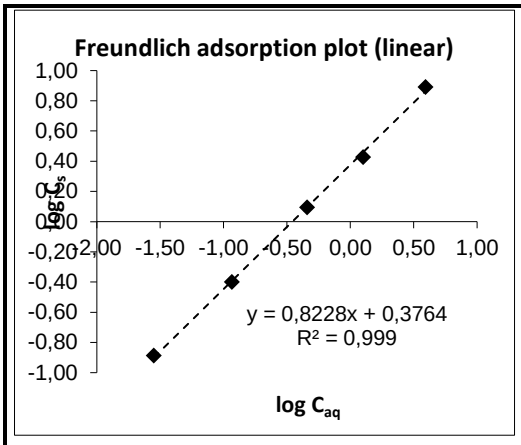


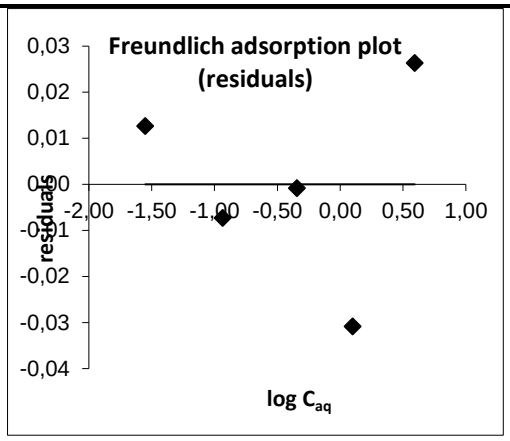
Elmton



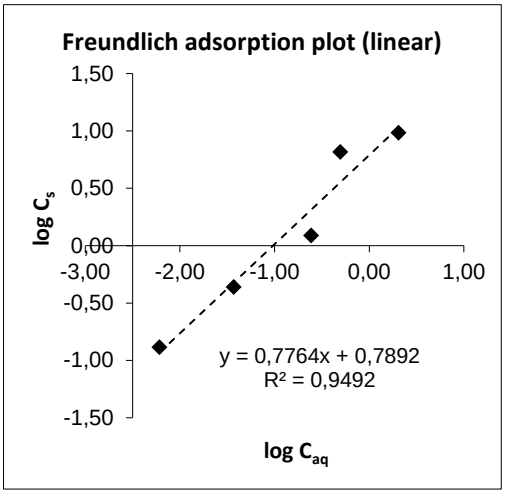


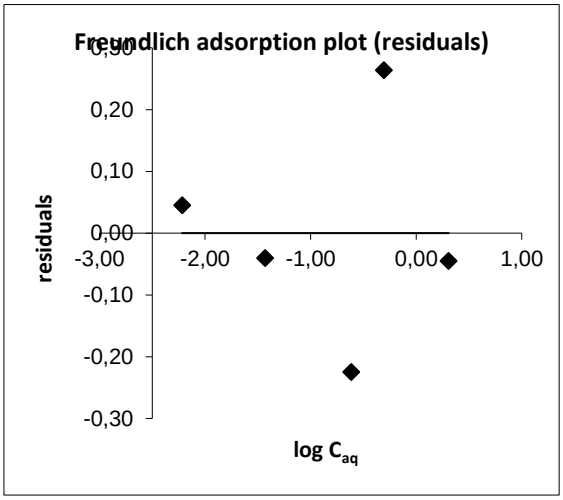
Warsop





Calke





Outcome and conclusion of the study:
The study is not valid as it was considered in the assessment of the confirmatory data.

A new adsorption-desorption study has been performed and is summarised below.

1. **Information on the study**

Data point	CA 7.1.3.1.1/02
Report author	A. Cashmore
Report year	2021
Report title	Thymol: Adsorption/Desorption in Soil
Report No.	Smithers ERS Ltd No. 3203040
Document No.	-
Guidelines followed in study	OECD 106
Deviations from current test guideline	None
Previous evaluation	No
GLP/Officially recognised testing facilities	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability	Yes

2. Full summary

The adsorption of Thymol was studied in four different soils: RefeSol 01-A (sandy loam; Northrhine-Westphalia, Germany), RefeSol 03-G (silty clay loam; Northrhine-Westphalia, Germany), Kenslow (clay loam; Derbyshire, UK) and La Reina (clay; Cordoba, Spain) using a standard batch equilibrium method, in the dark at 20 ± 2°C. The test was performed on sterilised soils under sterile conditions.

The test item was added to soil: aqueous slurries (resulting in a final nominal composition of 5 g of soil : 25 mL aqueous 0.01M calcium chloride in all four soils to achieve five nominal rates of application (5.0, 1.5, 0.5, 0.15 and 0.05 µg/mL)). The soil adsorption coefficients K_d and K_{OC}, together with the Freundlich adsorption constants K_F and K_{FOC}, were determined for each soil.

Following preliminary tests, an adsorption equilibration time of 24 hours was selected for the definitive test for RefeSol 01-A and RefeSol 03-G soils, and 3 hours for Kenslow and La Reina soils. Recovery of Thymol was determined, at the highest test item concentration for each soil, by high performance liquid chromatography mass spectroscopy (LC/MS-MS) analysis of the adsorption supernatants and soil extracts. Analysis of samples from all soils was performed by the direct method of calculation.

Findings

Method Validation
Mean recoveries from fortified samples were within the acceptable range of 70-120% at each concentration for supernatants and soil pellets for each soil. Precision (%RSD) was within the acceptable limit of ≤ 20% at each concentration for the supernatants and soil pellets for each soil.

Control (untreated) supernatants and soil pellets were free from components that interfered with the analysis (i.e. no peaks were present at > 30% of the LOQ at the retention time of Thymol). Therefore, the analytical procedures were considered to be specific.

A summary of the key adsorption and desorption values of Thymol in soil are shown below:

Soil	Texture class	Soil pH ($CaCl_2$)	OC (%)	Adsorption			Desorption		
				K_f	K_{foc}	1/n	K_f	K_{foc}	1/n
RefeSol 01-A	Sandy loam	5.4	1.0	1.710	171.0	0.908	2.507	250.7	0.907
RefeSol 03-G	Silty clay loam	6.0	4.6	5.361	116.6	0.929	5.816	126.4	0.881
Kenslow	Clay loam	5.0	3.8	4.137	108.9	0.958	5.206	137.0	0.978
La Reina	Clay	7.7	1.1	1.021	92.8	0.830	1.624	147.6	0.885

Using the McCall Classification scale to assess a chemical's potential mobility in soil (based on its mean K_{OC}), Thymol can be classified as having a "high" potential mobility in RefeSol 03-G, Kenslow and La Reina soils, and "medium" potential mobility in RefeSol 01-A soil.

I. MATERIALS AND METHODS

A MATERIALS

Test material 1

Name:	Thymol
Description:	Colourless crystals
Lot/Batch no.:	THY/02/2019-20
Chemical Purity:	99.58%
Storage:	Room temperature

Four soils were chosen to cover a wide range of agriculture soil types. RefeSol 01-A and RefeSol 03-G from Germany, Kenslow from UK and La Reina from Spain. Prior to use the soils were air dried and passed through a 2 mm sieve.

Table 8.1.3.1.1-13: Characterisation details for the soil used to investigate the adsorption desorption of thymol

Soil parameter				
Source	RefeSol 01-A	RefeSol 03-G	Kenslow	La Reina
Location	North Rhine-Westphalia, Germany	North Rhine-Westphalia, Germany	Derbyshire, UK	Cordoba, Spain
Particle size distribution, UK % Sand (63 µm-2 mm) % Silt (2 µm-63 µm) % Clay (< 0.002µm)	 73 20 7	 18 58 25	 28 47 25	 24 29 47
Textural classification, UK	Sandy loam	Silty clay loam	Clay loam	Clay
Soil pH (water)	6.2	6.5	5.6	8.5
Soil pH (0.01M $CaCl_2$)	5.4	6.0	5.0	7.7
Organic carbon (%)	1.0	4.6	3.8	1.1
Organic matter (%)	1.7	7.9	6.6	1.9
Cation exchange capacity, (meq/100 g soil)	6.2	24.3	21.2	20.7
Moisture at pF2.0 (w/w %)	13.0	39.1	33.5	34.2

B STUDY DESIGN

Experimental conditions

Soils were dispensed into pre-weighed Teflon™ tubes and were equilibrated by shaking with 0.01M calcium chloride solution overnight before the day of the experiment.

All tests were performed in a temperature-controlled room (20 ± 2°C) in the dark, with the exception of the solubility test which was performed at ambient temperature and not kept in the dark.

Preliminary investigations were conducted to determine the solubility, adsorption to containers, soil ratio, validation of analytical methods, adsorption equilibrium times and to check the stability prior to the isotherm determination

Definitive Test

The test was performed on sterilised soils under sterile conditions, as a precaution, as Thymol was expected to be unstable in non-sterile soil. Gamma-irradiated soils were used. 0.01M calcium chloride was autoclaved before use. Dispensing and application were performed in a laminar flow hood.

Four vessels were prepared for each concentration for each soil, using a 1:5 w/v soil: 0.01M calcium chloride solution ratio (5 g of soil and 25 mL of 0.01M calcium chloride).

Four procedural recovery samples and two untreated control samples were prepared for each of the four soils.

Soils were dispensed into pre-weighed Teflon® centrifuge tubes and were equilibrated by shaking with 0.01M calcium chloride solution overnight before the day of the experiment. The procedural recovery and control samples were centrifuged at *ca* 5000 *g* (4750 rpm) for 34 minutes and separated into adsorption supernatants and soil pellets.

RefeSol 01-A and RefeSol 03-G samples were mixed for 24 hours. Kenslow and La Reina samples were mixed for 3 hours. Test vessels, procedural recovery and control samples were mixed on a reciprocating shaker (20 ± 2°C in the dark). Test vessels, control and procedural recovery vessels containing supernatant and soil were centrifuged at *ca* 5000 *g* (4750 rpm) for 34 minutes. Test vessels were weighed before and after removal of the supernatant.

After addition of an internal standard (2,6-diisopropylphenol), supernatants were mixed with 1M ammonium hydroxide, extracted with ethyl acetate: hexane (1:1 v/v) and analysed by Gas Chromatography with Mass Spectrometry detection (GC-MS).

After addition of an internal standard (2,6-diisopropylphenol), soil pellets were extracted with acetone: hexane (1:1 v/v). The extracts were washed with water to remove the acetone. The hexane extract was dried with sodium sulphate, diluted 1:1 v/v with ethyl acetate and analysed by GC-MS.

The pH of each adsorption supernatant was measured.

Parameter		Description
Soil condition		Air dried, passed through 2 mm sieve, treated with gamma radiation.
Soil sample weight		5 g
Equilibrium solution		0.01M calcium chloride (25 mL), autoclaved
Control and procedural recovery conditions		Soil equilibrated with 0.01M calcium chloride, separated into supernatant and soil pellet, treated with test item at the same time as the samples, shaken with the samples during adsorption/desorption equilibration.
Number of replicates	Controls	2 adsorption supernatants, 1 adsorption soil pellet, 1 desorption supernatant, 1 desorption soil pellet per soil.
	Procedural recoveries	4 adsorption supernatants, 2 adsorption soil pellets, 2 desorption supernatants, 2 desorption soil pellets per soil.
	Treated samples	2 per soil at each concentration
Test apparatus		Teflon® tubes and seals, sprayed with denatured ethanol and allowed to evaporate in a laminar flow cabinet.
Test item application	Identity of solvent	Dosed in acetonitrile
	Volume of test solution used/treatment	0.025 mL
	Application method	Positive displacement pipette, in a laminar flow cabinet.
	Evaporation of application solvent	No
Test item concentration	Nominal application rates	5.0, 1.5, 0.5, 0.15 and 0.05 µg/mL
Soil : solution ratio		1:5
Indication of test item adsorbing to walls of test apparatus		No
Adsorption and desorption equilibrium conditions	Temperature (°C)	20 ± 2°C
	Time	24 hours (RefeSol 01-A and RefeSol 03-G), 3 hours (Kenslow and La Reina)
	Continuous darkness (Yes/No)	Yes
	Shaking method	Reciprocal shaker
Method of separation of supernatant		Centrifugation
Centrifugation	Speed	*ca.* 5000 *g*
	Duration	34 minutes
	Method of separating supernatant	Decanting

II RESULTS

A PRELIMINARY TEST

Test details	Result
Solubility test Solubility was tested at 50 µg/mL in 0.01M calcium chloride solution.	Mean recovery of 31.4% indicated observed solubility of approximately 15 µg/mL, therefore acetonitrile application solutions were used.
Adsorption to container test Adsorption to Teflon® from 0.01M calcium chloride solution at a concentration of 0.05 µg/mL	Mean recovery of 83.2% at 0 hours and 78.4% at 24 hours were comparable with procedural recoveries and indicated no significant adsorption.
Ratio of soil to aqueous phase test Performed at 5 µg/mL test item concentration, in Teflon® tubes and the following ratios: 1:5 ratio 5 g soil : 25 mL 0.01 M calcium chloride solution 1:25 ratio 1 g soil : 25 mL 0.01 M calcium chloride solution 1:50 ratio 0.5 g soil : 25 mL 0.01 M calcium chloride solution Four soils were tested.	At the 1:5 ratio 27.7 to 63.8% of the applied test item was adsorbed to the soil, therefore this ratio was selected.
Adsorption Equilibrium time test Performed at 5 µg/mL test item concentration and a 1:5 w:v soil	Adsorption equilibrium times of 24 hours were selected for RefeSol 01-A and RefeSol 03-G soils.

ratio with sampling intervals at 3, 6, 24 and 48 hours. Four soils were tested.	Adsorption equilibrium times of 3 hours were selected for Kenslow and La Reina soils.
Stability test Adsorption supernatants and soil extracts from the adsorption equilibrium time test were analysed by GC-MS.	Stability was acceptable for RefeSol 01-A and RefeSol 03-G soils, therefore adsorption equilibrium times of 24 hours were selected. Stability was not acceptable for Kenslow and La Reina soils, therefore shorter adsorption equilibrium times of 3 hours were selected.

Solubility Test
The solubility test was performed using an application solution in 0.01M calcium chloride at ten times the highest proposed treatment concentration of 5 µg/mL (50 µg/mL), which would allow for the maximum treatment volume of 10% of the total aqueous phase.
Two procedural recovery samples and one untreated control sample of 0.01M calcium chloride (Rec 1, Rec 2 and Con 1) were subjected to the same analytical procedure as the samples (without centrifugation and dilution).
Solubility was considered acceptable if the mean sample recovery was between 90-110% of the applied test item.
Solubility was demonstrated to be at least two times the highest nominal treatment concentration of 5 µg/mL (10 µg/mL).

Adsorption to Container Test
No significant absorption was considered to have occurred if the mean sample recovery was between 90-110% of the applied amount of test item and within ± 20% of the mean procedural recovery result from this test.

Ratio Test
The ratio test was performed in all four soils at the highest treatment concentration (5 µg/mL). One procedural recovery sample and one untreated control sample were prepared using the 1:5 ratio for each of the four soils. The supernatants from the procedural recovery vessels were fortified at the same time as the test vessels were treated.
The test vessels, controls and procedural recovery samples were shaken in the dark at 20°C ± 2°C for 24 hours.
The percentage of applied test item in the supernatant was calculated from the measured supernatant concentration. The percentage of applied test item in the soil was calculated by difference. The ratio test and procedural recovery results are given in the following tables

Table 8.1.3.1.1-14: Percent of thymol absorbed to soil following 24 hours mixing at soil to aqueous phase ratios of 1:5, 1:25 and 1:50 w/v.
1:5 (w/v) Ratio

Soil	Sample ID	% of Applied in Supernatant	% of Applied in Soil(by calculation)
RefeSol 01-A (CS18/18)	SSR 1	71.4	28.6
	SSR 2	71.6	28.4
	Mean	**71.5**	**28.5**
RefeSol 03-G (CS19/18)	SSR 3	43.0	57.0
	SSR 4	41.2	58.8
	Mean	**42.1**	**57.9**
Kenslow (CS20/18)	SSR 5	36.3	63.7
	SSR 6	36.1	63.9
	Mean	**36.2**	**63.8**
La Reina (CS08/19)	SSR 7	70.2	29.8
	SSR 8	74.4	25.6
	Mean	**72.3**	**27.7**

1:25 (w/v) Ratio

Soil	Sample ID	% of Applied in Supernatant	% of Applied in Soil (by calculation)
RefeSol 01-A (CS18/18)	SSR 9	94.9	5.1
	SSR 10	90.2	9.8
	Mean	**92.5**	**7.5**
RefeSol 03-G (CS19/18)	SSR 11	81.3	18.7
	SSR 12	80.5	19.5
	Mean	**80.9**	**19.1**
Kenslow (CS20/18)	SSR 13	75.7	24.3

	SSR 14	74.9	25.1
	Mean	**75.3**	**24.7**
La Reina (CS08/19)	SSR 15	89.6	10.4
	SSR 16	96.5	3.5
	Mean	**93.1**	**6.9**

1:50 (w/v) Ratio

Soil	**Sample ID**	**% of Applied in Supernatant**	**% of Applied in Soil (by calculation)**
RefeSol 01-A (CS18/18)	SSR 17	97.4	2.6
	SSR 18	98.5	1.5
	Mean	**97.9**	**2.1**
RefeSol 03-G (CS19/18)	SSR 19	88.2	11.8
	SSR 20	93.1	6.9
	Mean	**90.7**	**9.3**
Kenslow (CS20/18)	SSR 21	87.6	12.4
	SSR 22	91.1	8.9
	Mean	**89.3**	**10.7**
La Reina (CS08/19)	SSR 23	101.0	-1.0[a]
	SSR 24	95.6	4.4
	Mean	**98.3**	**1.7**

[a] A negative result was calculated due to % of applied in supernatant being slightly > 100%.

Table 8.1.3.1.1-15: Procedural Recovery (Supernatants)

Soil	**Sample ID**	**Ratio**	**Fortified Concentration (µg/mL)**	**Calculated Concentration (µg/mL)**	**Accuracy (%)**
CS18/18 RefeSol 01-A	Con 4	1:5	N/A	0.000	N/A
CS19/18 RefeSol 03-G	Con 5	1:5	N/A	0.000	N/A
CS20/18 Kenslow	Con 6	1:5	N/A	0.000	N/A
CS08/19 La Reina	Con 7	1:5	N/A	0.000	N/A
CS18/18 RefeSol 01-A	Rec 7	1:5	1	0.943	94.3
CS19/18 RefeSol 03-G	Rec 8	1:5	1	1.056	106
CS20/18 Kenslow	Rec 9	1:5	4	4.072	102
CS08/19 La Reina	Rec 10	1:5	4	4.033	101

The 1:5 w/v ratio was the only ratio where the adsorption was greater than 20% for all four soils, therefore the 1:5 w/v ratio was used in the subsequent tests and for validation of the analytical procedure SM 3203040-01V.C.

Adsorption Equilibrium Time Test

The adsorption equilibrium time test was performed in all four soils at the highest treatment concentration (5 µg/mL). Duplicate vessels were prepared for each time point for each soil, using a 1:5 w/v soil: 0.01M calcium chloride solution ratio (5 g of soil and 25 mL of 0.01M calcium chloride). One procedural recovery sample and one untreated control sample were prepared for each of the four soils. The supernatants and soil pellets from the procedural recovery vessels were fortified at the same time as the test vessels were treated.

The percentage of applied test item in the supernatant was calculated from the measured supernatant concentration. The percentage of applied test item in the soil was calculated by difference.

The adsorption equilibrium time test and procedural recovery results are given in the following tables

Table 8.1.3.1.1-16: Percentage of thymol in supernatant and soil (by calculation). RefeSol 01-A Soil

Sampling Interval (Hours)	**Sample ID**	**% of Applied in Supernatant**	**% of Applied in Soil (by calculation)**
	AEQ 1	76.8	23.2
3	AEQ 2	74.7	25.3
	Mean	**75.8**	**24.2**

6	AEQ 3	61.9	38.1
	AEQ 4	61.8	38.2
	Mean	**61.8**	**38.2**
24	AEQ 5	57.7	42.3
	AEQ 6	57.4	42.6
	Mean	**57.6**	**42.4**
48	AEQ 7	58.1	41.9
	AEQ 8	59.9	40.1
	Mean	**59.0**	**41.0**

Table 8.1.3.1.1-17: Percentage of thymol in supernatant and soil (by calculation). RefeSol 03-G Soil

Sampling Interval (Hours)	Solution Identification	% of Applied in Supernatant	% of Applied in Soil (by calculation)
3	AEQ 9	41.1	58.9
	AEQ 10	40.8	59.2
	Mean	**40.9**	**59.1**
6	AEQ 11	34.5	65.5
	AEQ 12	33.3	66.7
	Mean	**33.9**	**66.1**
24	AEQ 13	31.4	68.6
	AEQ 14	32.8	67.2
	Mean	**32.1**	**67.9**
48	AEQ 15	31.6	68.4
	AEQ 16	33.3	66.7
	Mean	**32.4**	**67.6**

Table 8.1.3.1.1-18: Percentage of thymol in supernatant and soil (by calculation). Kenslow Soil.

Sampling Interval (Hours)	Solution Identification	% of Applied in Supernatant	% of Applied in Soil (by calculation)
3	AEQ 17	45.8	54.2
	AEQ 18	46.2	53.8
	Mean	**46.0**	**54.0**
6	AEQ 19	38.7	61.3
	AEQ 20	38.5	61.5
	Mean	**38.6**	**61.4**
24	AEQ 21	28.3	71.7
	AEQ 22	26.5	73.5
	Mean	**27.4**	**72.6**
48	AEQ 23	25.5	74.5
	AEQ 24	24.7	75.3
	Mean	**25.1**	**74.9**

Table 8.1.3.1.1-19: Percentage of thymol in supernatant and soil (by calculation). La Reina Soil.

