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

EUGENOL
2-methoxy-4-(prop-2-en-1-yl)phenol

Volume 3 – B.8 (AS)

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

Version History

When	What
July 2022	Initial RAR prepared by RMS
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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B.8. 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	Eugenol: Aerobic soil metabolism
Report No.	Envigo Study No. PIF0002
Document No.	
Guidelines followed in study	OECD: Guideline 307, Aerobic and Anaerobic Transformation in Soil, 2002
Deviations from current test guideline	Five vessels for microbiology analysis were not prepared for all soil types. For Brierlow soil only three vessels were prepared and for Calke and Empingham only four vessels were prepared for each soil type. Only three vessels were required for analysis. The identity of the pH indicator added to the potassium hydroxide trapping solutions was not recorded in the study data.
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 eugenol were studied in four soils in the laboratory under aerobic conditions. The soils were selected to represent a range of textural characteristics, pH (5.0 - 7.7) and organic carbon contents (1.6 – 3.6 %). Soil samples were set up and allowed to acclimatise before being treated with [^{14}C]-eugenol at a nominal concentration of 0.52 mg/kg, approximately equivalent to a use rate of 520 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 81.2 – 116.1% 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 46.2 – 74.4% AR in each soil at zero-time to mean values of 7.6 – 14.2% AR after 3 days incubation before plateau through the remainder of the incubation period. Non-extractable radioactivity accounted for mean values of 65.6 – 70.4% AR after 120 days. Volatile radioactivity, characterised as $^{14}CO_2$, represented 19.1 – 20.2% AR after 120 days. Eugenol declined rapidly in each soil type. The DT_{50} and DT_{90} values were <1 day and <2 days respectively.
Eugenol degraded to multiple 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.

I. MATERIALS AND METHODS

A MATERIALS

Test material 1 (used in standard rate tests)
Name: [Phenyl-U-^{14}C]-eugenol

Description: In