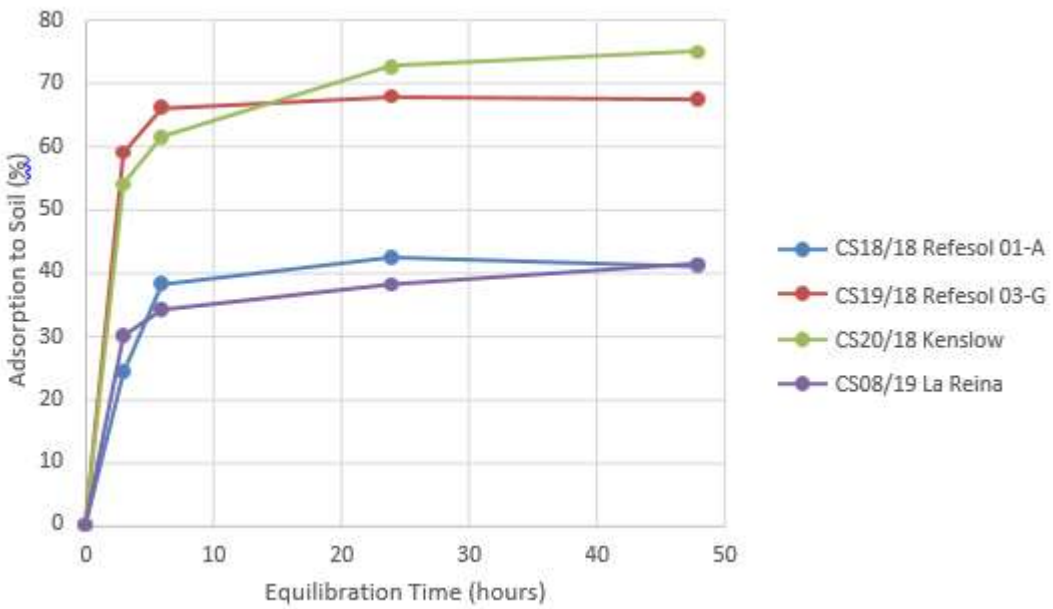
Sampling Interval (Hours)	Solution Identification	% of Applied in Supernatant	% of Applied in Soil (by calculation)
3	AEQ 25	71.5	28.5
	AEQ 26	68.7	31.3
	Mean	**70.1**	**29.9**
6	AEQ 27	65.6	34.4
	AEQ 28	66.2	33.8
	Mean	**65.9**	**34.1**
24	AEQ 29	60.5	39.5
	AEQ 30	63.4	36.6
	Mean	**61.9**	**38.1**
48	AEQ 31	55.1	44.9
	AEQ 32	62.2	37.8
	Mean	**58.7**	**41.3**

Table 8.1.3.1.1-20: Procedural Recovery (Supernatants)

Soil	Sample ID	Adsorption equilibrium time (hr)	Fortified Concentration (µg/mL)	Calculated Concentration (µg/mL)	Accuracy (%)
RefeSol 01-A (CS18/18)	Con 8 (C)	3	N/A	0.000	N/A
RefeSol 03-G (CS19/18_	Con 9 (C)	6	N/A	0.000	N/A
Kenslow (CS20/18)	Con 10 (C)	24	N/A	0.000	N/A
La Reina (CS08/19)	Con 11 (C)	48	N/A	0.000	N/A
RefeSol 01-A (CS18/18)	Rec 11 (C)	3	4	4.733	118
RefeSol 03-G (CS19/18)	Rec 12 (C)	6	1	0.944	94.4
Kenslow (CS20/18)	Rec 13 (C)	24	1	0.910	91.0
La Reina (CS08/19)	Rec 14 (C)	48	4	3.703	92.6

N/A = Not Applicable.

The following graph shows the calculated % adsorption of Thymol to soils over time.



The calculated adsorption to soil appeared to plateau between 6 and 48 hours for RefeSol 01-A and RefeSol 03-G soils, therefore an intermediate equilibrium time of 24 hours was selected for the definitive test for these two soils.

The calculated adsorption to soil appeared to increase over the test period for Kenslow and La Reina soils. The stability test indicated degradation was responsible for the apparent increase, therefore a short equilibrium time of 3 hours was selected for the definitive test for these two soils.
The desorption equilibrium times were selected to be the same as the adsorption equilibrium times.

Stability Test
The levels and amounts of Thymol measured in the supernatant extracted from the soil pellets from each sampling interval from the adsorption equilibrium time test are shown in the following tables:

Table 8.1.3.1.1-21: Percentage of thymol in supernatant and soil. RefeSol 01-A Soil

Sampling Interval (Hours)	Sample ID	% of Applied in Supernatant removed	% of Applied extracted from Soil pellet	Total % of Applied Recovered
	AEQ 1	72.0	27.8	99.8
3	AEQ 2	70.5	25.4	95.9
	Mean	**71.2**	**26.6**	**97.8**
	AEQ 3	57.8	32.4	90.1
6	AEQ 4	58.0	27.8	85.8
	Mean	**57.9**	**30.1**	**88.0**
	AEQ 5	54.0	30.4	84.4
24	AEQ 6	53.6	28.5	82.2
	Mean	**53.8**	**29.5**	**83.3**
	AEQ 7	54.3	33.5	87.9
48	AEQ 8	56.4	31.5	87.9
	Mean	**55.3**	**32.5**	**87.9**

Table 8.1.3.1.1-22: Percentage of thymol in supernatant and soil. RefeSol 03-G Soil

Sampling Interval (Hours)	Sample ID	% of Applied in Supernatant removed	% of Applied extracted from Soil pellet	Total % of Applied Recovered
	AEQ 9	35.9	55.4	91.3
3	AEQ 10	35.6	49.0	84.6
	Mean	**35.7**	**52.2**	**87.9**
	AEQ 11	30.6	51.5	82.1
6	AEQ 12	29.0	53.9	82.9
	Mean	**29.8**	**52.7**	**82.5**
	AEQ 13	27.2	53.9	81.2
24	AEQ 14	28.3	51.5	79.8
	Mean	**27.8**	**52.7**	**80.5**
	AEQ 15	27.5	50.8	78.3
48	AEQ 16	29.0	50.8	79.7
	Mean	**28.2**	**50.8**	**79.0**

Table 8.1.3.1.1-23: Percentage of thymol in supernatant and soil. Kenslow Soil.

Sampling Interval (Hours)	Sample ID	% of Applied in Supernatant removed	% of Applied extracted from Soil pellet	Total % of Applied Recovered
	AEQ 17	40.2	45.4	85.6
3	AEQ 18	40.4	44.5	84.8
	Mean	**40.3**	**44.9**	**85.2**
	AEQ 19	34.5	43.9	78.4
6	AEQ 20	34.0	44.7	78.7
	Mean	**34.2**	**44.3**	**78.5**
	AEQ 21	24.8	38.3	63.0
24	AEQ 22	23.3	36.6	59.8
	Mean	**24.0**	**37.4**	**61.4[a]**
	AEQ 23	22.4	33.4	55.8
48	AEQ 24	21.6	31.7	53.3
	Mean	**22.0**	**32.5**	**54.5[a]**

[a] Due to test vessel recoveries < 70% at the 24 and 48 hour sampling intervals, a short adsorption equilibrium time of 3 hours was selected.

Table 8.1.3.1.1-24: Percentage of thymol in supernatant and soil. La Reina Soil.

Sampling Interval (Hours)	Sample ID	% of Applied in Supernatant removed	% of Applied extracted from Soil pellet	Total % of Applied Recovered
	AEQ 25	63.5	26.0	89.6

3	AEQ 26	61.4	28.7	90.0
	Mean	**62.4**	**27.4**	**89.8**
6	AEQ 27	58.2	27.9	86.1
	AEQ 28	58.7	27.5	86.3
	Mean	**58.5**	**27.7**	**86.2**
24	AEQ 29	53.6	25.6	79.2
	AEQ 30	56.3	26.6	82.9
	Mean	**54.9**	**26.1**	**81.1**
48	AEQ 31	48.8	20.8	69.6
	AEQ 32	55.1	25.9	81.1
	Mean	**52.0**	**23.4**	**75.3**

Due to procedural recovery < 70% at the 48 hour sampling interval, a short adsorption equilibrium time of 3 hours was selected.

Table 8.1.3.1.1-25: Procedural Recovery (Soil Pellets)

Soil	Sample ID	Adsorption equilibrium time (hr)	Analyte Concentration (mg/kg)	Calculated Concentration (mg/kg)	Accuracy (%)
RefeSol 01-A (CS18/18)	Con 8 (S)	3	N/A	0.000	N/A
RefeSol 03-G (CS19/18)	Con 9 (S)	6	N/A	0.000	N/A
Kenslow (CS20/18)	Con 10 (S)	24	N/A	0.000	N/A
La Reina (CS08/19)	Con 11 (S)	48	N/A	0.000	N/A
RefeSol 01-A (CS18/18)	Rec 11 (S)	3	5	3.883	77.7
RefeSol 03-G (CS19/18)	Rec 12 (S)	6	20	17.376	86.9
Kenslow (CS20/18)	Rec 13 (S)	24	20	14.196	71.0
La Reina (CS08/19)	Rec 14 (S)	48	5	2.045	40.9[a]

[a] Due to procedural recovery of 40.9% at the 48 hour sampling interval, a short adsorption equilibrium time of 3 hours was selected for La Reina soil.
N/A = Not Applicable.

For RefeSol 01-A soil, the mean percentage recovery of the test vessels ranged from 83.3 to 97.8%, so appeared to be stable over the test period. The procedural recovery result for the soil pellet was 77.7% after 3 hours. Therefore, an adsorption equilibrium time of 24 hours was selected for the definitive test for RefeSol 01-A soil.

For RefeSol 03-G soil, the mean percentage recovery of the test vessels decreased only slightly from 87.9 to 79.0% over the test period. The procedural recovery result for the soil pellet was 86.9% after 6 hours. Therefore, an adsorption equilibrium time of 24 hours was selected for the definitive test for RefeSol 03-G soil.

For Kenslow soil, the mean percentage recovery of the test vessels decreased from 85.2 to 54.5% over the test period. The procedural recovery result for the soil pellet was 71.0% after 24 hours. Therefore, an adsorption equilibrium time of 3 hours was selected for the definitive test for Kenslow soil.

For La Reina soil, the mean percentage recovery of the test vessels decreased from 89.8 to 75.3% over the test period. The procedural recovery result for the soil pellet was 40.9% after 48 hours. Therefore, an adsorption equilibrium time of 3 hours was selected for the definitive test for La Reina soil.

B DEFINITIVE ISOTHERMS

The test was performed at a soil to aqueous phase ratio of 1:5 w/v, with a 24-hour adsorption equilibrium period for RefeSol 01-A and RefeSol 03-G soils, and a 3-hour adsorption equilibrium period for Kenslow and La Reina soils, based upon the results of the preliminary tests.
The test was performed on sterilised soils under sterile conditions, as a precaution, as Thymol was expected to be unstable in non-sterile soil. Gamma-irradiated soils were used. 0.01M calcium chloride was autoclaved before use. Dispensing and application were performed in a laminar flow hood.

Table 8.1.3.1.1-26: Recovery of Thymol following the adsorption phase

Soil	Nominal Concentration (µg/mL)	Sample Identification Code	Amount (µg) Supernatant	Amount (µg) Soil	% Recovery Supernatant	% Recovery Soil	% Recovery Total	% Recovery Mean
RefeSol 01-A (CS18/18)	5	ISO 9	91.7	32.1	73.3	25.7	99.0	**98.5**
	5	ISO 10	88.0	34.5	70.4	27.6	98.0	
RefeSol 03-G (CS19/18)	5	ISO 29	36.8	54.8	29.4	43.8	73.3	**73.7**
	5	ISO 30	39.5	53.2	31.6	42.6	74.2	
Kenslow (CS20/18)	5	ISO 49	45.9	47.7	36.8	38.2	74.9	**75.6**
	5	ISO 50	46.5	49.0	37.2	39.2	76.3	
La Reina (CS08/19)	5	ISO 69	92.7	26.8	74.2	21.4	95.6	**94.7**
	5	ISO 70	88.2	29.2	70.5	23.3	93.8	

RefeSol 01-A and RefeSol 03-G used a 24 hour adsorption phase. Kenslow and La Reina used a 3 hour adsorption phase.
RefeSol 03-G and Kenslow had a mass balance < 90% but > 70%. This was considered acceptable because the compound is known to be unstable, and the direct method of calculation was used to calculate the Freundlich coefficients.

Concentrations of Thymol in the adsorption supernatants and adsorbed to the soil are shown in the following tables. Soil concentrations were calculated using the direct method (from extraction of soil pellets, corrected for the entrained aqueous phase).

Table 8.1.3.1.1-27: Concentrations of Thymol in supernatants and associated with RefeSol 01-A soil

		Adsorption		Desorption		
Nominal Concentration (µg/mL)	Sample Identification Code	Ce (µg/mL)	X/m (µg/g)	Ce (µg/mL)	C1 (µg/mL)	X1/m (µg/g)
0.05	ISO 1 / ISO 11	0.0278	0.0674	0.0282	0.0094	0.0374
0.05	ISO 2 / ISO 12	0.0294	0.0653	0.0305	0.0099	0.0367
	Mean	0.0286	0.0664	0.0293	0.0097	0.0371
0.15	ISO 3 / ISO 13	0.0964	0.1873	0.0876	0.0301	0.1125
0.15	ISO 4 / ISO 14	0.0936	0.1971	0.0833	0.0319	0.1078
	Mean	0.0950	0.1922	0.0854	0.0310	0.1102
0.5	ISO 5 / ISO 15	0.2960	0.6315	0.2852	0.1106	0.3140
0.5	ISO 6 / ISO 16	0.3052	0.6050	0.2987	0.1070	0.3502
	Mean	0.3006	0.6182	0.2919	0.1088	0.3321
1.5	ISO 7 / ISO 17	0.9563	1.7657	0.9986	0.3292	0.8839
1.5	ISO 8 / ISO 18	0.9697	1.7190	0.9634	0.3267	0.8950
	Mean	0.9630	1.7424	0.9810	0.3280	0.8894
5	ISO 9 / ISO 19	3.8869	5.2067	3.5484	1.1434	2.9213
5	ISO 10 / ISO 20	3.7552	5.6628	3.3837	1.0596	2.6603
	Mean	3.8210	5.4347	3.4661	1.1015	2.7908

Table 8.1.3.1.1-28: Concentrations of Thymol in supernatants and associated with RefeSol 03-G soil

		Adsorption		Desorption		
Nominal Concentration (µg/mL)	Sample Identification Code	Ce (µg/mL)	X/m (µg/g)	Ce (µg/mL)	C1 (µg/mL)	X1/m (µg/g)
0.05	ISO 21 / ISO 31	0.0143	0.0959	0.0168	0.0048	0.0534

0.05	ISO 22	/ ISO 32	0.0140	0.0983	0.0135	0.0052	0.0579
		Mean	0.0141	0.0971	0.0152	0.0050	0.0556
0.15	ISO 23	/ ISO 33	0.0519	0.3840	0.0497	0.0000	0.0219
0.15	ISO 24	/ ISO 34	0.0474	0.3878	0.0508	0.0203	0.1738
		Mean	0.0496	0.3859	0.0503	0.0102	0.0979
0.5	ISO 25	/ ISO 35	0.1622	1.0104	0.1837	0.0803	0.5627
0.5	ISO 26	/ ISO 36	0.1884	0.9606	0.1830	0.0532	0.5168
		Mean	0.1753	0.9855	0.1834	0.0667	0.5397
1.5	ISO 27	/ ISO 37	0.5346	2.7127	0.5060	0.2629	1.7839
1.5	ISO 28	/ ISO 38	0.5282	2.6360	0.4797	0.2469	1.6391
		Mean	0.5314	2.6743	0.4928	0.2549	1.7115
5	ISO 29	/ ISO 39	1.6797	9.8998	1.9924	1.0230	5.9082
5	ISO 30	/ ISO 40	1.7918	9.5149	2.0061	0.9187	5.6160
		Mean	1.7358	9.7074	1.9993	0.9709	5.7621

Table 8.1.3.1.1-29: Concentrations of Thymol in supernatants and associated with Kenslow soil

			Adsorption		Desorption		
Nominal Concentration (µg/mL)	Sample Identification Code		C_e (µg/mL)	X/m (µg/g)	C_e (µg/mL)	C_1 (µg/mL)	X_1/m (µg/g)
0.05	ISO 41	/ ISO 51	0.0162	0.0712	0.0151	0.0057	0.0354
0.05	ISO 42	/ ISO 52	0.0152	0.0839	0.0147	0.0057	0.0318
		Mean	0.0157	0.0775	0.0149	0.0057	0.0336
0.15	ISO 43	/ ISO 53	0.0506	0.2373	0.0523	0.0174	0.0947
0.15	ISO 44	/ ISO 54	0.0514	0.2510	0.0536	0.0189	0.1048
		Mean	0.0510	0.2442	0.0530	0.0182	0.0998
0.5	ISO 45	/ ISO 55	0.1771	0.7384	0.1797	0.0769	0.4404
0.5	ISO 46	/ ISO 56	0.1787	0.7817	0.1787	0.0754	0.4368
		Mean	0.1779	0.7600	0.1792	0.0762	0.4386
1.5	ISO 47	/ ISO 57	0.5543	2.6710	0.5619	0.2673	1.3704
1.5	ISO 48	/ ISO 58	0.6293	2.4434	0.6955	0.2591	1.4389
		Mean	0.5918	2.5572	0.6287	0.2632	1.4047
5	ISO 49	/ ISO 59	2.0716	8.3000	2.0502	1.0073	5.2048
5	ISO 50	/ ISO 60	2.1038	8.4650	2.0472	0.9954	5.1186
		Mean	2.0877	8.3825	2.0487	1.0013	5.1617

Table 8.1.3.1.1-30: Concentrations of Thymol in supernatants and associated with La Reina soil

			Adsorption		Desorption		
Nominal Concentration (µg/mL)	Sample Identification Code		C_e (µg/mL)	X/m (µg/g)	C_e (µg/mL)	C_1 (µg/mL)	X_1/m (µg/g)
0.05	ISO 61	/ ISO 71	0.0253	0.0528	0.0272	0.0070	0.0191
0.05	ISO 62	/ ISO 72	0.0257	0.0456	0.0268	0.0069	0.0185
		Mean	0.0255	0.0492	0.0270	0.0069	0.0188
0.15	ISO 63	/ ISO 73	0.0767	0.1180	0.0797	0.0244	0.0597
0.15	ISO 64	/ ISO 74	0.0707	0.1359	0.0870	0.0234	0.0578
		Mean	0.0737	0.1269	0.0833	0.0239	0.0588
0.5	ISO 65	/ ISO 75	0.2982	0.3515	0.3453	0.0725	0.2099
0.5	ISO 66	/ ISO 76	0.3236	0.3145	0.3132	0.0894	0.2022
		Mean	0.3109	0.3330	0.3293	0.0809	0.2061
1.5	ISO 67	/ ISO 77	1.0345	0.9819	1.0571	0.3314	0.5285
1.5	ISO 68	/ ISO 78	1.0150	1.1114	1.0521	0.2957	0.5323
		Mean	1.0248	1.0467	1.0546	0.3135	0.5304
5	ISO 69	/ ISO 79	4.0674	3.1374	3.7382	1.1530	1.6405
5	ISO 70	/ ISO 80	3.8652	3.6736	3.8983	0.9406	1.7193

	Mean	3.9663	3.4055	3.8183	1.0468	1.6799

The coefficients K_d and K_{OC} were determined at each concentration, for the adsorption phase.

Parameter	RefeSol 01-A (CS18/18)	RefeSol 03-G (CS19/18)	Kenslow (CS20/18)	La Reina (CS08/19)
Method	Direct	Direct	Direct	Direct
Texture (UK)	sandy loam	silty clay loam	clay loam	clay
pH (0.01M $CaCl_2$)	5.4	6.0	5.0	7.7
%OC	1.0	4.6	3.8	1.1
Adsorption				
K_F* (95% confidence intervals)	1.710 (1.599-1.828)	5.361 (4.671-6.154)	4.137 (3.799-4.505)	1.021 (0.907-1.149)
K_{FOC}*	**171.0**	**116.6**	**108.9**	**92.8**
1/n (95% confidence intervals)	0.908 (0.876-0.941)	0.929 (0.873-0.984)	0.958 (0.923-0.992)	0.830 (0.776-0.885)
r^2	0.998	0.995	0.998	0.994
K_d (mean)	1.93	6.19	4.48	1.32
K_{OC} (mean)	192.82	134.6	117.81	120.33
Mass balance (highest concentration)	98.5	73.7	75.6	94.7
Desorption				
K_F*	2.507	5.816	5.206	1.624
K_{FOC}*	250.7	126.4	137.0	147.6
1/n	0.907	0.881	0.978	0.885
r^2	0.999	0.998	1.000	0.995
K_d (mean)	3.14	8.13	5.53	2.22
K_{OC} (mean)	313.91	176.70	145.49	201.59
Mass balance (highest concentration)	98.1	79.0	77.6	98.7

K_F, K_{FOC}, Kd and K_{OC} all have the units L/kg.
* From linear isotherms.
RefeSol 03-G and Kenslow had a mass balance < 90% but > 70%. This was considered acceptable because the compound is known to be unstable, and the direct method of calculation was used to calculate the Freundlich coefficients.

C OECD 106 QUALITY CHECKS

All relevant quality checks were performed by the applicant as part of confirming the acceptability of the study and of the reported endpoints. The results summary is provided below.

Table 8.1.3.1.1-31: Summary of OECD quality checks for thymol

Soil	Units	RefeSol 01-A (CS 18/18)	RefeSol 03-G (CS19/18)	Kenslow (CS 20/18)	La Reina (CS 08/19)
Adsorption method	-	Direct measurement of aqueous and soil phases			
Soil:solution ratio	g dw/mL	1:5	1:5	1:5	1:5
Mass balance of test item (at highest tested conc.)*	%	98.5	73.7	75.6	94.7
Adsorbed percentage	%	20.7-26.8	35-51.7	28.2-35.3	12.5-21
K_d × (soil: solution ratio)	-	4.19-7.50	7.46-11.78	5.80-8.39	1.39-3.93
$^{ads}K_F$ (95% confidence interval)	L/kg dw	1.710 (1.599-1.828)	5.361 (4.671-6.154)	4.137 (3.799-4.505)	1.021 (0.907-1.149)
$^{ads}1/n$ (95% confidence interval)	L/kg dw	0.908	0.929	0.958	0.830
$^{ads}R^2$	-	0.998	0.995	0.998	0.994
$^{ads}K_{F, OC}$	L/kg OC	171.0	116.6	108.9	92.8
K_{fE} /K_f	-	Not required for direct method			

* Mass balance in OECD 106 is defined as the percentage of test substance which can be analytically recovered and does not include non-extracted residues or degradation products
** Calculated as % Concentration on soil/Initial concentration x 100

No pH dependence was observed between K_{FOC} and pH ($CaCl_2$). No correlation was seen between K_F and OC. Overall, this study is deemed acceptable according to OECD.

III CONCLUSION

The adsorption properties of Thymol were studied in four sterilised soils: namely RefeSol 01-A, RefeSol 03-G, Kenslow and La Reina soil using the direct method of determination.

Mean values for partition coefficients (K_d) per soil were in the range 1.32 to 6.19 L/kg for adsorption and 2.22 to 8.13 L/kg for desorption. When corrected for organic carbon, the corresponding K_{OC} values were in the range 117.81 to 192.82 L/kg for adsorption and 145.49 to 313.91 for desorption.
Freundlich coefficients (K_F) were in the range 1.021 to 5.361 L/kg for adsorption and 1.624 to 5.816 L/kg for desorption. When corrected for organic carbon, the corresponding K_{FOC} values were in the range of 92.8 to 171.0 L/kg for adsorption and 126.4 to 250.7 L/kg for desorption. The corresponding 1/n values ranged from 0.830 to 0.978.
Using the McCall Classification scale to assess a chemical's potential mobility in soil (based on its mean K_{OC}), Thymol can be classified as having a "high" potential mobility in RefeSol 03-G, Kenslow and La Reina soils, and "medium" potential mobility in RefeSol 01-A soil.

3. Assessment and conclusions

Assessment and conclusion by applicant:
This study was performed on the non-radiolabelled Thymol, due to issues with radiosynthesis. This study was performed well and passes the OECD quality checks. The K_{FOC} ranges from 92.8 to 171.0 L/kg. The data from this study was used for the updated risk assessments in MCP-9 for Mevalone.

Assessment and conclusion by RMS:
A new adsorption/desorption according to OECD 106 has been submitted since the previous one was not considered valid in the assessment of the confirmatory data.
Cashmore, 2021 studied the adsorption of thymol in four different soils: RefeSol 01-A, RefeSol 03-G, Kenslow and La Reina using a standard batch equilibrium method, in the dark at 20 ± 2°C. The test was performed with non-radiolabelled thymol and direct method was employed.

Regarding to the preliminary test, the instability of thymol was confirmed being more evident in Kenslow soil. The selected soil:solution ratio was 1:5 in all cases. At this ratio the adsorption was greater than 20% for all soils. It is noted that for this preliminary test, the percentage of applied test item in the soil was calculated by difference with the concentration in the supernatant. Therefore, the possible degradation of thymol in soil was not accounted. However, even taking into account that this adsorbed percentage could be overestimated, it is considered that the reached adsorbed percentages are high enough (28-63.8%) in order to obtain reliable adsorption parameters.

The selection of equilibrium times for the definitive tests were considered appropriate with 24 for RefeSol 01-A and RefeSol 03-G and 3 hours for Kenslow and La Reina soil. As in the case of the soil:solution ratio preliminary test, the adsorbed percentage of thymol in soil was theoretically calculated. Since Kenslow and La Reina soil were seen to be more instable, the increase in the adsorption could be due to the degradation of thymol, therefore shorter equilibrium times was selected for these soils (3 hours); In case that at 3 hours, the equilibrium would not have been reached, it would be a worst case of adsorption.

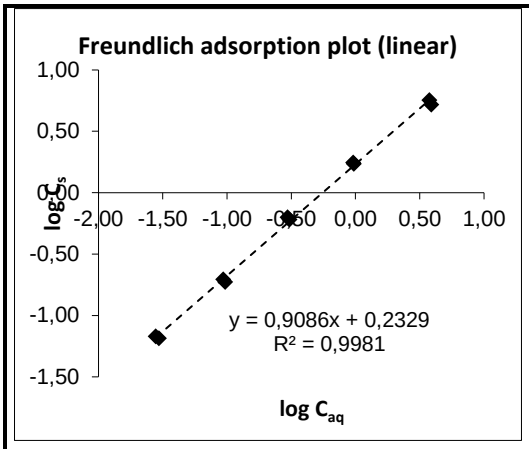
Regarding to the definitive test, the mean mass balance was 98.5% for RefeSol 01-A, 73.7% for RefeSol 03-G, 75.6% for Kenslow and 94.7% for La Reina soils. These percentages are within the normal range considering that the direct method was followed. It is noted that since non-radiolabel thymol was used for the experiment, the non-extractable residues could not be determined.
From the study report, the Kd × (soil: solution ratio) were calculated to be above the recommended limit of 0.3 for all soils. The percent adsorbed in the definitive test ranged from 12.5 to 51.7 (tables 11 to 14 from document K). It is noted that, calculation of % adsorbed to the soil was calculated from the quantification of the test item in calcium chloride and theoretical calculation of the amount of test item soil. Considering that the weight of thymol adsorbed to soil has been reported for each sampling point, it is not clear why the actual percentage of thymol adsorbed in soil was not reported. Since the adsorbed percentage in soil is part of the OECD quality checks, this information should be addressed.

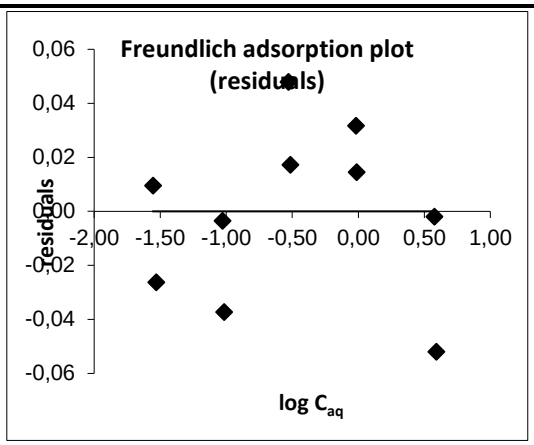
Freundlich coefficients (KF) were in the range 1.021 to 5.361 L/kg for adsorption and 1.624 to 5.816 L/kg for desorption. When corrected for organic carbon, the corresponding KFOC values were in the range of 92.8 to 171.0 L/kg for adsorption and 126.4 to 250.7 L/kg for desorption. The corresponding 1/n values ranged from 0.830 to 0.958.
Using the McCall Classification scale to assess a chemical's potential mobility in soil (based on its mean KOC), Thymol can be classified as having a "high" potential

GC-MS methods were used to analyse supernatants (SM 3203040-01V.C) and soil pellets (SM 3203040-01V.S) for Thymol. The validation samples were prepared at the chosen ratio of 1:5 (soil: solution w/v) for Kenslow and La Reina soils, following selection of the optimum ratio during the preliminary tests.
The LOQ was 0.0025 µg/mL for supernatants. For La Reina soil pellets, one confirmatory ion (*m/z* 115.1) failed at 0.0125 mg/kg, therefore the effective LOQ for *m/z* 115.1 was 2.5 mg/kg and the LOQ for the primary ion (*m/z* 135.1) and another confirmatory ion (*m/z* 150.1) was 0.0125 mg/kg. This is acceptable because only the primary ion is required for an OECD 106 study.

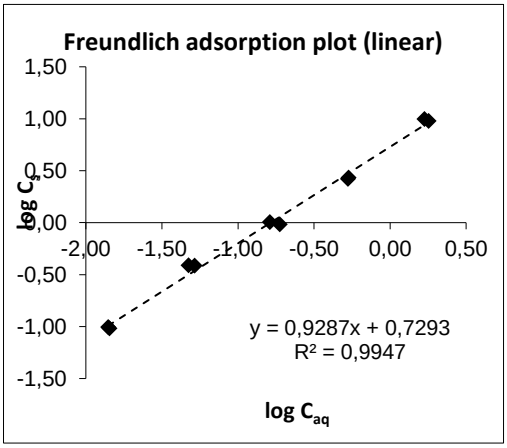
Finally, the graphical fits of the Freundlich equation are presented below based on the standard linear regression form using log-log transformed data alongside the associated residual plots. The R2 of the standard linear regressions ranged from 0.994 to 0.998.
There was a strong positive correlation between KF and % organic carbon. There was no correlation observed between KFOC and pH.

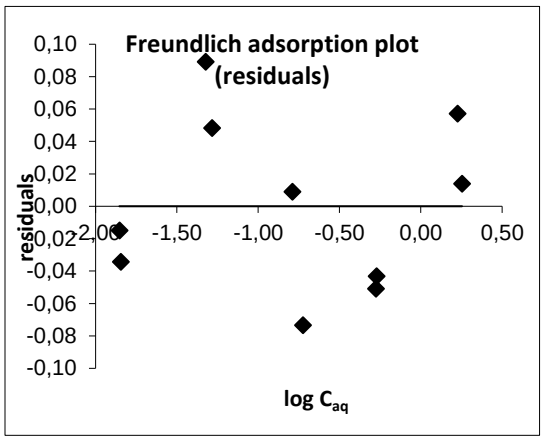
RefeSol 01-A



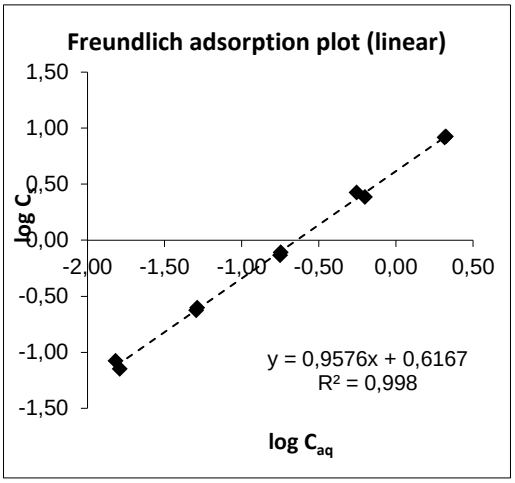


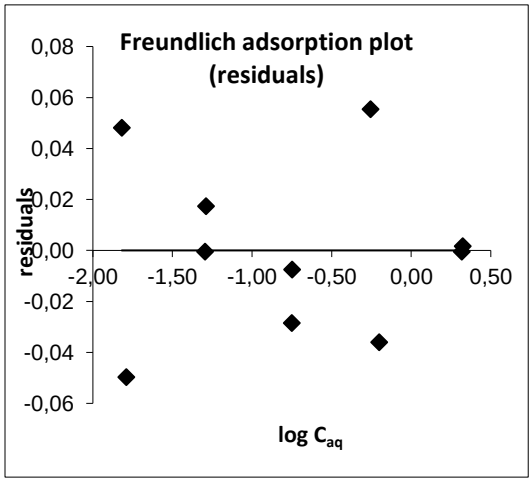
RefeSol 03-G



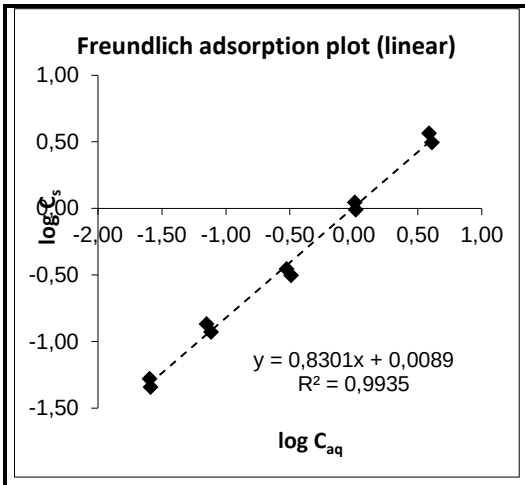


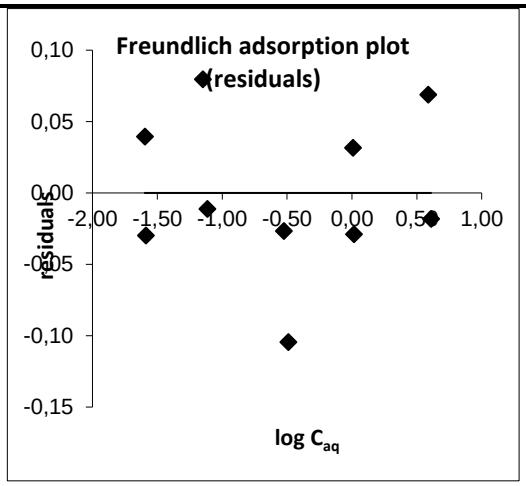
Kenslow





La Reina





Outcome and conclusion of the study:
The study is considered acceptable.
Clarifications should be added to include the actual adsorbed percentage of thymol reached in the definitive test.

B.8.1.3.1.2. Adsorption and desorption of metabolites, breakdown and reaction products
From the data generated under CA 7.1.1.1, in studies designed to investigate the route and rate of aerobic soil degradation of thymol, no major soil metabolites or degradation products are observed at levels exceeding 10% of the applied amount, nor at above 5% of the applied amount for two sequential measurements, nor above 5% of the applied amount at the final measurement.
Therefore, data for metabolites are not required and no studies have been performed or submitted.

B.8.1.3.2 Aged sorption
Aged sorption studies are not required for EU approval.

B.8.1.4 Mobility in soil

B.8.1.4.1 Column leaching studies

B.8.1.4.1.1 Column leaching of the active substance
From the data generated under CA 7.1.3.1.1, reliable sorption data has been generated for the active substance and column leaching data is not required.

B.8.1.4.1.2. Column leaching of metabolites, breakdown and reaction products
From the data generated under CA 7.1.1.1, in studies designed to investigate the route and rate of aerobic soil degradation of thymol, no major soil metabolites or degradation products are observed at levels exceeding 10% of the applied amount, nor at above 5% of the applied amount for two sequential measurements, nor above 5% of the applied amount at the final measurement.
Therefore, data for metabolites are not required and no studies have been performed or submitted

B.8.1.4.2. Lysimeter studies
No data submitted.

B.8.1.4.3. Field leaching studies
Field leaching studies are not required for EU approval. Sufficient data are available for modelling of leaching using standard EU scenarios and are submitted under CP 9.2.4.

B.8.2. FATE AND BEHAVIOUR IN WATER AND SEDIMENT

B.8.2.1. Route and rate of degradation in aquatic systems (chemical and photochemical degradation)

B.8.2.1.1. Hydrolytic degradation

1. Information on the study

Data point	CA 7.2.1.1/01
Report author	Kelly, D. and Bluemink, S.
Report year	2021
Report title	Thymol – Hydrolysis of thymol in Sterile Buffer at pH 4,7 and 9
Report No.	Smithers ERS Ltd No. 3202788
Document No.	-
Guidelines followed in study	OECD 111
Deviations from current test guideline	None
Previous evaluation	No
GLP/Officially recognised testing facilities	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability	Yes

2. Full summary

The hydrolysis of thymol was studied in the dark in sterile aqueous buffered solutions at pH 4 (acetate buffer), pH 7 (phosphate buffer) and pH 9 (borate buffer). The aqueous solutions were analysed by gas chromatography-mass spectrometry (GC-MS) analysis using a calibration curve.
The specificity and efficiency of the analytical method was tested at each sampling interval by the inclusion of control (untreated) and recovery samples (fortified after sampling with a known amount of thymol), which were quantified in the same way as the test samples.
The method was validated with regards to accuracy, precision, linearity, specificity and limit of quantification (LOQ). The limit of detection (LOD) was calculated and the buffer matrix effects were assessed.

Tier I Test
Solutions of aqueous buffers were prepared at pH 4, 7 and 9 at 50 ± 0.5°C then sterilised by autoclaving. Thymol (in acetonitrile, 25 µL), was applied to glass vials containing buffer (3 mL) to achieve a final concentration of 1.0 mg thymol /L. Duplicate vessels were analysed at the start and after incubation at 50°C for 0.1, 1 and 5 days.
Additional vials, treated with thymol, were incubated concurrently with the test samples and were used to confirm pH and sterility at the end of the incubation.
Tier II Test
Solutions of aqueous buffer were prepared at pH 9 at 40, 50 and 60 ± 0.5°C. Thymol (in acetonitrile, 25 µL), was applied to glass vials containing buffer (3 mL) to achieve a final concentration of 1.0 mg thymol /L. Duplicate vessels at each of seven sampling intervals (including 0 DAT), were incubated for up to 31 days.
Additional vials, treated with thymol, were incubated concurrently with the test samples and were used to confirm pH and sterility at the end of the incubation.
Degradation rates were determined by single first order (SFO) kinetics according to FOCUS kinetic modelling.
Findings

Tier I Test
In the Tier I test, thymol was shown to be stable at 50 ± 0.5°C in pH 4 and 7 buffers, and unstable in pH 9 buffer.
The results of the Tier I test are summarised below:

pH	Recovery of thymol	
	0 Day	5 Day
4	101.5	92.1
7	94.5	88.2
9	100.1	89.3

In pH 4 and 7 buffers, no significant change in the levels of thymol was observed (< 10%). Tier II test was performed at pH 9 as > 10% decrease in the levels of thymol was observed.

Tier II Test
The temperature generally remained constant throughout each of the incubation periods and there was no significant variation in the pH values of the buffered solutions. The samples also remained sterile throughout the study.
Thymol decreased from mean values of 86.8 to 87.8% at 0 DAT to between 46.3% at 60°C and 81.0% at 40°C at the end of incubation.
The $DegT_{50}$ and $DegT_{90}$ values were calculated using non-linear regression and first-order kinetics (SFO) and are summarised below:

Temperature (°C)	pH 9 - SFO				
	$DegT_{50}$ (days)	$DegT_{90}$ (days)	k (day^{-1})	Chi^2	r^2
40	228	759	0.003035	1.08	0.5886
50	82.4	274	0.008411	1.94	0.8583
60	36.1	120	0.01919	2.43	0.9296

At pH values of 4 and 7, Tier I testing showed < 10% hydrolysis of thymol occurred at 50 ± 0.5°C after 5 days (representing a $DegT_{50}$ at 25°C > 1 year) and therefore the test substance was considered hydrolytically stable at pH 4 and 7.
In the Tier I test at pH 9, there was > 10% hydrolysis of thymol at 50 ± 0.5°C after 5 days. Tier II testing at 40, 50 and 60 ± 0.5°C was performed. From the results of these elevated temperature tests, the degradation half-life at 25°C was calculated as 1048.7 days indicating that thymol should also be considered hydrolytically stable at environmentally relevant temperatures under basic conditions.

I. MATERIALS AND METHODS
A MATERIALS
Test material 1

Name:	Thymol
Description:	Colourless crystals
CAS:	89-83-8
Lot/Batch no.:	THY/02/2019-20
Purity:	99.58%
Storage:	Room temperature

Internal standard

Name:	2,6-Diisopropylphenol
Description:	Dark yellow liquid
Lot/Batch no.:	MKBK7900V
Purity:	99.0%
CAS #:	2078-54-8
Storage/stability:	Room temperature

B STUDY DESIGN
Experimental conditions
Test System

Preparation of Buffers
Buffers were prepared at room temperature.
Acetate buffer, pH 4
0.01M Acetate buffer solution was prepared by mixing 0.1M acetic acid solution with 0.1M sodium acetate solution and diluting with Milli-Q water.
Phosphate buffer, pH 7
0.01M Phosphate buffer was prepared by dissolving potassium dihydrogen orthophosphate in Milli-Q water.
Borate buffer, pH 9
0.01M Borate buffer was prepared by addition of boric acid and sodium hydroxide solution (2M) and diluted in Milli-Q water.

Tier I

The incubation vessels were prepared by dispensing 3 mL of the appropriate sterile buffer solution into a sterilised (by autoclaving) hydrolysis vessel and sealing with a crimp cap. The dispensing was performed under aseptic conditions in a laminar flow hood.

Incubation Conditions and Sampling Intervals
With the exception of two vessels per pH that were used for 0 day analyses (A1, A2, B1, B2, C1 and C2), all prepared samples were placed in a water bath set at 50°C. The incubation was performed in the dark. Measured temperatures were recorded daily throughout the incubation period using a calibrated thermometer.
Following incubation at 50°C, samples were removed for analysis.

Tier II test
The incubation vessels were prepared by dispensing 3 mL of the sterile pH 9 borate buffer solution into a sterilised (by autoclaving) hydrolysis vessel and sealing with a crimp cap. The dispensing was performed under aseptic conditions in a laminar flow hood.

Test Substance Application
On each dosing occasion, 25 µL of Application Solution 2 was applied into vessels containing buffer solution under aseptic conditions. Test substance application occurred on multiple occasions, where samples were added to each incubation, such that their incubation terminated with the 30 ± 1 DAT samples.
Vessels, containing appropriate buffer solution intended for post dose pH and sterility were treated with 25 µL of Application Solution 2 under aseptic conditions and were incubated for 30 ± 1 DAT.
Each vessel was gently mixed.
Incubation Conditions and Sampling Intervals
Samples were incubated in a water bath at the required temperature for up to 30 ± 1 days under dark conditions. Measured temperatures were recorded daily throughout the incubation period using a calibrated thermometer.
Duplicate samples at each temperature were incubated for 0, 5±1, 10±1, 15±1, 20±1, 25±1 and 30±1 days.
Duplicate pH samples were taken at the start and end of the test and sterility samples were taken at the end of the test only.
Analysis was performed by GC-MS method.

II RESULTS
A Tier I
The results of the Tier I test are summarised below:

Table 8.2.1.1-1: Recovery of thymol in buffers incubated at 50°C expressed as a percentage of nominal concentration 1.0 µg/mL (Tier I Test)

	Recovery of thymol Time after application (days)											
	0			0.1			1			5		
pH	A	B	**Mean**	A	B	**Mean**	A	B	**Mean**	A	B	**Mean**
4	102.0	101.0	**101.5**	96.7	95.3	**96.0**	95.6	94.8	**95.2**	89.9	94.2	**92.1**
7	89.0	100.0	**94.5**	85.4	90.5	**88.0**	102.0	103.0	**102.5**	92.3	84.0	**88.2**
9	101.0	99.2	**100.1**	90.6	98.4	**94.5**	89.5	91.7	**90.6**	91.6	87.0	**89.3**

Table 8.2.1.1-2: Concentration of thymol in buffers incubated at 50°C (Tier I Test)

	Concentration of thymol in µg/L Time after application (days)											
	0			0.1			1			5		
pH	A	B	**Mean**	A	B	**Mean**	A	B	**Mean**	A	B	**Mean**
4	1.019	1.007	**1.013**	0.967	0.953	**0.960**	0.956	0.948	**0.952**	0.899	0.942	**0.921**
7	0.890	1.001	**0.945**	0.854	0.905	**0.879**	1.023	1.026	**1.024**	0.923	0.840	**0.882**
9	1.015	0.992	**1.003**	0.906	0.984	**0.945**	0.895	0.917	**0.906**	0.916	0.870	**0.893**

In pH 4 and 7 buffers, only minor degradation (< 10%) was observed during the 5-day incubation period and therefore no Tier II testing was required. Tier II testing was performed at pH 9 as > 10% decrease in the levels of thymol was observed.

B Tier II

A summary of the GC-MS analysis is shown below:

Table 8.2.1.1-3: Mean recovery of thymol in pH 9 samples expressed as a percentage of nominal concentration 1.0 µg/mL (Tier II Test)

Nominal Sampling Interval (days)	0	5	10	15	20	25	30
Replicate	**Mean**	**Mean**	**Mean**	**Mean**	**Mean**	**Mean**	**Mean**
Temperature (°C)							
40	86.8	87.1	83.4	81.8	80.0	80.0	81.0
50	86.9	84.7	78.2	76.9	69.4	72.3	67.7
60	87.8	75.7	69.9	67.0	59.5	53.6	46.3

Table 8.2.1.1-4: Mean concentration of thymol in pH 9 buffer (Tier II Test)

Nominal Sampling Interval (days)	0	5	10	15	20	25	30
Replicate	**Mean**	**Mean**	**Mean**	**Mean**	**Mean**	**Mean**	**Mean**
Temperature (°C)							
40	**0.867**	**0.871**	**0.834**	**0.818**	**0.800**	**0.800**	81.0
50	**0.868**	**0.847**	**0.782**	**0.769**	**0.694**	**0.723**	67.7
60	**0.878**	**0.757**	**0.699**	**0.670**	**0.594**	**0.535**	46.3

C Rate of degradation

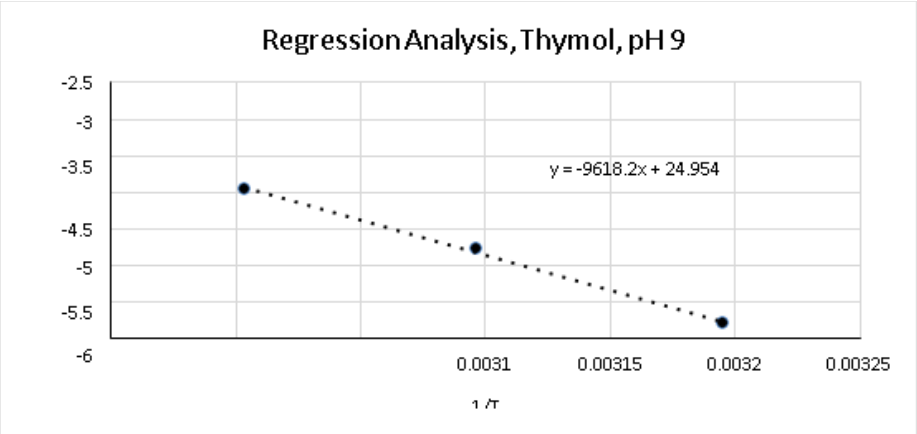
The degradation rate ($DegT_{50}$) of thymol was determined using non-linear regression and single first order kinetics model. SFO kinetics describes the degradation of thymol adequately.

The $DegT_{50}$ values at pH 9, calculated by CAKE were:

Table 8.2.1.1-5: Summary of kinetics

Temperature (°C)	SFO					
	$DegT_{50}$ (days)	$DegT_{90}$ (days)	k (day^{-1})	Chi^2	r^2	Prob > t
pH 9						
40	228	759	0.003035	1.08	0.5886	6.61E-4
50	82.4	274	0.008411	1.94	0.8583	1.01E-6
60	36.1	120	0.01919	2.43	0.9296	1.97E-8

A plot of 1/T (in Kelvin) against the natural log of the rate constant resulted in the following straight-line plot:



Using an Arrhenius plot, estimation of the rate constant (k) and the $DegT_{50}$ at 25°C was performed. For thymol, the $DegT_{50}$ at 25°C was estimated as 1048.7 days, at pH 9.

III CONCLUSION

At pH values of 4 and 7, Tier I testing showed < 10% hydrolysis of thymol occurred at 50 ± 0.5°C after 5 days (representing a $DegT_{50}$ at 25°C > 1 year) and therefore the test substance was considered hydrolytically stable at pH 4 and 7.

In the Tier I test at pH 9, there was > 10% hydrolysis of thymol at 50 ± 0.5°C after 5 days. Tier II testing at 40, 50 and 60 ± 0.5°C was performed. From the results of these elevated temperature tests, the degradation half-life at 25°C was calculated as 1048.7 days indicating that thymol should also be considered hydrolytically stable at environmentally relevant temperatures under basic conditions.

3. Assessment and conclusions

Assessment and conclusion by applicant:

This study was performed on the non-radiolabelled Thymol, due to issues with radiosynthesis. It shows that thymol is stable to hydrolysis at environmental temperatures.

Assessment and conclusion by RMS:

The hydrolysis of thymol was studied in the dark in sterile aqueous buffered solutions at pH 4 (acetate buffer), pH 7 (phosphate buffer) and pH 9 (borate buffer). The study was performed with non-radiolabelled thymol

Regarding to the analytical method, the LOQ which was based upon the lowest level that was successfully validated was confirmed to be 0.05 µg/mL for thymol in pH 7 buffer.

According to the study report, in the Tier I test, thymol was shown to be stable at 50 ± 0.5°C in pH 4 and 7 buffers, and unstable in pH 9 buffer. Tier II was only performed at pH 9 since > 10% decrease in the levels of thymol was observed. However, it seems that at pH 7 the behaviour could be considered as similar (see **Table 0-1**) and it is thought that tier II should be also carried out at this pH.

At tier II (pHh 9), thymol decreased from mean values of 86.8 to 87.8% at 0 DAT to between 46.3% at 60°C and 81.0% at 40°C at the end of incubation. The degradation rate at 25ºC was calculated to be 1048.7 days.

The study did not include the identification of any degradation products, however, since thymol could be considered stable under natural conditions, no metabolites are expected to appear at concentrations of significance.

Outcome and conclusion of the study:

The study is considered acceptable.

For thymol, the $DegT_{50}$ at 25°C was estimated as 1048.7 days, at pH 9. Thymol is considered hydrolytically stable at pH 4 and 7.

B.8.2.1.2. Direct photochemical degradation

1. Information on the study

Data point	CA 7.2.1.2/01
Report author	Yeomans, P.
Report year	2022
Report title	Thymol: Photodegradation of Thymol in Sterile, Aqueous Solution
Report No.	Smithers ERS Ltd No. 3202789
Document No.	-
Guidelines followed in study	OECD 316
Deviations from current test guideline	None
Previous evaluation	No
GLP/Officially recognised testing facilities	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability	Yes

2. Full summary

The UV-VIS absorption spectrum of the test substance in pH7 buffer, was measured and used to calculate the maximum possible loss due to photolysis over 30 days. The value of 100% indicated that more than 50% could degrade in sunlight and therefore, in accordance with the guideline, it was appropriate to perform the further tests.

The photodegradation of Thymol was investigated in sterile aqueous buffered solutions. Thymol was applied, at a rate of 2 µg/mL, to sterile pH 7 buffer solutions in individual photolysis vessels or serum vials for incubation in the dark. The treated irradiated buffer solutions were continuously irradiated using light from a xenon arc lamp.

The light source was from Suntest Accelerated Exposure machines. These instruments remove light with a wavelength of < 290 nm and produce light with a similar spectrum to sunlight. The irradiation intensity was adjusted so that the light received per day was approximately equivalent to one day of UK/US summer sunlight (*ca* 25 Watts/m^2, 300-400 nm).

In the test, treated vessels were maintained at *ca* 25 ± 2°C in either dark or light conditions. Duplicate samples were taken from the dark vessels for analysis immediately after treatment and from both dark and light vessels at the other sampling times. Vessels were treated on six occasions and sampled on two occasions to allow incubation times of 0, 1, 3, 8, 14, 21 and 28 days.

The buffered water in the photolysis vessel or serum vial was analysed by adding 2,6-diisopropylphenol (internal standard), ammonium hydroxide and ethyl acetate: hexane (1:1 v/v). The mixture was shaken by hand and an aliquot of the upper organic layer was then transferred to a volumetric flask. The buffer sample in the vessel was then transferred to a centrifuge tube. This was aided by rinsing with further ethyl acetate:hexane. The mixture in the centrifuge tube was shaken for a further 2 minutes and layers allowed to separate. The upper organic layer was then transferred to the volumetric flask combining the extracts. The mixture was diluted to the 10 mL mark with ethyl acetate: hexane (1:1 v/v), mixed and an aliquot transferred to a vial for GC/MS analysis. The limit of quantitation (LOQ) of the procedure was 0.1 µg/mL of Thymol in buffered water with the upper limit of quantitation (ULOQ) of 2.4 µg/mL.

Recoveries of thymol ranged from 91.3% at 0 day to 69.1% at 28 days (irradiated unamended) and from 91.3% at 0 days to 112.2% at 28 days (dark control unamended).

Observation of the plotted data for dark samples showed four sampling times below the calculated degradation curve followed by three sampling times above the calculated degradation curve which corresponded to the two analysis occasions covering four and three incubation times respectively. The mean fortification recoveries for these analysis occasions were 111.4% and 88.3%. For these samples, it appeared that the results were determined by the analysis occasion. As a result, the data (concentration and percent of nominal concentration) were normalised using the mean fortification recovery for the relevant analysis occasion.

Recoveries of thymol ranged from 103.4% at 0 day to 62.0% at 28 days (irradiated normalised) and from 103.4% at 0 days to 100.7% at 28 days (dark control normalised).

The rate of transformation of Thymol in pH 7 buffer under dark and irradiated conditions, was modelled using single first-order (SFO) kinetics and the results were accepted based on visual fit and chi^2 values.

Thymol degraded very slowly in the dark but more rapidly when irradiated with light simulating sunlight. The $DegT_{50}$ value for photolysis only was 59.5 days using unamended data and 33.3 days using normalised data.

Single first-order kinetics provided a good fit for the data. The corrected $DegT_{50}$ values for photolysis of Thymol applicable to Europe and North America (latitudes 30°, 40° and 50°) were 60.6 and 33.9 days for unamended and normalised values respectively. In Japanese spring sunlight the corrected $DegT_{50}$ values for Thymol in irradiated samples were 196 and 110 days for unamended and normalised values respectively.

I. MATERIALS AND METHODS

A MATERIALS

Test material 1 (used in standard rate tests)

Name:	Thymol
CAS:	89-83-8
Description:	Colourless crystals
Lot/Batch no.:	THY/02/2019-20
Purity:	99.58%
Storage:	Room temperature / dark

Test System

0.01 M Phosphate buffer solutions (KH_2PO_4) were prepared at pH 7.0 ± 0.2 using purified water and 0.01 M sodium hydroxide. A UV/VIS spectrum of the buffer and electrical conductivity were measured. The electrical conductivity of the buffer was 1407 µS/cm. The buffer was sterilised by autoclaving prior to use in the study.

B EXPERIMENTAL CONDITIONS

The application rate of 2 µg/mL, equivalent to 50 µg per unit, was calculated as less than half the water solubility at 20°C and gave a decadic absorbance of less than 0.02 between 290 nm and 800 nm. A solution of Thymol (SSP-9, 50 µL) was dispensed dropwise over the surface of each treated buffer and the vessels were swirled to disperse the test substance.

Vessels were then incubated at *ca* 25°C under xenon lamp or in the dark for up to 28 days.

All preliminary and definitive test samples (0.01M sterile phosphate buffer, 25 mL) were dispensed under sterile conditions.

0 DAT samples were analysed following application. All remaining samples were maintained at 25 ± 2°C. In order to maintain the temperature of the irradiated samples they were placed in an aluminium block, designed to enable cooling water to circulate through it. A thermocouple was placed in an untreated sample containing RO water and positioned in the block alongside the treated samples. Temperature measurements were taken daily throughout the photolysis period. The non-irradiated samples were maintained in an incubator.

Vessels used for photodegradation tests were specially designed vessels of internal diameter *ca* 28 mm, with a sample depth of *ca* 42 mm, and had quartz glass lids, inlets and outlets. Some vessels had an additional injection port, sealed by a PTFE lined crimped top. The side arms were covered with PTFE tape and connected by Marprene tubing so that no air flow occurred and were not connected to flow through traps.

Non-irradiated and zero-time samples were contained in glass serum vials sealed with crimped PTFE-lined rubber caps. They had no air flow and were not connected to traps.

Vessels containing samples for checking pH and sterility at the end of the incubation periods were equivalent to irradiated or non-irradiated vessels.

Vessels were treated on six occasions and sampled on two occasions to allow incubation times of 0, 1, 3, 8, 14, 21 and 28 days.

Atlas Suntest accelerated exposure machines (Atlas Materials Testing Technology, Bicester, UK) were used as the light source.

Sample Analysis

The procedure involved analysing 25 mL of buffered water in a photolysis vessel or serum vial (100 mL) by adding 100 µL of 10 µg/mL 2,6-Diisopropylphenol (internal standard), 1 mL of 0.5 M ammonium hydroxide and 5 mL of ethyl acetate: hexane (1:1 v/v). The mixture was shaken by hand for 2 minutes. An aliquot of the upper organic layer was then transferred to a 10 mL volumetric flask. The buffer sample in the vessel was then transferred to a 50 mL polypropylene centrifuge tube. This was aided by rinsing with a further 5 mL of ethyl acetate: hexane (1:1 v/v). The mixture in the centrifuge tube was shaken for a further 2 minutes and layers allowed to separate. The upper organic layer was then transferred to the same 10 mL volumetric flask combining the extracts. The mixture was diluted to the 10 mL mark with ethyl acetate: hexane (1:1 v/v), mixed and an aliquot transferred to a vial for GC/MS analysis. The limit of quantitation (LOQ) of the procedure was 0.1 µg/mL of Thymol in buffered water with the upper limit of quantitation (ULOQ) of 2.4 µg/mL.

II RESULTS

A Analytical procedures

Linearity

Calibration standards were prepared over the ranges of 0.05 to 10 µg/mL. The line contained eight concentration levels. The correlation coefficients were all acceptable at ≥ 0.99. Residual plots demonstrated visual acceptability of the calibration function.
Matrix Assessment
Matrix matched and non-matrix matched calibration standards were prepared in triplicate at 5 µg/mL and their peak areas compared. Matrix effects were found to be insignificant (< 20%), therefore non-matrix calibration lines were used.

Specificity
Two control samples were analysed in the same sequence as the samples fortified at the LOQ. There were no peaks at the retention time of Thymol > 30% of the LOQ. Therefore the analysis was considered to be specific.
Precision and Accuracy
Five samples were fortified at 0.1 and 2.4 µg/mL. Precision and accuracy were acceptable with an RSD of ≤ 20% and a mean recovery of 70-120% at each level.

Limit of Detection (LOD)
The LOD was defined as the sample concentration equivalent to the lowest calibration standard. As there was no sample dilution, the LOD was 0.05 µg/mL.
Limit of Quantification (LOQ)
The LOQ was the lowest validation level where the validation criteria were met, which was 0.1 µg/mL.

In-Life Sample Analysis
Linearity
Calibration lines were quadratic with 1/x weighting. The correlation coefficients were all acceptable at ≥ 0.99. Residual plots demonstrated visual acceptability of the calibration function.
Specificity
The control buffer extracts were free from components that interfered with the analysis of Thymol. There were no interferences at the retention time of Thymol > 30% of the LOD, therefore, the analytical procedure was considered specific for Thymol.
Accuracy
The mean recoveries at both fortification levels (0.1 and 2.4 µg/mL) were within the acceptable limits (70-120%), confirming the robustness of the analytical procedure.
Light Intensity Measurements
The overall mean light intensity was 25.6 W/m^2 in the 300 to 400 nm range.

B RECOVERIES

Table 8.2.1.2-1: Recovery (%) of Thymol Unamended results

Condition	Time (days)	0	1	3	8	14	21	28
Irradiated	Concentration (µg/mL)	1.8258	1.9014	1.9212	1.5661	1.6554	1.4155	1.3817
	% recovery	91.3	95.1	96.1	78.3	82.8	70.8	69.1
Dark control	Concentration (µg/mL)	1.8258	1.8211	1.8004	1.7354	2.2483	2.1133	2.2443
	% recovery	91.3	91.1	90.0	86.8	112.4	105.7	112.2

Observation of the plotted data for dark samples showed four sampling times below the calculated degradation curve followed by three sampling times above the calculated degradation curve and corresponding to the two analysis occasions covering four and three incubation times respectively. The mean fortification recoveries for these analysis occasions were 88.3% and 111.4%. For these samples, it appeared that the results were determined by the analysis occasion. As a result, the data (percent of nominal concentration) were normalised using the mean fortification recovery for the relevant analysis occasion as follows:

Normalised value = Unamended value × 100 / Mean fortification recovery

Table 8.2.1.2-2: Recovery (%) of Thymol Normalised results

Condition	Time (days)	0	1	3	8	14	21	28
Irradiated	Concentration (µg/mL)	2.0677	2.1533	2.1757	1.7736	1.4860	1.2706	1.2403

	% recovery	103.4	107.7	108.8	88.7	74.3	63.5	62.0
Dark control	Concentration (µg/mL)	2.0677	2.0624	2.0390	1.9653	2.0182	1.8970	2.0146
	% recovery	103.4	103.1	101.9	98.3	100.9	94.9	100.7

C Kinetics

The rate of transformation of Thymol in pH 7 buffer in the dark and irradiated, was modelled using single first-order (SFO) kinetics which was accepted based on visual fit and chi^2 values.

Table 8.2.1.2-3: Degradation rates for thymol - Unamended data

Test System	$DegT_{50}$ [days]	$DegT_{90}$ [days]	χ^2%	r^2
Irradiated (experimental result)	59.5	198	3.81	0.779
Non-irradiated	3.07×10^{12}	1.02×10^{13}	8.37	0.6779
Photolysis	59.5	198	-	-
Corrected $DegT_{50}$ and $DegT_{90}$ for different latitudes				
Summer Sunlight 30-50ºN (OECD)	60.6	202	--	--
Tokyo Spring Sunlight	196	652	--	--

Table 8.2.1.2-4: Degradation rates for thymol - Normalised data

Test System	$DegT_{50}$ [days]	$DegT_{90}$ [days]	χ^2%	r^2
Irradiated (experimental result)	30.8	102	4.15	0.9194
Non-irradiated	413	1370	1.78	0.2321
Photolysis	33.3	111	-	-
Corrected $DegT_{50}$ and $DegT_{90}$ for different latitudes				
Summer Sunlight 30-50ºN (OECD)	33.9	113	--	--
Tokyo Spring Sunlight	110	365	--	--

III CONCLUSION

Photodegradation of Thymol occurred in pH 7 aqueous buffer solutions exposed to simulated sunlight. Degradation in non-irradiated samples was minor.

Single first-order kinetics provided a good fit for the data. The corrected $DegT_{50}$ values for photolysis of Thymol applicable to Europe and North America (latitudes 30°, 40° and 50°) were 60.6 and 33.9 days for unamended and normalised values respectively. In Japanese spring sunlight the corrected $DegT_{50}$ values for Thymol in irradiated samples were 196 and 110 days for unamended and normalised values respectively.

3. Assessment and conclusions

Assessment and conclusion by applicant:
Due to problems with synthesis of the radiolabelled thymol, this study was performed with non-labelled thymol. SFO kinetics gave DT_{50} photolysis values of 60.6 and 33.9 days in Summer Sunlight at 30-50°N for unamended and normalised values respectively.
This study passed all the analytical criteria and was performed well.

Assessment and conclusion by RMS:

This new study was provided in support of the assessment for the renewal of this active substance according to Regulation (EC) No 1107/2009.

The photodegradation of Thymol was investigated in sterile aqueous buffered solutions according to OECD 316. In the test, vessels were treated and maintained at *ca* 25 ± 2°C in either dark or light conditions. The treated irradiated buffer solutions were continuously irradiated using light from a xenon arc lamp. The study was performed with non-radiolabelled thymol.

The validation of the analytical method was assessed against the linearity, matrix assessment, specific, extract stability, precisions and accuracy. The LOD was defined as the sample concentration equivalent to the lowest calibration standard. As there was no sample dilution, the LOD was 0.05 µg/mL. The LOQ was the lowest validation level where the validation criteria were met, which was 0.1 µg/mL.

The mean fortification recoveries were 111.4% (sampling intervals 14, 21 and 28 DAT) and 88.3% (sampling intervals 0,1, 3 and 8 DAT), which is in the range of recoveries for non-labelled chemicals (70-120%). The LOQ was the lowest validation level where the validation criteria were met, which was 0.1 µg/mL.

Recoveries of thymol ranged from 103.4% at 0 day to 62.0% at 28 days (irradiated normalised) and from 103.4% at 0 days to 100.7% at 28 days (dark control normalised).

Normalization of the data was done in view of the dark controls plot, since the sampling points, below and above the degradation curve, could have varied with the fortification's recoveries for these measurements.

As a result, the data (percent of nominal concentration) were normalised using the mean fortification recovery for the relevant analysis occasion as follows:

Normalised value = Unamended value × 100 / Mean fortification recovery

Below, the visual fit of non-normalized and normalized for the degradation of thymol::

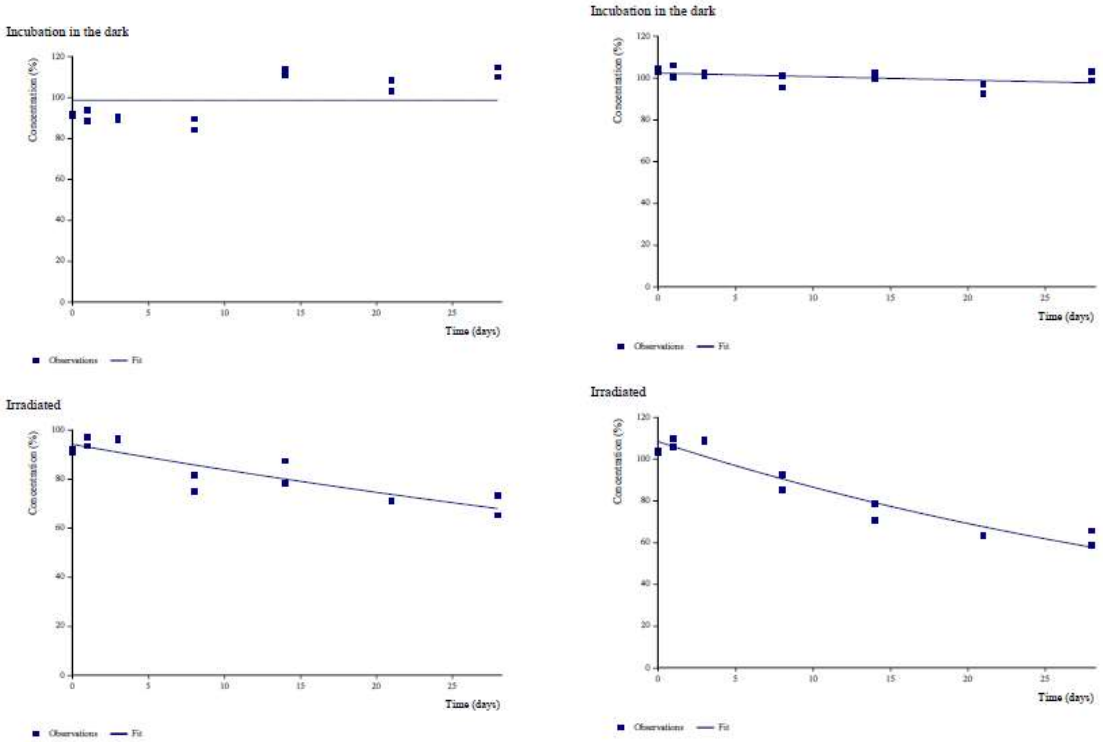


Figure 0-1: Degradation of Thymol in pH 7 Buffer, Unamended Data (left) and Normalised Data (right) under irradiated (bottom) and non-irradiated conditions (up).

Single first-order kinetics provided a good fit for the data. The corrected DegT50 values for photolysis of Thymol applicable to Europe and North America (latitudes 30°, 40° and 50°) were 60.6 and 33.9 days for unamended and normalised values respectively. In Japanese spring sunlight the corrected DegT50 values for Thymol in irradiated samples were 196 and 110 days for unamended and normalised values respectively.

Some deviations were found with respect to the OECD 316 guideline. First of all, the guide states that the major degradation products should be isolate and identify. Additionally, reference substances should be used for the

characterisation and/or identification of phototransformation products by spectroscopic and chromatographic methods. No other reference standard than thymol was employed and therefore, the study did not investigate the possible photodegradation products. Labelled material is required for studying the pathway of phototransformation and for establishing a mass balance.

According to Regulation 283/2013, the identity of breakdown products formed which exceed 10 % of the applied test substance at any time during the study, a mass balance to account for at least 90 % of the applied radioactivity, as well as photochemical half-life (DT50) shall be reported.

Outcome and conclusion of the study:
Supplementary information.
This study is valid to stablish the rate of degradation under irradiated conditions but not the route.
The DT50 of thymol in sunlight was 33.9 days.
A new direct photochemical study with radio-labelled thymol is requested in order to address the route of degradation in water.
The following comment was added by applicant during the co-RMS-applicant´s initial revision: A new study will be performed and submitted.

B.8.2.1.3. Indirect photochemical degradation

Data on indirect photochemical degradation are not required under Commission Regulation (EU) No 283/2013.

B.8.2.2. Route and rate of biological degradation in aquatic systems

B.8.2.2.1. "Ready biodegradability"

The ready biodegradability of thymol has been previously evaluated, and the study remains valid. Thymol is readily biodegradable.

1. Information on the study

Data point	CA 7.2.2.1/01
Report author	B. Seyfried
Report year	2007
Report title	Thymol: Ready Biodegradability in a Manometric Respirometry Test
Report No.	RCC Study number B34435
Document No.	
Guidelines followed in study	OECD: Guideline 301F, Ready Biodegradability: Manometric Respirometry Test, 1992 EU Commission Directive 92/69 EEC, C.4-D, Manometric Respirometry Test, 1992
Deviations from current test guideline	None
Previous evaluation	Yes, DAR 2011
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Yes

2. Full summary

The test item, thymol was investigated for its ready biodegradability in a manometric respirometry test over 28 days. The percent biodegradation of the test item was calculated based on the theoretical oxygen demand (ThOD) of 2.77 mg O_2/mg test item.
The biochemical oxygen demand (BOD) of thymol in the test media significantly increased from about Exposure Day 3 until test termination after 28 days. At the end of the 28-day exposure period, the mean biodegradation of thymol amounted to 87%. Consequently, thymol was found to be biodegradable under the test conditions within 28 days.

Moreover, the pass level for ready biodegradability, i.e. biodegradation of at least 60% of the ThOD in a 10-day window within the 28-day period of the test, was reached.
In conclusion, Thymol can be classified as readily biodegradable under the test conditions.
In the toxicity control, containing both thymol and the reference item sodium benzoate, biodegradation was >25% within 14 days of exposure. Thus, thymol had no inhibitory effect on the activity of activated sludge microorganisms at the concentration of 98 mg/L.
In the procedure controls, the reference item sodium benzoate was degraded by an average of 94% by exposure day 14, and reached an average biodegradation of 99% by the end of the test (day 28), thus confirming suitability of the activated sludge.

I. MATERIALS AND METHODS

A MATERIALS

Test material 1 (used in standard rate tests)

Name:	Thymol
Description:	White solid
Lot/Batch no.:	94747
Purity:	99.7%
Storage :	At room temperature 20°C, in the dark

Reference Item

Name:	Sodium benzoate
Lot/Batch no.:	1274106
Purity:	100.0%
CAS #:	
Storage/stability:	Room temperature at 20°C

B STUDY DESIGN

Experimental conditions

Test system
The study performed with aerobic activated sludge from a wastewater treatment plant (ARA Ergolz II, Füllinsdorf, Switzerland) treating predominantly domestic wastewater.
Calculated amounts of wet sludge were suspended in test water to obtain a concentration equivalent to 4g (±10%) dry material per litre. The sludge was aerated at room temperature for 1 day prior to use. The sludge was thoroughly mixed and then diluted with test water to a concentration of 1 g per litre (dry weight basis). Based on the determined dry weight of this diluted activated sludge defined amounts were added to test water to obtain a final concentration of 30 mg dry material per litre.

Test water
The test water was prepared according to the testing guidelines. Analytical grade salts were dissolved in purified water to obtain the following stock solutions:

1) KH_2PO_4 8.50 g/L; K_2HPO_4 21.75 g/L; Na_2HPO_4 x $2H_2O$ 33.40 g/L; NH_4Cl 0.50 g/L pH of this solution 7.4

2) MgSO4 x $7H_2O$ 22.50 g/L

3) $CaCl_2$ x $2H_2O$ 36.40 g/L

4) $FeCl_3$ x $6H_2O$ 0.25 g/L, stabilised with one drop of concentrated HCl per litre.

To obtain the final test water, 10 mL of stock solution a) and 1 mL each of stock solution b) -d), were combined and made up to 1000 mL with purified water. The pH was adjusted from 7.8 to 7.4 with a diluted hydrochloric acid solution.

Test concentration

A stock solution containing 2.5 g/L sodium benzoate test water was prepared. From this stock solution, 10 mL aliquots were added to the corresponding flasks containing test water that were used as procedure control. Test item solution was at 103 mg/L. Activated sludge was then added to each flask with a final test volume of 250 mL per test flask.

Table 8.2.2.1-1: Test concentrations

Identification	Replicate	Amount of test item		Amount of reference item	
		mg/L	ThOD	mg/L	ThOD
Test item	1	103	285	-	NA
Test item	2	103	284	-	NA
Inoculum control	1	-	NA	-	NA
Inoculum control	2	-	NA	-	NA
Procedure control	1	-	NA	100	167
Procedure control	2	-	NA	100	167
Toxicity control	1	98	271	100	167

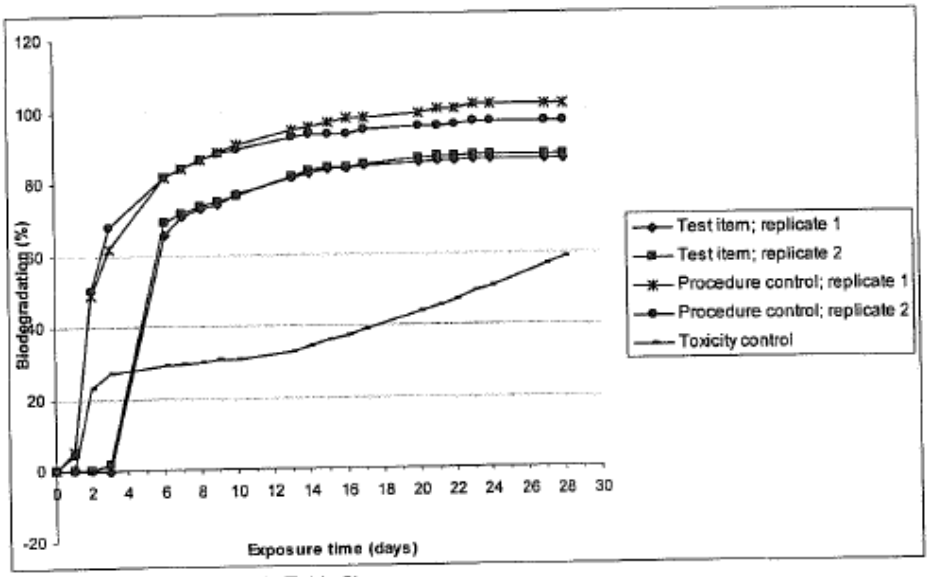
ThOD – theoretical oxygen demand
NA- not available

Test conditions

The test flasks (500mL Erlenmeyer flasks) were incubated under continuous stirring in a SAPROMAT D12. Oxygen consumption was recorded manually by taking a daily reading on each working day. The test duration was for 28 days in the dark at 22°C. Prior to the test, the pH was measured in each test flask before the addition of the activated sludge inoculum. The pH was between 7.2 and 7.3 in all test flasks and thus was not adjusted. At the end of incubation, the pH was measured again in each test flask.

II. RESULTS

The percent biodegradation of the test item, thymol, was calculated based on the theoretical oxygen demand (ThOD) of 2.77 mg O_2/mg test item.
The biochemical oxygen demand of thymol in the test media significantly increased from about Exposure Day 3 until test termination after 28 days. At the end of the 28 day exposure period, the mean biodegradation of thymol was 87%. Consequently, thymol was found to be biodegradable under the test conditions within 28 days and had reached at least 60% of the ThOD in a 10-day window withing the 28-day period.
In the procedure controls, the reference item sodium benzoate was degraded by an average of 94% by exposure day 14, thus confirming suitability of the activated sludge.
The toxicity control, contained both thymol and the reference item sodium benzoate, showed that the test item had no inhibitory effect on activated sludge microorganisms at the tested concentration of 98 mg/L, because biodegradation in the toxicity control was >25% within 14 days.



(based on values presented in Table 2)

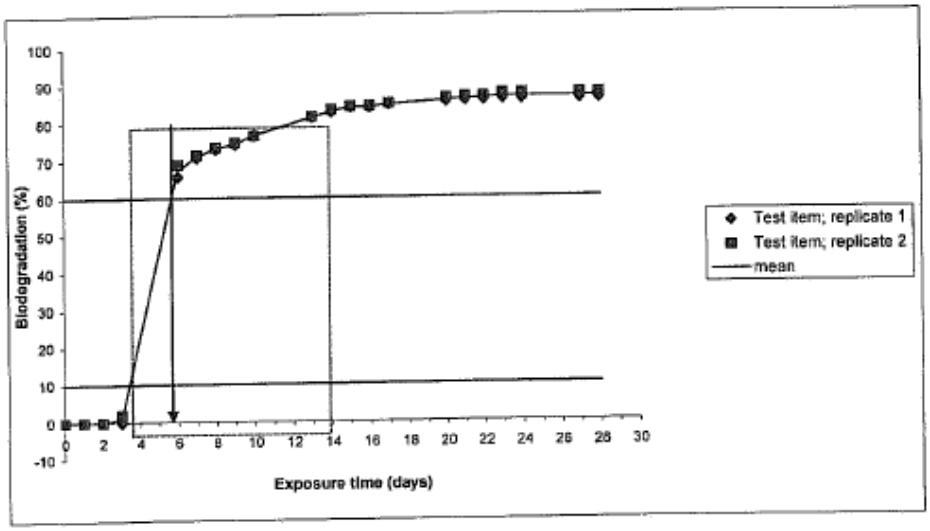


Figure 8.2.2.1 1: Biodegradation in the test flasks during the incubation period. Overview over the whole test series (up). 10-day window for the biodegradation of the test item (down).

III. CONCLUSION

The test item, thymol was readily biodegradable under the conditions of the test.

3. Assessment and conclusions

Assessment and conclusion by applicant:
Results are acceptable from previous evaluation and show thymol is ready biodegradable.

Assessment and conclusion by RMS:
This study was previously evaluated and accepted in DAR 2011.
The ready biodegradability of thymol was determined in a manometric respirometry test over 28 days in accordance with OECD test guideline 301 F and to GLP. Thymol was readily biodegradable under the conditions of the test, the mean degradation was 87% in 28 days.
Outcome and conclusion of the study:
The RMS considers the study to be acceptable.

1. Information on the study

Data point	CA 7.2.2.1/02
Report author	Nabeoka, R, Taruki, M., Kayashima, T., Yoshida, T., Kameya, T.
Report year	2016
Report title	Effect of test concentration in the ready biodegradability test for chemical substances: Improvement of OECD test guideline 301C
Report No.	-
Document No.	Environmental Toxicology and Chemistry, Vl.35, No.1, pp.84-90,2016
Guidelines followed in study	OECD 301C
Deviations from current test guideline	-
Previous evaluation	No
GLP/Officially recognised testing facilities	No
Acceptability/Reliability	2, supporting data

2. Full summary

ABSTRACT (copied from original literature)
In Japan, understanding the environmental persistence of chemicals is very important for risk assessment, and ready biodegradability tests are mainly conducted according to the Organisation for Economic Co-operation and Development test guideline 301C. However, the highest test concentration specified in test guideline 301C, 100 mg/L, may cause microbial toxicity and incomplete biodegradation. The authors performed test guideline 301C tests at test concentrations of 30 mg/L for 13 substances that were readily biodegradable in ready biodegradability tests but not in test guideline 301C tests. Of the 5 substances with potential to cause microbial toxicity at 100 mg/L, the percentage of biodegradation of sodium dimethyldithiocarbamate, 4-chloro-3-cresol (CC), thymol (THY), and p-tert-butyl-a-methylbenzenepropionaldehyde measured by biochemical oxygen demand (BOD) increased in the test guideline 301C test at 30 mg/L, suggesting a reduction in toxicity effects. Furthermore, CC and THY met the criteria for ready biodegradability, which are more than 60% of biodegradation by BOD and a 10-d window. Of the 8 substances with a low potential for causing microbial toxicity at 100 mg/L, the percentage of biodegradation of only 2-(diethylamino)ethanol increased in the test guideline 301C test at 30 mg/L. Employing a lower test concentration in the standard test guideline 301C test will contribute to improvement of consistency between results of a test guideline 301C test and other ready biodegradability tests.

I. MATERIALS AND METHODS
A MATERIALS
Test material

Name:	Thymol
CAS number:	89-83-8
Purity:	>95%

Reference material

Name:	Aniline
CAS number:	62-53-3
Purity:	>99%

Test Conditions
Activated sludge was prepared and cultivated according to the protocol specified in guideline 301C. None of the prepared activated sludge was acclimated to the test substances. Mineral medium at pH7 was prepared according to test guideline 301C.
The test solutions were prepared by using 30 mg/L and 100 mg/L of thymol, incubated at 25±1°C under dark conditions for 28 d while being continuously stirred with a stir bar. Biochemical oxygen demand was continuously measured using a manometric respirometer. Theoretical oxygen demand was calculated on the assumption that no nitrification had occurred.
To investigate the primary degradation quantitative analyses were performed at the end of the incubation period by HPLC with photodiode array detection.

II. RESULTS
The validity criteria concerning the oxygen uptake of the blank control and the reference substance reaching the pass levels by day 14, as stipulated by the OECD guideline, were met for all test substances. The results of the validity test were acceptable with sodium benzoate and aniline degrading at >80%.
At 30 mg/L, thymol degraded under the OECD 301C test method with a biodegradation based on BOD of 82±15.6%, and primary degradation of 100±0%. Thymol showed toxicity to the activated sludge at the higher concentration of 100 mg/L and the primary degradation over the study was 1%.

Assessment and conclusion
Reliability assessment
For full details and justification, please refer to Document K.
This paper confirms the results of the Ready Biodegradation Test, that thymol is readily degraded.
Proposed category: 2 supporting data

Assessment and conclusion by RMS:
This study has been included after the open literature review. The ready biodegradability of thymol was determined in accordance with the OECD test guideline 301C, but at a lower concentration (30 g/L). Thymol was readily biodegradable under the conditions of the test; the mean degradation was 82% in 28 days.
Outcome and conclusion of the study:
Not GLP study.
Supplementary information.

1. Information on the study

Data point	CA 7.2.2.1/03
Report author	N. Nakada, T. Tanishima, H. Shinohara, K. Kiri and H. Takada
Report year	2006
Report title	Pharmaceutical chemicals and endocrine disrupters in municipal wastewater in Tokyo and their removal during activated sludge treatment
Report No.	NA
Document No.	Water Research 40 (2006) 3297-3303
Guidelines followed in study	None
Deviations from current test guideline	None
Previous evaluation	No
GLP/Officially recognised testing facilities	No
Acceptability/Reliability	Reliability 2 - supplementary information

2. Full summary

ABSTRACT (copied from original literature)

We measured six acidic analgesics or anti-inflammatories (aspirin, ibuprofen, naproxen, ketoprofen, fenoprofen, mefenamic acid), two phenolic antiseptics (thymol, triclosan), four amide pharmaceuticals (propyphenazone, crotamiton, carbamazepine, diethyltoluamide), three phenolic endocrine disrupting chemicals (nonylphenol, octylphenol, bisphenol A), and three natural estrogens (17b-estradiol, estrone, estriol) in 24-h composite samples of influents and secondary effluents collected seasonally from five municipal sewage treatment plants in Tokyo. Aspirin was most abundant in the influent, with an average concentration of 7300 ng/L (n ¼ 16), followed by crotamiton (921 ng/L), ibuprofen (669 ng/L), triclosan (511 ng/L), and diethyltoluamide (503 ng/L). These concentrations were 1 order of magnitude lower than those reported in the USA and Europe. This can be ascribed to lower consumption of the pharmaceuticals in Japan. Aspirin, ibuprofen, and thymol were removed efficiently during primary+secondary treatment (490% efficiency). On the other hand, amide-type pharmaceuticals, ketoprofen, and naproxen showed poor removal (o50% efficiency), which is probably due to their lower hydrophobicity (log Kowo3). Because of the persistence of crotamiton during secondary treatment, crotamiton was most abundant among the target pharmaceuticals in the effluent. This is the first paper to report ubiquitous occurrence of crotamiton, a scabicide, in sewage. Because crotamiton is used worldwide and it is persistent during secondary treatment, it is a promising molecular marker of sewage and secondary effluent.

I. MATERIALS AND METHODS

Twenty-four-hour composite samples of plant influent and secondary effluent were taken from five municipal STPs in Tokyo. Samples were filtered through pre-baked glass fibre filters and store at 5°C until solid-phase extraction (SPE). Before storage, part of the filtrates were acidified.
Thymol was extracted using SPE under neutral conditions. 1 litre of filtrate was passed through the cartridge at a flow rate of 15 mL/min. Compounds retained on the cartridge were eluted with 20 mL methanol.
The eluents were methylated with BF3/MeOH (10% [w/w] boron trifluoride in MeOH, for 5 h at 80°C. Methylated derivatives were liquid–liquid-extracted with n-hexane and purified by 5% H_2O-deactivated silica gel column chromatography. The column (1 cm x 9 cm) was eluted with 20mL of hexane/DCM (75:25; v/v), 40mL of DCM, and 30mL of DCM/acetone (70:30; v/v). Thymol was eluted in the DCM fraction.
Compounds in the DCM fraction were derivatized to acetates with pyridine and acetic anhydride before GC-MS analysis. Acetates were extracted with hexane, concentrated to dryness, and redissolved in 50–1000 mL of the IIS isooctane solution for GC-MS analysis.

II. RESULTS

The recovery of thymol in spiked STP effluent was 56% and concentrations were not corrected for recovery.

Thymol was detected in the tens to hundreds ng/L in the influent and was reduced to sub-ng to a few ng/L (<20 ng) in the effluents showing that the removal efficiency of thymol during the treatment in the STP was around 95%.

Assessment and conclusion
Reliability assessment
For full details and justification, please refer to Document K (study x).
Proposed category: 2 reliable with restrictions, supplementary data only
This paper confirms the results of the Ready Biodegradation Test, that thymol is readily degraded in the STP.

Assessment and conclusion by RMS:
This study has been included after the open literature review. The reduction in the concentration of thymol observed between the data measurements in the influent and effluent of the STPs, supports that thymol is readily degraded. Removal efficiency was around 95%.

Outcome and conclusion of the study:
Not GLP study.
Supplementary information.

B.8.2.2.2. Aerobic mineralisation in surface water

1. Information on the study

Data point	CA 7.2.2.2/01
Report author	Simmonds, L
Report year	2021
Report title	Thymol: Aerobic Mineralisation in Surface Water
Report No.	Smithers ERS Ltd No. 3202790
Document No.	-
Guidelines followed in study	OECD 309
Deviations from current test guideline	None
Previous evaluation	No
GLP/Officially recognised testing facilities	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability	Yes

2. Full summary

The degradation rate of thymol in a surface water test system (Oak Beck) was studied at 20 ± 2°C in the dark. The test was performed using two application rates (95 and 10 µg/L). Sterilised samples were also tested at the higher application rate.
After test substance application (thymol dissolved in acetonitrile) to the test system, moistened air was bubbled under the surface of the water under negative pressure and mechanically agitated (stirrer bar) continuously throughout the incubation period, to maintain aerobic conditions.
Sub-samples of non-sterile water were removed for analysis at 0, 1, 2, 4, 7, 15, 21 and 30 DAT (days after treatment) at the low application rate and 0, 1, 2, 4, 7, 15, 21, 30 DAT and 35 DAT at the high application rate. The sterile vessels were sampled at 35 DAT.
Internal standard (2,6-diisopropylphenol) was added to water samples prior to liquid-liquid extraction by shaking with 1M ammonium hydroxide and 1:1, (v/v) ethyl acetate: hexane. A portion of the upper layer was analysed for thymol and internal standard by GC-MS. The ratio of thymol to internal standard was used to quantify thymol against a calibration line.
The specificity and efficiency of the analytical method was tested at each sampling interval by the inclusion of blank control (untreated) and recovery samples (fortified after sampling with a known amount of thymol), which were incubated and processed in the same way as the test samples.
The analytical method was validated with regards to accuracy, precision, linearity, specificity and limit of quantification (LOQ). The limit of detection (LOD) was estimated and the surface water (test system) matrix effects were assessed.
The microbial viability of the test system was assessed by the inclusion of reference substance/ solvent control samples.
The water samples treated with reference substance (sodium [^{14}C]-benzoate) and acetonitrile (blank treatment vehicle) were attached to sodium hydroxide traps (to capture evolved carbon dioxide, $^{14}CO_2$) and incubated under test conditions

for 14 DAT. The extent of mineralisation of the reference substance and mass balance was determined by liquid scintillation counting (LSC).
Matrix effects were insignificant when comparing mean peak areas (< 20% difference from non-matrix standards), therefore non-matrix matched calibration standards were used.
Blank control (untreated) surface water samples were free from components that interfered with the analysis (i.e. no peaks were present at > 30% of the LOQ at the retention time of thymol). Therefore, the analytical procedures were considered to be specific.
Mean recoveries from fortified samples were within the acceptable range of 70-120% and precision (%RSD) was within the acceptable limit of ≤ 20% at each fortification level.
The mineralisation of sodium [^{14}C]-benzoate to $^{14}CO_2$ from the surface water exceeded 50% AR by 7 DAT, the surface water used was microbially active, thus validating the study.
The aerobic degradation of thymol in surface water data did not fit SFO models well but were fitted to HS models.
Under non-sterile conditions, the results showed a lag phase (tB) with relatively slow degradation (k_1) followed by a sudden rapid degradation (k_2). The end of the lag phase varied between the two vessels at each application rate; at the low rate it was 5 and 14 days and at the high rate it was 15 and 21 days.
The existence of a lag phase required modelling of individual vessels using HS kinetics.
The degradation of thymol was significantly slower under sterile conditions. Levels of thymol were <LOD at the end of the respective incubation periods (30 DAT and 35 DAT at the low and high application rates, respectively) in the non-sterile vessels and present at 80% (nominal application rate) at 35 DAT in the sterile vessels.
Aerobic degradation of thymol in a surface water test system was characterised by a variable length lag phase, with slow degradation, followed by a sudden and much more rapid degradation. The data did not fit SFO models well but were fitted to HS models to obtain the following DT_{50} values for the slow and rapid phases for the two application rates tested.
Lag phase duration was 5 and 14 days for the low application rate vessels and was 15 and 21 days for the high application rate vessels.
Degradation was significantly slower under sterile conditions showing that the degradation was primarily microbial.

I. MATERIALS AND METHODS

A MATERIALS

Test material 1 (used in standard rate tests)

Name:	Thymol
CAS:	89-83-8
Description:	Colourless crystals
Lot/Batch no.:	THY/02/2019-20
Purity:	99.58%
Storage:	Room temperature / dark

Reference material

Name:	Benzoic Acid
Description:	Colourless to pale yellow
Lot/Batch no.:	171201
Purity:	99%
Specific activity:	130 mCi/mmol

Natural water was collected on from the Oak Beck stream in Harrogate, UK. The test system was named 'Oak Beck'. Prior to use, the water was stored in the dark in an environmental chamber routinely maintained at 4 ± 2°C, with free access to air. Water was passed through a 100 µm sieve prior to use and subsequent characterisation.

Table 8.2.2.2-1: Characterisation details for the water used to investigate the aerobic mineralisation of thymol

Soil parameter	Value
Source	Oak Beck
Test System code	CS108/21
pH	7.45
Sampling water O2 content mg/L	9.78
Total Organic carbon (TOC) (mg/L)	9.59
Total Phosphorus (mg/L)	0.177
Ammonia (mg/L)	0.124
Total Nitrogen (mg/L)	2.33

Suspended solids (mg/L)	6
Alkalinity (mg/L)	84
Hardness (mg/L $CaCO_3$)	103
Nitrite (mg/L)	<0.015
Nitrate (mg/L)	0.921

B STUDY DESIGN

Experimental conditions

Each incubation vessel was labelled with the study number and a unique sample code.

Water samples were maintained under experimental conditions (continuously stirred at 20 ± 2°C in the dark) for 3 days prior to treatment.

For each vessel (Incubation groups A-E), moistened air was bubbled just under the surface of the sample water via a dip-tube under negative pressure and the effluent air expelled via the vacuum. 0.2 µm bacterial air filters were inserted before and after the incubation vessel for the sterile vessels (group C) to maintain sterility.

Incubation group F: Moistened air was passed over the surface of the water under negative pressure. The effluent air from each vessel was be passed through an individual series of traps, containing 2M NaOH solution for the capture of liberated $^{14}CO_2$.

Vessels in each incubation group were maintained in the dark in a temperature-controlled room at 20 ± 2°C. The contents of the incubation vessels were agitated by continuous stirring with a PTFE magnetic stirrer bar. Blank control vessels were incubated in the same way.

The units were treated with nominal application rates of 10 µg/L (Low rate) and 95 µg/L (High rate).

Non-sterile incubated water vessels were sub-sampled at 0, 1, 2, 4, 7, 14, 15, 21 and 30 DAT at the low application rate (group A) and at 0, 1, 2, 4, 7, 14, 15, 21 and 35 DAT at the high application rate (group B). The sterile vessels (group C) were only sampled at the end of the incubation period (35 DAT). On the day of each sampling interval, the analytical control vessel (group D) was sampled.

The reference/ solvent control vessels (group F) were removed for analysis along with their associated sodium hydroxide traps at 14 DAT. During this incubation period, the sodium hydroxide trapping solutions were removed for analysis and replenished at 2 and 7 DAT.

Sample Analysis

Water Samples

Aliquots (5 mL) from the incubation vessels (group A, B, and C) were transferred into glass tubes with caps. 0.02 mL of 5 µg/mL 2,6-diisopropylphenol (internal standard), 0.25 mL of 1M ammonium hydroxide and 5 mL of ethyl acetate: hexane (1:1 v/v) was added to each sample. The vessels were shaken well by hand and the layers allowed to settle. A portion of the upper layer was analysed by GC-MS.

Reference Vessels / Solvent Controls

The contents of the incubation vessel (excluding the stirrer bar) were transferred into a pre-weighed glass jar labelled 'Extract 1'. The incubation vessel was rinsed with RO water (10 mL) and the rinsate transferred to the Extract 1 jar. The incubation vessel was rinsed again with ethanol (10 mL) and transferred into a pre-weighted jar labelled 'Extract 2'.

The contents of the 2M sodium hydroxide traps were transferred into pre-weighed vials at the time of sampling.

The radioactivity in samples (Extract 1, Extract 2) and traps was determined by measuring the total sample weight and assaying duplicate aliquots by LSC. Weighed portions of these samples were added directly to scintillant (*ca* 5 mL) prior to LSC quantification.

Analytical Procedure

An attempt was made to validate the analytical method at the proposed LOQ of 0.5 ng/mL (5 µg/L) which failed the acceptance criteria due to unacceptable interference in the controls. The validation was repeated at the higher concentration of 1 ng/mL (1 µg/L) where all acceptance criteria were met.

Assay Accuracy and Precision

The assay accuracy and precision (recovery) were determined by the analysis of replicate aliquots of surface water fortified with the test substance, together with a calibration series of standard solutions.

Samples were prepared at the following fortification levels:

- 1 × reagent blank (Milli-Q water in place of surface water)
- 2 × control samples (no fortification with thymol, but with internal standard added)
- 5 × LOQ (Limit of Quantification) at 0.001 µg/mL (1 µg/L)
- 5 × ULOQ (Upper Limit of Quantification) at 0.12 µg/mL (120 µg/L)

The data were considered acceptable when the following criteria were met:

- Calibration standards met the criteria given in the linearity section.
- Amounts found in control samples, at the retention time of the analyte peak, were < 30% of the limit of quantification (LOQ) peak area response.
- The peak area ratio response of the fortified samples was within the calibration range
- The target assay accuracy (defined as the mean percentage determined concentration/actual concentration) at the two validation levels had recoveries between 70 and 120%
- The target assay precision, which is defined as the relative standard deviation (%RSD) of the mean determined concentration at each fortification level, and overall, was ≤ 20%

The identities of thymol and 2,6-diisopropylphenol were confirmed by analysis of the mass spectra collected by the GC-MS detection. The GC-MS system used selected ion monitoring at *m/z* 135.1 for thymol and *m/z* 163.1 for 2,6-diisopropylphenol (internal standard). The internal standard ratio was used for the quantification of thymol

II. RESULTS

A Analytical Procedure

Linearity
The GC-MS detector response for thymol was found to be linear in the range of 0.00015 µg/mL to 0.015 µg/mL and 0.005 to 15 µg/m. The correlation coefficients were all acceptable at ≥ 0.98.

Matrix Assessment
No significant matrix effect (< 20%) was observed, therefore non-matrix matched calibration lines were used.

Specificity
One reagent blank and two control samples were analysed in the same sequence as the samples fortified at the LOQ. There was a small peak in the reagent blank and control samples close to the retention time of thymol (appearing as a split peak shoulder in the LOQ samples). As it was ≤ 30% of the LOQ peak height at the retention time of thymol, it was not considered to interfere with the analysis.

Assay Accuracy and Precision
Five samples were fortified at 0.001 and 0.12 µg/mL. Precision and accuracy were acceptable with an RSD of ≤ 20% and a mean recovery of 70-120% at each level.

Limit of Quantification (LOQ)
The LOQ was the lowest validation level where the validation criteria were met, which was 0.001 µg/mL (equivalent to 1 µg/L).

Limit of Detection (LOD)
The LOD was defined as the sample concentration equivalent to the lowest calibration standard. As the sample dilution factor was 1, the LOD was equivalent to 0.00015 µg/mL (equivalent to 0.15 µg/L). The LOD expressed as nominal application rate (%) at the low and high application rates are 0.2% and 1.5%, respectively.

Accuracy
The mean recoveries at both fortification levels (1 µg/L and 120 µg/L) were within the acceptable limits (70-110%), confirming the robustness of the analytical procedure.

B Recovery and concentrations of thymol

Characterisation details for the water used to investigate the aerobic mineralisation of thymol.

Table 8.2.2.2-1: Recovery (%) of thymol in surface over time

Application Rate		Recovery (%) of Thymol in Surface over time (days)						
	Vessel Codes	0	4	7	15	21	30	35
Low 10 µg/L (Non-Sterile)	A5	102	95	73	24	3	<LOD	NA
	A6	100	88	86	53	10	<LOD	NA
	Mean	**101**	**92**	**80**	**39**	**6**	**<LOD**	**NA**
High 95 µg/L (Non-Sterile)	B5	91	89	84	61	1	1	<LOD
	B6	100	94	84	79	68	1	<LOD
	Mean	**96**	**91**	**84**	**70**	**34**	**1**	**<LOD**
High 95 µg/L (Sterile)	C5	NS	NS	NS	NS	NS	NS	80
	C6	NS	NS	NS	NS	NS	NS	79
	Mean	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**80**

NS = Not sampled. Sterile vessels were sampled at 35 DAT only.
NA = Not applicable. The final sampling interval for the low application rate vessels was at 30 DAT.

Table 8.2.2.2-2: Concentration (µg/L) of thymol in surface over time

Application Rate		Recovery (%) of Thymol in Surface over time (days)						
	Vessel Codes	0	4	7	15	21	30	35
Low 10 µg/L (Non-Sterile)	A5	10.2	9.5	7.3	2.4	0.3	<LOD	NA
	A6	10.0	8.8	8.6	5.3	1.0	<LOD	NA
	Mean	**10.1**	**9.2**	**8.0**	**3.9**	**0.6**	**<LOD**	**NA**
High 95 µg/L (Non-Sterile)	B5	86.6	84.4	79.6	58.4	1.0	1.0	<LOD
	B6	95.1	89.1	79.5	75.0	64.6	1.1	<LOD
	Mean	**90.8**	**86.8**	**79.5**	**66.7**	**32.8**	**1.0**	**<LOD**
High 95 µg/L (Sterile)	C5	NS	NS	NS	NS	NS	NS	76.1
	C6	NS	NS	NS	NS	NS	NS	75.3
	Mean	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**75.7**

NS = Not sampled. Sterile vessels were sampled at 35 DAT only.
NA = Not applicable. The final sampling interval for the low application rate vessels was at 30 DAT.

The degradation of thymol was significantly slower under sterile conditions. Levels of thymol were <LOD at the end of the respective incubation periods (30 DAT and 35 DAT at the low and high application rates, respectively) in the non-sterile vessels and present at 80% (nominal application rate) at 35 DAT in the sterile vessels.
Under non-sterile conditions, the results showed a lag phase with relatively slow degradation followed by a sudden rapid degradation. The end of the lag phase varied between the two vessels at each application rate, at the low rate it was 5 and 14 days and at the high rate it was 15 and 21 days.
The existence of a lag phase required modelling of individual vessels using HS kinetics.

C Kinetics

Concentrations of thymol (% nominal application rate) for individual vessels (0 and 30 DAT for the low application rate and 0 and 35 DAT for the high application rate) were fitted to SFO and HS kinetic models in accordance to FOCUS guidelines.
The degradation of thymol fitted to SFO kinetics resulted in a poor visual fit to the data. Relatively high chi squared values (χ^2) and low r^2 values were obtained and the results are summarised in the table below.

Application Rate	Sample	Single First Order		
		DT_{50}	χ^2	r^2
Low (10 µg/L)	A5	7.9	13.8	0.9412
	A6	10.7	14.2	0.9090
High (95 µg/L)	B5	10.7	21.9	0.8637
	B6	16.3	20.4	0.7904

Note: SFO: single first order kinetics. DT_{50}: Calculated degradation half-life of thymol. χ^2: chi-square statistical value. r^2: coefficient of determination.

The degradation rates for thymol fitted to HS kinetics (model of best fit) are summarised in the following table. There was a good visual fit to the data and χ^2 and r^2 values were much improved relative to SFO. The lag phase was very variable between vessels but overall was shorter at the low application rate and the rapid phase degradation rate was more rapid at the low application rate.

Application Rate	Sample	Hockey Stick						
		DT_{50} (days)						
		Slow Phase (k_1)	Rapid Phase (k_2)	Slow Phase k_1 (day^{-1})	Rapid Phase k_2 (day^{-1})	Lag Phase tB (days)	χ^2	r^2
Low (10 µg/L)	A5	53.6	4.34	0.01294	0.1598	5.499	4.14	0.9958
	A6	32.0	2.49	0.02165	0.2782	13.85	1.70	0.9989
	Mean	**42.8**	**3.42**	**0.01730**	**0.2190**	**9.675**	**2.92**	**0.9974**
High (95 µg/L)	B5	73.6	1.01	0.00942	0.6844	14.61	3.89	0.9964
	B6	46.9	1.47	0.01479	0.4711	20.95	3.23	0.9957
	Mean	**60.3**	**1.24**	**0.01210**	**0.5778**	**17.78**	**3.56**	**0.9961**

Note: DT_{50}: Calculated degradation half-life of thymol. χ^2: chi-square statistical value. r^2: coefficient of determination. k1:.initial rate constant. k2: second rate constant. tB: the time at which the rate constant changed

D Reference Substance

The mineralisation of sodium [^{14}C]-benzoate to $^{14}CO_2$ from surface water exceeded 50% AR by 7 DAT. This demonstrated that the acetonitrile in the application solution did not inhibit the microbial degradation of sodium [^{14}C]-benzoate and the surface water used was microbially active, thus validating the study.
At the end of the 14 day incubation period, the radioactivity present in the treated surface water was determined, allowing a total recovery of radioactivity to be calculated. Values obtained (95.5% AR) indicated complete recovery of radioactivity.

III. CONCLUSION

The degradation of thymol was significantly slower under sterile conditions. Levels of thymol were <LOD at the end of the respective incubation periods (30 DAT and 35 DAT at the low and high application rates, respectively) in the non-sterile vessels and present at 80% (nominal application rate) at 35 DAT in the sterile vessels.
Aerobic degradation of thymol in a surface water test system was characterised by a variable length lag phase, with slow degradation, followed by a sudden and much more rapid degradation. The data did not fit SFO models well but were fitted to HS models to obtain the following DT_{50} values for the slow and rapid phases for the two application rates tested.

Application Rate	DT_{50} (days)	
	Slow phase	Rapid phase
Low (10 µg/L)	**42.8**	**3.42**
High (95 µg/L)	**60.3**	**1.24**

Lag phase duration was 5 and 14 days for the low application rate vessels and was 15 and 21 days for the high application rate vessels.
Degradation was significantly slower under sterile conditions showing that the degradation was primarily microbial

3. Assessment and conclusion

Assessment and conclusion by applicant:

Due to problems with synthesis of the radiolabelled thymol, this study was performed with non-labelled thymol. This meant that released CO_2 (mineralisation) could not be assessed.

Degradation of thymol showed that 50% of thymol was degraded in <21 days. SFO kinetics gave DT_{50} values of 7.9-16.3 days. However, HS kinetics appears to be a better fit with a lag phase seen for both the high and low concentration samples.

This study passed all the analytical criteria and was performed well.

Assessment and conclusion by RMS:

This new study was provided in support of the assessment for the renewal of this active substance according to Regulation (EC) No 1107/2009.

The degradation rate of thymol in a surface water test system (Oak Beck) was studied at 20 ± 2°C in the dark. The test was performed using two application rates (95 and 10 µg/L). The study was performed with non-radiolabelled thymol. The validation of the analytical method was assessed against the linearity, matrix assessment, specific, extract stability, precisions and accuracy. The LOQ was the lowest validation level where the validation criteria were met, which was 0.001 µg/mL.

Mean recoveries from fortified samples were within the acceptable range of 70-120% and precision (%RSD) was within the acceptable limit of ≤ 20% at each fortification level.

The degradation of thymol was significantly slower under sterile conditions. Levels of thymol were <LOD at the end of the respective incubation periods (30 DAT and 35 DAT at the low and high application rates, respectively) in the non-sterile vessels and present at 80% (nominal application rate) at 35 DAT in the sterile vessels.

Concentrations of thymol (% nominal application rate) for individual vessels (0 and 30 DAT for the low application rate and 0 and 35 DAT for the high application rate) were fitted to SFO and HS kinetic models in accordance to FOCUS guidelines. The existence of a lag phase required modelling of individual vessels using HS kinetics.

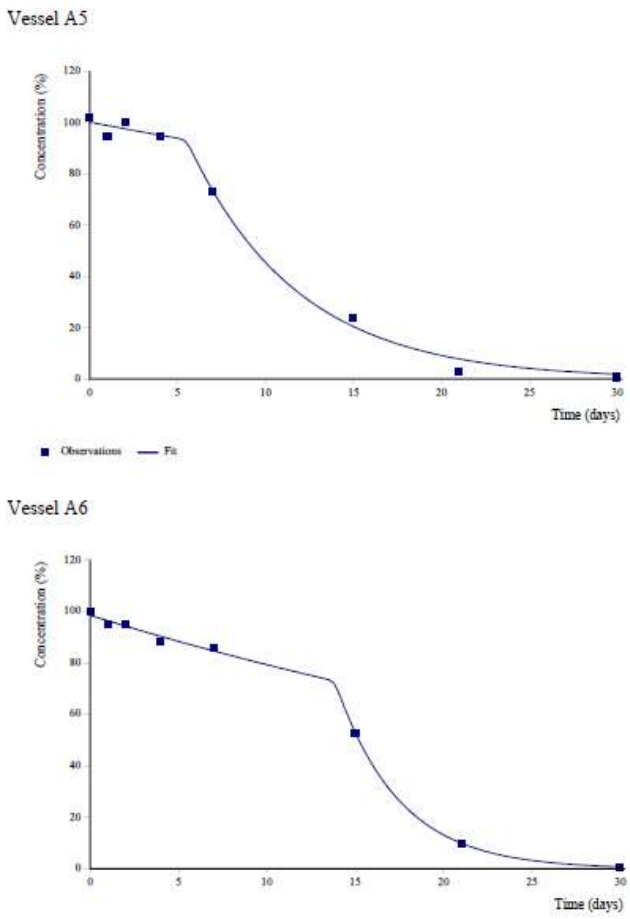


Figure 0-1: Modelling fit: Low application rate (HS)

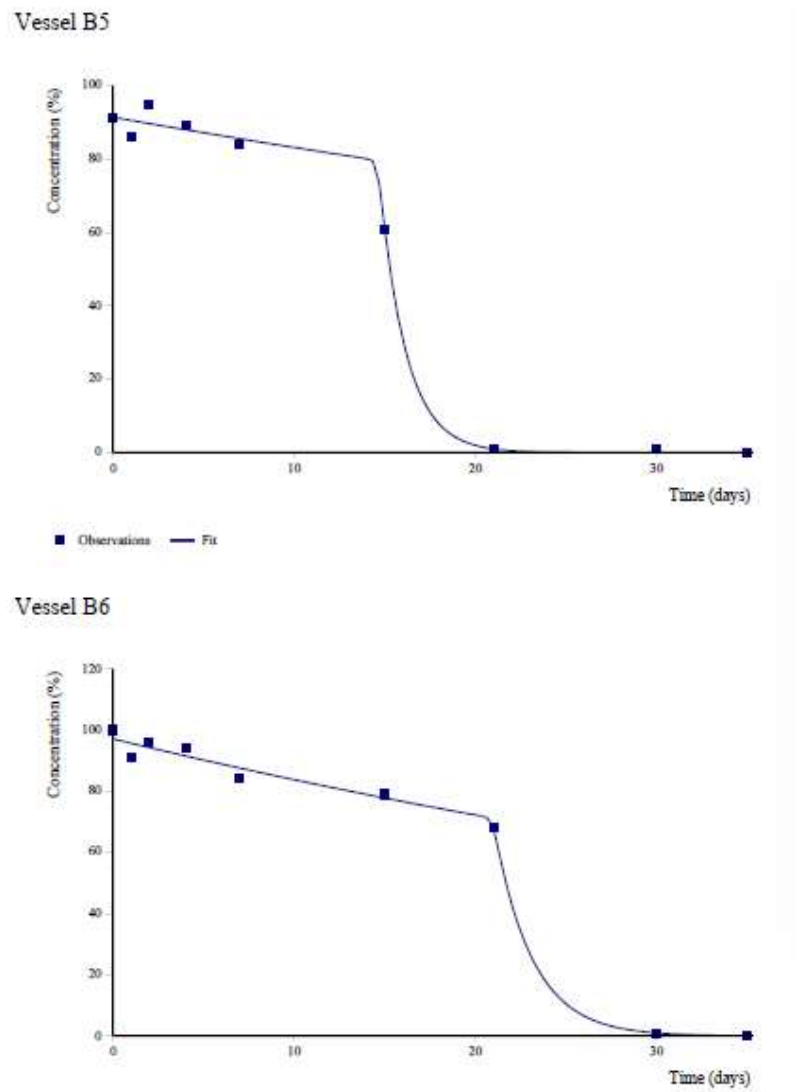


Figure 0-2: Modelling fit: High application rate (HS)

The mineralisation of sodium [14C]-benzoate to 14CO2 from the surface water exceeded 50% AR by 7 DAT, the surface water used was microbially active. However, the extent of mineralization of thymol could not be calculated since non-radiolabelled thymol was used.

According to Regulation 283/2013, the rate of degradation and the pathway or pathways shall be reported either for a 'pelagic' test system or for a 'suspended sediment' system.
Results obtained shall be presented in the form of schematic drawings showing the pathways involved, and in the form of balance sheets which show the distribution of radio-label in water and, where relevant, sediment as a function of time, as between: (a) active substance; (b) CO2; (c) volatile compounds other than CO2; and (d) individual identified transformation products. Therefore, it is considered that this data requirement is not fulfilled.

Outcome and conclusion of the study:
Supplementary information.
This study could be valid to stablish the rate but not the degradation pathway. The levels of reached CO2 could not been determined.
A new aerobic mineralization study using radio-labelled thymol is required.
The following comment was added by applicant during the co-RMS-applicant´s initial revision: A new study will be performed and submitted.

B.8.2.2.3. Water/sediment studies

No data has been submitted.
According to section 7.2.2.3 of Regulation (EU) No. 283/2013, *the water/sediment study shall be reported unless the applicant shows that contamination of surface water will not occur*. **Consequently, RMS has identified a data gap for a water/sediment study.**

1. **Information on the study**

Data point	CA 7.2.2.3/01 (and CA 7.1.2.1.1/02)
Report author	D. Hu and J. Coats
Report year	2008
Report title	Evaluation of the environmental fate of thymol and phenethyl propionate in the laboratory
Report No.	NA
Document No.	Pest Management Science 64:775-779 (2008)
Guidelines followed in study	Not stated
Deviations from current test guideline	^{3}H used, open water system, no trapping of volatiles
Previous evaluation	No
GLP/Officially recognised testing facilities	No
Acceptability/Reliability	2, supporting data

2. **Full summary**

ABSTRACT (copied from original literature)
BACKGROUND: The natural monoterpenoid pesticides thymol and phenethyl propionate (PEP) are used indoors and outdoors, but their fate in the environment has not been reported. In order better to understand their impact on the environment, water metabolism and soil metabolism studies were conducted with thymol and PEP at a concentration of 10 µg g^{-1} in water and in soil under laboratory conditions.
RESULTS: Dissipation half-lives of thymol and PEP were 16 and 5 days in water and 5 and 4 days in soil. 2-Phenylethanol and 2-(4-hydroxyphenyl)ethanol were detected as primary degradation products of PEP. Over time, a considerable volatilization loss of thymol, but not of phenethyl propionate, was found in the 1 month study under the experimental conditions used. Less than 6% of thymol and PEP were detected as bound residues, and less than 3% were mineralized during the 30 day study.
CONCLUSION: In order to maximize the pesticidal effect, more attention should be paid to the temperature for thymol than for PEP when they are being applied, owing to the high volatility of the former. Thymol and PEP pose low risks to the ecosystem because of their rapid dissipation and low bound residues in the environment.

I. MATERIALS AND METHODS
A MATERIALS
Test material

Name:	(^{3}H)-Thymol
CAS number:	NS
Purity:	NS
Specific Activity:	27.7 mCi $mmol^{-1}$

Synthesized by PerkinElmer Life Sciences and Analytical Sciences (Boston, MA)
Test Conditions
Pond water was collected from the Iowa State University Horticulture Farm pond (Ames, IA). The pH of the water was 7.3, the alkalinity was 91 mg mL^{-1} and the total hardness was 182 mg mL^{-1}. Pond water (100 mL) was kept in open 250 mL French square bottles and spiked with ^{3}H-thymol in 300 µL of acetone carrier solvent to result in a concentration of 10 µg mL^{-1} in the pond water. The total radioactivity of thymol was 1.2 µCi. The samples were incubated in dark and in light separately to determine the effect of photodegradation, and were maintained at a constant temperature of 25 ± 2 ◦C throughout the study in the environmental chamber with a 12 h diurnal light cycle of 5000 foot candles of fluorescent light. There were four replications each for the dark group and the light group. Aluminum foil wrapping was used to

prevent 'dark' incubations from having exposure to light. Samples (0.5 mL) were taken at days 0, 0.25, 0.5, 1, 3, 7, 14, 21 and 30 post-treatment, and were stored in the −20 ◦C freezer prior to analysis.
The dissipation half-lives (DT_{50}) were calculated using a first-order dissipation model.

II. RESULTS

Thymol and related degradation products were quantified by a HPLC method coupled with LSC. Recoveries of ^{3}H-thymol were more than 98% from water, as determined by spike-recovery experiments at the initial time point. All measured concentrations of thymol were corrected for their recovery in the study.
The dissipation half-live (DT_{50}) of thymol in water were calculated using the first-order dissipation model. For the water metabolism study, the dissipation half-lives of thymol were determined from the pooled data of the light group and the dark group, because no significant difference in dissipation rates was found for thymol between these two groups. This also indicated that photodegradation was not the major contribution of breakdown in water. The dissipation in water was rapid, with a DT_{50} of 16 days. The total radioactivity in the pond water was directly measured by LSC, and an accumulated loss of 65% of total radioactivity was found in 1 month, based on the difference of the spiked and the recovered amount of thymol from the pond water. This indicated that volatilization was the major factor in thymol dissipation in the open testing system of the water metabolism study.

3. Assessment and conclusions

Assessment and conclusion by applicant:

Reliability assessment

For full details and justification, please refer to Document K.
This paper confirms that thymol degrades fairly rapidly in water with expected volatilisation, as supports the DT50 of 15 days used in the surface water modelling.
Proposed category: 2 supporting data

Assessment and conclusion by RMS:
This study has been included after the open literature review.
The fate of thymol and PEP in pond water and agronomic soil was investigated to determine the dissipation half-life and the identity and quantity of degradation products that form in water or soil. As a part of the environmental fate studies, volatilization, mineralization and bound residues in soil were also determined.
Pond water was collected from the Iowa State University Horticulture Farm Pond and spiked with 3H-thymol or 3H-PEP in 300 µL of acetone carrier solvent to result in a concentration of 10 µg mL−1 in the pond water. The samples were incubated in dark and in light separately to determine the effect of photodegradation, and were maintained at a constant temperature of 25 ± 2 ◦C.
The dissipation of thymol in water was rapid, with a DT50 of 16 days. Volatilization was the major factor in thymol dissipation in the open testing system of the water metabolism study.
In conclusion, thymol dissipates rapidly in water and in soil under aerobic conditions.

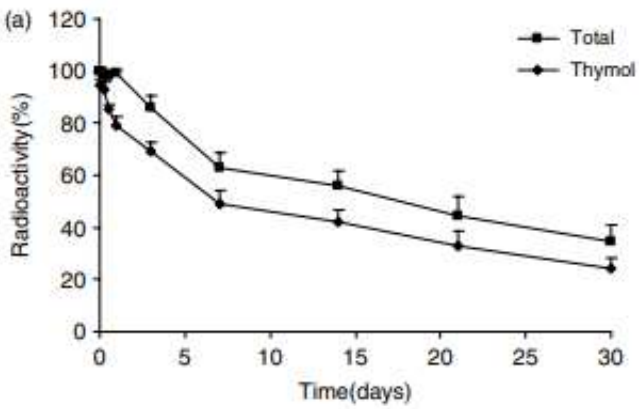


Figure 8.2.2.3-1: Dissipation profile of thymol in water

Outcome and conclusion of the study:
Not GLP study.
Supplementary information.

The following comment was added by applicant during the co-RMS-applicant´s initial revision: A new study will be performed and submitted.

B.8.2.2.4. Irradiated water/sediment

Studies on irradiated water/sediment systems are not required for EU approval.

B.8.2.3. Degradation in the saturated zone

Based on the information described under CP 9.2.4 for the representative product, thymol is not expected to leach through the soil profile to the saturated zone in significant quantities. Therefore, no further studies have been carried out.

B.8.3. FATE AND BEHAVIOUR IN AIR

Adequate data to assess the route and rate of degradation of thymol in air were evaluated during the first EU review and no further data are considered necessary.
The vapour pressure of thymol is 3.4 Pa at 20°C and the water solubility at 20°C is 596 mg/l. A Henry's Law constant of 0.86 $Pa.mol^{-1}.m^3$ was derived. A DT_{50} of 1.197 hours was derived by the Atkinson model (v1.92) using a 12-hour/day OH concentration of 1.5×10^6 OH/cm³.

Table 8.3-1: Summary thymol degradation in air

Process	Rate constant (cm^3, $molecule^{-1}$, s^{-1})	DT_{50} in the troposphere (12 hr sunlight, days)
Direct photodegradation	-	-
Reaction with hydroxy radicals	107.2489×10^{-12}	0.100
Reaction with $O(^3P)$ species	-	-
Direct photodegradation and reaction with hydroxyl radicals	-	-
Direct photodegradation and reaction with hydroxyl radicals and reaction with $O(^3P)$ species	-	-

B.8.3.1. Route and rate of degradation in air

1. Information on the study

Data point	CA 7.3.1/01
Report author	V. Thomas
Report year	2007
Report title	Thymol: Estimated of Atmospheric Oxidation Rate
Report No.	EDR/02/01a
Document No.	-
Guidelines followed in study	US EPA, 2000; Meylan and Howard, 1993
Deviations from current test guideline	None
Previous evaluation	Yes, DAR 2011
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability	Yes

2. Full summary

The atmospheric oxidation rate for thymol was estimated using the Atmospheric Oxidation Program (AOPWIN 1.92). This estimates the rate constant for the atmospheric, gas-phase reaction, between photochemically produced hydroxyl radicals and organic chemicals. It was estimated that the atmospheric half-life for thymol due to gas phase hydroxyl radical reaction was 0.100 days (1.197 hours) under environmental conditions with a diurnal cycle of 12 hours. On this basis, it can be concluded that thymol is impersistent in the atmosphere and would not be subject to significant concerns related to long-range atmospheric transport and atmospheric accumulation.

I. METHODS

The atmospheric oxidation rate of thymol was estimated using the Atmospheric Oxidation Program (AOPWIN v1.92), which is included in the EPI Suite software (v3.2, US EPA 2007). Specifically, the model estimates the rate constant for the atmospheric, gas-phase reaction between photochemically produced hydroxyl radicals and organic chemicals.

The rate constants estimated by the program are then commonly used to calculate atmospheric half-lives for organic compounds based upon average atmospheric concentrations of hydroxyl radicals and ozone.

Assumed hydroxyl radical and ozone concentrations were as follows:

Hydroxyl radical concentration: 1.5×10^6 OH radical/cm^3 (assumed to be an average concentration during daylight, based on experimental observations).

Ozone concentration: 7×10^{11} ozone mol/cm^3 (assumed to be the concentration of ozone in unpolluted air in the lower troposphere)

The AOPWIN program is an estimated method in which structural components are characterised with respect to potential for interaction with hydroxyl radicals and ozone. Input of the structure takes the form of SMILES notation.

SMILES: Cc1cc(O)c(C(C)C)cc1

Molecular formula: $C_{10}H_{14}O$

II. RESULTS

AOPWIN estimated the following rate constants for the various processes.

Hydrogen abstraction	2.4106 x 10^{-12} cm^3 $molecule^{-1}$ sec^{-1}
Reaction with N, S and OH	0.14 x 10^{-12} cm^3 $molecule^{-1}$ sec^{-1}
Addition to triple bonds	No reaction
Addition to olefinic bonds	No reaction
Addition to aromatic rings	104.6984 x 10^{-12} cm^3 $molecule^{-1}$ sec^{-1}
Addition to fused rings	No reaction

The half-life due to reaction with hydroxyl radicals is therefore calculated to be 1.197 hours, equivalent to 0.100 days under environmental conditions with a diurnal cycle of 12 hours.

III. CONCLUSION

On this basis, it is concluded that thymol is impersistent in the atmosphere and would not be subject to significant concerns related to long-range atmospheric transport and atmospheric accumulation.

3. Assessment and conclusions

Assessment and conclusion by applicant:

This study was previously accepted and indicated that thymol degrades quickly in the atmosphere with DT_{50} of 0.1 days with hydroxyl radicals.

Assessment and conclusion by RMS:

This study was previously evaluated and accepted in DAR 2011. The atmospheric half-life as calculated by the Atkinson method was 0.1 days.

Outcome and conclusion of the study:

Accepted.

B.8.3.2. Transport via air

An estimation of the photochemical oxidative degradation rate (using the Atkinson equation, see CA 7.3.1/01) has estimated that the expected half-life in air to be 1.197 hours. This indicates that any volatilised thymol will be extremely short-lived in the atmosphere. Any thymol that does reach air will be rapidly degraded before significant transport can occur.

B.8.3.3. Local and global effects

An estimation of the photochemical oxidative degradation rate (using the Atkinson equation, see CA 7.3.1/01) has estimated that the expected half-life in air to be 1.197 hours. This indicated that any volatilised thymol will be extremely short-lived in the atmosphere, therefore there be no local or global effects. Any thymol that does reach air will be rapidly degraded before significant transport can occur.

B.8.4. DEFINITION OF RESIDUE

B.8.4.1. Definition of the residue for risk assessment

Soil: Thymol
Groundwater: Thymol
Surface water: Thymol
Sediment: Thymol
Air: Thymol

B.8.4.2. Definition of the residue for monitoring

Soil:	Thymol
Groundwater:	Thymol
Surface water:	Thymol
Sediment:	Thymol
Air:	Thymol

B.8.5. MONITORING DATA CONCERNING FATE AND BEHAVIOUR OF THE ACTIVE SUBSTANCE, METABOLITES, DEGRADATION AND REACTION PRODUCTS

No monitoring data is available and based on the short half-life of thymol is not required.

B.8.6. REFERENCES RELIED ON

B.8.6.1. Open literature review

B.8.6.1.1 Summary

This data point reviewed the literature data relating to the active substance thymol, as required by Article 8(5) of Regulation (EC) No 1107/2009 on the placing of plant protection products on the market.
It has been written according to:
- EFSA (2011). Guidance of EFSA, Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009, EFSA Journal 2011;9(2):2092.
- AGES (2013). External scientific report, Case studies for the application of the Guidance of EFSA on Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009, using substances for which dossiers are submitted under Regulation (EU) No 1141/2010, EFSA supporting publication 2013:EN-511.

In this literature search, the aim was to find scientific peer-reviewed open literature on thymol dealing with toxicological and toxicokinetic studies, operator exposure, residues, fate and behaviour in the environment and ecotoxicological studies which were published between 1/1/2005 and 16/02/2021 for thymol.

To achieve this goal, a wide range of reference collections was consulted utilising the online various databases.
The following initial search strategy was used, in line with Section 5.2.2 of EFSA Journal 2011;9(2):2092:
- The search terms were defined, initially using terms related to the active substance, its metabolites and plant protection products containing the active substance, including synonyms.
- Specific search terms were then used relating to the specific data requirements in question (i.e. for toxicology, human exposure, residues, environmental fate and behaviour and ecotoxicology) to refine the search.
- The search of publications was conducted, using the two-stage approach outlined above. A list of titles, authors and dates were extracted. Any duplicate summary records were removed, before proceeding.

The remaining summary records were then assessed for relevance in a two-step process, in line with Section 5.3 of EFSA Journal 2011;9(2):2092:
- Firstly, a rapid assessment for relevance was conducted based on the titles and abstracts, to exclude summary records which were obviously irrelevant to the specific data requirements in question.
- Secondly, a detailed assessment of full-text documents was conducted to identify potentially relevant, irrelevant or relevant and reliable records that were not excluded in the first step.

All literature papers that were considered relevant to the risk assessment following this process are summarised in the corresponding MCA/MCP sections of this supplementary dossier, where an assessment of their reliability is also presented in line with Section 5.4 of EFSA Journal 2011;9(2):2092.
The results of this search were as follows:

Study selection process	Thymol

	Toxicology (including human exposure)	Residues	Environmental fate and behaviour	Ecotoxicology
Total number of documents retrieved (with duplicates) (global search results related to the active substance, its metabolites and plant protection products containing the active substance)	35 229			
Total number of documents retrieved (without duplicates) after focused search strategy (combining terms for active substance and specific data requirements)	494	303	510	1120
Number of publications excluded after rapid assessment for relevance (non-relevant, excluded literature)	481	284	507	983
Number of publications for which detailed assessment of full-text required after rapid assessment for relevance	13	19	3	137
Number of publications excluded from further consideration after detailed assessment for relevance (non-relevant, excluded literature)	3	9	0	93
Number of publications considered relevant following the two-step process (i.e. after detailed assessment of full-text)	**10**	**10**	**3**	**44***

* Includes 41 papers, plus 3 papers for which full-text was not available. In absence of the full-text, these 3 papers are considered relevant and the available abstract has been provided as a summary (but cannot be assessed for reliability).

For residue and environmental fate: the 10 and 3 publications, respectively, considered relevant following the two-step process are summarized in respective MCAs.

Aim is to find scientific peer-reviewed open literature, as required by Article 8(5) of Regulation (EC) No 1107/2009 on the placing of plant protection products on the market, on thymol dealing with toxicological and toxicokinetic studies, operator exposure, residues, fate and behaviour in the environment and ecotoxicological articles which are published within the last fifteen years from a wide range of reference collections consulted through online wide and various databases.

Details on these various reference collections are provided hereafter (see CA 9.2) fulfilling requirements from EFSA and AGES on the bibliographic database (in terms of quality and quantity). The search strategy leading to the inclusion or exclusion of publications is described below (see CA 9.2, CA 9.3 and CA 9.4) and summarized in two workflow charts (see CA 9.6.1 Appendix I).

B.8.6.1.2. Search strategy

The following search strategy was used, in line with Section 5.2.2 of EFSA Journal 2011;9(2):2092:
The following initial search strategy was used, in line with Section 5.2.2 of EFSA Journal 2011;9(2):2092:

- The search terms were defined using terms related to the active substance, its metabolites and plant protection products containing the active substance, including synonyms.
- Specific search terms relating to the data requirements in question (i.e. for toxicology, human exposure, residues, environmental fate and behaviour and ecotoxicology) were then used to refine the search.
- These search terms are listed in Section 9.2.4 below.

The literature search was conducted between 2nd July 2020 and 16/17 February 2021. The search identified scientific peer-reviewed open literature published in the last 15 years (from 1/1/2005 to 16/02/2021) for thymol.

B.8.6.1.2.1. Bibliographic databases used in the literature review

In line with Section 5.2.1 of EFSA Journal 2011;9(2):2092, a number of different sources were used in the literature search with the aim to locate all relevant scientific peer-reviewed open literature. The reference collections searched were

AGRICOLA, AGRIS (FAO), PASCAL, PubMed (MEDLINE) inc. TOXNET, Science Direct, Springer, and Wiley. All sources and the rationale for their inclusion in the search are presented in the table below.

Table .8.6.1.2.1-1: Bibliographic databases used in the literature search

Database	Description/Rationale for Inclusion
1. AGRICOLA	Coverage of the database includes agricultural economics and rural sociology, agricultural production, animal sciences, chemistry, entomology, food and human nutrition, forestry, natural resources, pesticides, plant science, soils and fertilizers, and water resources. More than 6.7 million records (09/2019). 1970-present.
2. AGRIS (FAO)	AGRIS is one of the most comprehensive search engines in food and agricultural scientific literature providing free access to millions of bibliographic records in 90 different languages. Up to hundreds of organisations worldwide contribute to knowledge and data to the AGRIS platform, resulting in a multilingual bibliographic collection of food and agricultural scientific research with special attention to scientific information produced in the global south. Therefore, AGRIS is used by whoever is inclined to find literature on any of FAO's areas of interest.
3. PASCAL	The site is an archive of the PASCAL and FRANCIS bibliographic databases in exact, human and social sciences, produced by the Inist-CNRS from 1972 to 2015. It provides access to more than 20 millions references (articles, patents, maps, conferences, books, reports and thesis) in exact sciences and technology, in biological and medical sciences, in art, archaeology, economics, ethnology, geography, history of science and technology, literature, linguistics, administrative and legal sciences, education and religion sciences.
4. PubMed MEDLINE (including TOXNET)	MEDLINE contains information on every area of medicine. More than 30 million records (08/2019). 1946-present.
5. Science Direct	ScienceDirect provides access to more than 16 million articles, 2,500 journals, 370 full open access journals, 39,000 books and 330,000 topic pages to help researchers discover more insights, achieve more breakthroughs and move their research forward.
6. Springer	Springer is a leading global scientific, technical and medical portfolio, providing researchers in academia, scientific institutions and corporate R&D departments with quality content through innovative information, products and services. It handles more than 2,900 journals and 300,000 book.
7. Wiley	Wiley is one of the largest and most authoritative collections of online journals, books, and research resources, covering life, health, social, and physical sciences.

B.8.6.1.2.2. Input parameters (search terms) for the literature search

Any documents relating to the essential oils, their synonyms and other common names were collected. The reference collections were queried by name e.g. "thymol" in addition to common variants such as thymic acid, and "thyme campher". Where the number of records for, say, thymol alone exceeded individual reference collection download limits additional specific topics of interest, ecotoxicity, fate, residues, etc., were included. The tables below present the general search terms used:

Table 8.6.1.2.2-1: List search terms used to identify all literature related to the active substance (thymol), its metabolites and plant protection products containing the active substance

Common name	Thymol Thyme camphor Thymic acid Cymophenol, alpha- m-Thymol IPMP Isopropyl cresol

	Cymophenol, alpha- phenol
Chemical name (IUPAC and CA)	5-methyl-2-propan-2-ylphenol 2-Isopropyl-5-methylphenol 5-Methyl-2-isopropylphenol 1-Hydroxy-5-methyl-2-isopropylbenzene 1-Methyl-3-hydroxy-4-isopropylbenzene 2-Hydroxy-1-isopropyl-4-methylbenzene 2-Isopropyl-5-methylphenol 3-Hydroxy-1-methyl-4-isopropylbenzene 3-Hydroxy-p-cymene 3-Methyl-6-isopropylphenol 3-p-Cymenol 5-Methyl-2-(1-methylethyl)phenol 5-Methyl-2-isopropyl-1-phenol Methyl-2-isopropylphenol 6-Isopropyl-3-methylphenol 6-Isopropyl-m-cresol 6-Isopropyl-p-cresol Phenol, 5-methyl-2-(1-methylethyl)- Phenol, 2-isopropyl-5-methyl-
Metabolite(s)	-
Product name	Mevalone, Cedroz, Andromeda, 3Logym ES-00108, Trigemol, Cagenoleta, Eugeti
CAS No.	89-83-8
EC No.	201-944-8

Often an event or outcome is not explicitly described by the subject at the title or abstract level and it would be difficult to adequately describe the individual ecotox effects one can envisage using key words and/or subject headings in a complex search query. This granular information was captured during the text processing phase using customised gazetteer lists such as the extracts given in Table 9.4.2-2 below for aquatic invertebrates and birds. These case insensitive lists contain both generic terms such as “mollusc(s)” and both common and scientific names at the species level, e.g., pond snail (Lymnaea). In total the gazetteer list of terms describing aquatic invertebrates contained 47 entities and the birds gazetteer contained 63 entities (All subject gazetteer list files used in this project have been provided to Staphyt as a separate zip file KCA 9.2.4/01). On another hand, the subject lists were crossed with Effects lists for each section (All effect list files used in this project have been provided to Staphyt as a separate zip file KCA 9.2.4/02).

The tables below present examples of the subject and effect section specific search terms used:

Table 8.6.1.2.2-2: List of subject search terms used specific to a section (example)

Aquatic Invertebrate list (selected entries)	**Birds list (selected entries)**
chironomid	Anas
chironomids	avian
Chironomus	bird
Crassostrea	birds
crustacea	blackcap
crustacean	blackcaps
crustaceans	bluetit
Daphnia	bluetits
daphnid	bobwhite
…	…

Table 8.6.1.2.2-3: List of effect search terms used specific to a section (example)

Effect (ecotox)
absorption
acute
adrenal
adsorption
adverse
allergen
allergenic
allergens
allergic
…

B.8.6.1.3. Search results

The total numbers of records (before removing duplicates) for the search are summarised in the table below. The full results of the search (spreadsheets) are provided at KCA 9.3/01.
The peer-reviewed literature search strategy highlighted for first search (done in 2020), 33 715 documents, for second search (done in 2021), 1 514 documents, total 35 229 documents of potential interest for thymol to this review before the removal of duplicates occurring as a result of searching the individual reference collections separately.

Table 8.6.1.3-1: Numbers of records (before removing duplicates) for the search (based on search terms according to Table 9.2.4-1)

Databases (through online STN)	**Number of hits Thymol First search**	**Number of hits Thymol Second search**	**Number of hits Thymol Total**
1. AGRICOLA	-	88	88
2. AGRIS (FAO)	190	14	204
3. PASCAL	-	-	-
4. PubMed MEDLINE (including TOXNET)	17 870	223	18 093
5. Science Direct	7 424	219	7 643
6. Springer	4 701	575	5 276
7. Wiley	3 530	395	3 925
Total number of documents retrieved (with duplicates) (global search results related to the active substance, its metabolites and plant protection products containing the active substance)	**33 715**	**1 514**	**35 229**

To identify which of these records mentioned the aspects of interest to this particular project a text mining application was built and applied to each document in turn and only those which specifically mentioned, for example fate and behavior or the specified ecotox terms together with an essential oil or its common variants, were identified as a positive result.
The text mining application comprises a number of different steps each performing a different function in the application and in general terms the approach taken was to Tokenise (identify individual words and features) and Sentence split the documents; use the Gazetteer lists to identify any important key words and phrases such as sediment dwelling organisms; identify the Title and Abstract part of the document; look within the Title and Abstract for patterns matching the natural language expressions for, say, the ecotoxicity of thymol, an example being "Acaricidal activities of *Santolina africana* and *Hertia cheirifolia* essential oils against the two-spotted spider mite (*Tetranychus urticae*)."; and index the results.
After removing duplicates and text mining process, the total number of hits potentially relevant is presented below:

Table 9.11.1.3-2: Number of documents identified as potentially relevant after text mining and removal of duplicates

All databases	**Number of hits* Thymol**
Mammalian Toxicity	494
Residues	303
Environmental fate and behaviour	510
Ecotox	1120

*Total number of documents retrieved after removal of duplicates/triplicates (using general plus section specific terms)

B.8.6.1.4. Study selection

The remaining summary records were assessed for relevance in a two-step process, in line with Section 5.3 of EFSA Journal 2011;9(2):2092:

- Firstly, a rapid assessment for relevance was conducted based on the titles and abstracts, to exclude summary records which were considered irrelevant and the justification was recorded.
- Secondly, a detailed assessment relevance and reliability of full-text documents was conducted for any remaining summary records that were not excluded in the first step.

All literature papers that were considered relevant to the risk assessment following this two-step process are summarised in the corresponding MCA/MCP sections of this supplementary dossier, where an assessment of their reliability is also presented in line with Section 5.4 of EFSA Journal 2011;9(2):2092.

Crietria for relevance is included in the following table

Data requirement	Justification for Search Strategy
Fate and behaviour in the environment (OECD IIA 7.1 – 7.10)	
Environmental Fate and Behaviour in soil (OECD IIA 7.1 - 7.4)	• Content of the papers addresses data requirements for environmental fate and behaviour in soil • Well defined test material applied as active substance solution or plant protection product (not as a by-product/ingredient of a soil amendment) • Substrate: representative agriculture used soils with well-defined soil properties (e.g. pH, organic carbon content, microbial biomass etc.) also field studies • No previous contamination of the soil • Exposure through active substance applied as a solution or through a commercial formulation, no mixtures with other active substances
Environmental Fate and Behaviour in water and sediment (OECD IIA 7.5 - 7.9)	•Content of the papers addresses data requirements for environmental fate and behaviour in water •Well defined test material (including its purity and impurity profile) •Test material used are samples from representative European aquatic resources (no contamination) •Exposure through active substance solution or plant protection product, no mixtures with other active substances
Environmental Fate and Behaviour in air (OECD IIA 7.10)	•Content of the papers addresses data requirements for environmental fate and behaviour in air •Well defined test material (including its purity and impurity profile) •No analytical papers • Representative sampling in Europe

B.8.6.1.4.1. Rapid assessment

The total number of publications excluded after rapid assessment for relevance and the number of publications for which a detailed assessment of the full-text was required are summarised in the table below. Full details of the rapid assessment are presented in spreadsheet KCA 9.4/01 for thymol.

Table 8.6.1.4.1-1: Numbers of publications excluded/identified after rapid assessment for relevance for thymol

Study selection process	Thymol			
	Toxicology (including human exposure)	Residues	Environmental fate and behaviour	Ecotoxicology
Number of publications excluded after rapid assessment for relevance (non-relevant, excluded literature)	481	284	507	983
Number of publications for which detailed assessment of full-text required after rapid assessment for relevance	13	19	3	137

B.8.6.1.4.2. Detailed assessment

The total number of publications excluded and the final number of relevant papers identified after detailed assessment of full-texts for relevance are summarised in the tables below. Full details of the detailed assessment are presented in spreadsheet KCA 9.4/01 for thymol.

Table 8.6.1.4.2-1: Numbers of publications excluded/identified after detailed assessment for relevance for thymol

Study selection process	Thymol

	Toxicology (including human exposure)	**Residues**	**Environmental fate and behaviour**	**Ecotoxicology**
Number of publications excluded from further consideration after detailed assessment for relevance (non-relevant, excluded literature)	3	9	0	93
Number of publications considered relevant following the two-step process (i.e. after detailed assessment of full-text)	**10**	**10**	**3**	**44***

* Includes 41 papers, plus 3 papers for which full-text was not available. In absence of the full-text, these 3 papers are considered relevant and the available abstract has been provided as a summary (but cannot be assessed for reliability).

All publications excluded following the detailed assessment are presented in the tables below, with a reason for not including in the dossier. The full texts of non-relevant publications (pdfs) are inserted in KCA 9.4/02

Table 8.6.1.4.2-2: Publications excluded from the risk assessment after detailed assessment of full-text documents – results for thymol

Author(s)	Year	Title	Source	Reason for not including in dossier (based on relevance criteria)	RMS conclusion
TOXICOLOGY AND HUMAN EXPOSURE					
ENVIRONMENTAL FATE AND BEHAVIOUR					
None of the publications were excluded following detailed assessment of the full-text.					
ECOTOXICOLOGY					

All relevant publications identified following the detailed assessment are presented in the tables below, in order of data requirement and author, respectively. These relevant papers and reliable are summarised in the corresponding MCA/MCP sections of this supplementary dossier, where an assessment of their reliability is also presented in line with Section 5.4 of EFSA Journal 2011;9(2):2092. For MCA S5 and MCA S8 papers, the toxrtool has been used to categorize the reliability of relevant papers (KCA 9.4.2/01 Toxrtool tox and KCA 9.4.2/02 Toxrtool ecotox).

For residue and environmental fate: the 3 publications considered relevant following the two-step process are summarized in respective MCAs.

Table 8.6.1.4.2-3: Relevant studies included in the supplementary dossier after detailed assessment of full-text documents for relevance: sorted by data requirement(s) for thymol

Data requirement (indicated by the corresponding CA and CP data point)	Author(s)	Year	Title	Source
ENVIRONMENTAL FATE AND BEHAVIOUR				
CA 7.1.2.1.1/02 (and 7.2.2.3/01)	D. Hu and J. Coats	2008	Evaluation of the environmental fate of thymol and phenethyl propionate in the laboratory	Pest Management Science 64:775-779 (2008)
CA 7.2.2.1/02	Nabeoka, R, Taruki, M., Kayashima, T., Yoshida, T., Kameya, T	2016	Effect of test concentration in the ready biodegradability test for chemical substances: Improvement of OECD test guideline 301C	Environmental Toxicology and Chemistry, Vl.35, No.1, pp.84-90,2016
CA 7.2.2.1/03	N. Nakada, T. Tanishima, H. Shinohara, K. Kiri and H. Takada	2006	Pharmaceutical chemicals and endocrine disrupters in municipal wastewater in Tokyo and their removal during activated sludge treatment	Water Research 40 (2006) 3297-3303

Table 8.6.1.4.2-4: Relevant studies included in the supplementary dossier after detailed assessment of full-text documents for relevance: sorted by author(s) for thymol

Author(s)	Data requirement (indicated by the corresponding CA and CP data point	Year	Title	Source
ENVIRONMENTAL FATE AND BEHAVIOUR				
D. Hu and J. Coats	CA 7.1.2.1.1/02 (and 7.2.2.3/01)	2008	Evaluation of the environmental fate of thymol and phenethyl propionate in the laboratory	Pest Management Science 64:775-779 (2008)
Nabeoka, R, Taruki, M., Kayashima, T., Yoshida, T., Kameya, T	CA 7.2.2.1/02	2016	Effect of test concentration in the ready biodegradability test for chemical substances: Improvement of OECD test guideline 301C	Environmental Toxicology and Chemistry, Vl.35, No.1, pp.84-90,2016
N. Nakada, T. Tanishima, H. Shinohara, K. Kiri and H. Takada	CA 7.2.2.1/03	2006	Pharmaceutical chemicals and endocrine disrupters in municipal wastewater in Tokyo and their removal during activated sludge treatment	Water Research 40 (2006) 3297-3303

B.8.6.1.5. Conclusion

In this literature search for the active substance thymol, 35 229 references were identified and evaluated for their potential relevance for data requirements "toxicological and toxicokinetic studies, human exposure, residues, fate and behaviour in the environment and ecotoxicological studies".

After rapid and detailed assessments for relevance, the following publications were considered to be relevant:

	Number of publications considered to be relevant Thymol
Toxicology, including human exposure	10
Residues	10
Environmental fate	3
Ecotoxicology	44*

* Includes 41 papers, plus 3 papers for which full-text was not available. In absence of the full-text, these 3 papers are considered relevant and the available abstract has been provided as a summary (but cannot be assessed for reliability).

All relevant and reliable publications identified following the detailed assessment are summarised in the corresponding MCA/MCP sections of this supplementary dossier, where an assessment of their reliability is also presented in line with Section 5.4 of EFSA Journal 2011;9(2):2092. For MCA S5 and MCA S8 papers, the toxrtool has been used to categorize the reliability of relevant papers (KCA 9.4.2/01 Toxrtool tox and KCA 9.4.2/02 Toxrtool ecotox).

For environmental fate the 3 publications considered relevant following the two-step process are summarized in respective MCAs.

Assessment and conclusion by RMS:

The RMS has checked the review of literature data submitted by the applicant and considers the databases used for the search are acceptable, and the search strategy as well.

The following criteria for relevance in the detailed screening was used:

Data requirement	**Justification for Search Strategy**
Fate and behaviour in the environment (OECD IIA 7.1 – 7.10)	
Environmental Fate and Behaviour in soil (OECD IIA 7.1 - 7.4)	• Content of the papers addresses data requirements for environmental fate and behaviour in soil • Well defined test material applied as active substance solution or plant protection product (not as a by-product/ingredient of a soil amendment) • Substrate: representative agriculture used soils with well-defined soil properties (e.g. pH, organic carbon content, microbial biomass etc.) also field studies • No previous contamination of the soil • Exposure through active substance applied as a solution or through a commercial formulation, no mixtures with other active substances
Environmental Fate and Behaviour in water and sediment (OECD IIA 7.5 - 7.9)	•Content of the papers addresses data requirements for environmental fate and behaviour in water •Well defined test material (including its purity and impurity profile) •Test material used are samples from representative European aquatic resources (no contamination) •Exposure through active substance solution or plant protection product, no mixtures with other active substances
Environmental Fate and Behaviour in air (OECD IIA 7.10)	•Content of the papers addresses data requirements for environmental fate and behaviour in air •Well defined test material (including its purity and impurity profile) •No analytical papers • Representative sampling in Europe

For environmental fate, 3 publications with thymol were considered relevant and therefore summarized under their corresponding data requirement point.

- *Evaluation of the environmental fate of thymol and phenethyl propionate in the laboratory. D. Hu and J. Coats, 2008.*

- *Effect of test concentration in the ready biodegradability test for chemical substances: Improvement of OECD test guideline 301C. Nabeoka, R, et.al. 2016.*

- *Pharmaceutical chemicals and endocrine disrupters in municipal wastewater in Tokyo and their removal during activated sludge treatment. N. Nakada, et.al. 2006.*

Outcome and conclusion of the study:
RMS considers acceptable the review of open literature.

B.8.6.2. References relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previously used Y/N If yes, for
CA 7.1.1.1/01 CA 7.1.2.1.1/01	A, Jones	2015a	Thymol: Aerobic soil metabolism Envigo Study No. PIF0008 GLP Unpublished	N	N	-	Eden Research plc	Y In EFSA Confirmatory Data 2016
CA 7.1.2.1.1/02 CA 7.2.2.3/01	D. Hu and J. Coats	2008	Evaluation of the environmental fate of thymol and phenethyl propionate in the laboratory Pest Management Science 64:775-779 (2008) Non-GLP Published paper	N	N	-	Public	N
CA 7.1.3.1.1/01	A. Jones	2015b	Thymol: Adsorption/desorption in Five soils Envigo Study No. PIF0009 GLP Unpublished	N	N	-	Eden Research plc	Y In EFSA Confirmatory Data 2016
CA 7.1.3.1.1/02	A. Cashmore	2021	Thymol: Adsorption/Desorption in Soil Smithers ERS Ltd 3203040 GLP Not published	N	Y	Study not previously submitted	Eden Research plc	N
CA 7.2.1.1/01	D. Kelly & S. Bluemink	2021	Thymol – Hydrolysis of thymol in Sterile Buffer at pH 4,7 and 9	N	Y	Study not previously submitted	Eden Research plc	N

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previously used Y/N If yes, for
			Smithers ERS Ltd 3202788 GLP Not published					
CA 7.2.1.2/01	P. Yeomans	2022	Thymol: Photodegradation of Thymol in Sterile, Aqueous Solution Smithers ERS Ltd 3202789 GLP Not published	N	Y	Study not previously submitted	Eden Research plc	N
CA 7.2.2.1/01	B. Seyfried	2007	Thymol: Ready Biodegradability in a Manometric Respirometry Test RCC Study number B34435 GLP Not published	N	N	-	Eden Research plc	Y In DAR 2011
CA 7.2.2.1/02	Nabeoka, R, Taruki, M., Kayashima, T., Yoshida, T., Kameya, T.	2016	Effect of test concentration in the ready biodegradability test for chemical substances: Improvement of OECD test guideline 301C Environmental Toxicology and Chemistry, Vl.35, No.1, pp.84-90,2016 Non GLP Published Paper	N	N	-	Public	N
CA 7.2.2.1/03	N. Nakada, T. Tanishima, H. Shinohara, K. Kiri, H. Takada	2006	Pharmaceutical chemicals and endocrine disrupters in municipal wastewater in Tokyo and their removal during activated sludge treatment Water Research 40 (2006) 3297-3303 Non GLP Published paper	N	N	-	Public	N
CA 7.2.2.2/01	L. Simmonds	2021	Thymol: Aerobic Mineralisation in Surface Water	N	Y	Study not previously submitted	Eden Research plc	N

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previously used Y/N If yes, for
			Smithers ERS Ltd 3202790 GLP Not Published					
CA 7.3.1/01	V. Thomas	2007	Thymol: Estimated of Atmospheric Oxidation Rate EDR/02/01a No GLP required Not published	N	N	-	Eden Research plc	Y In DAR 2011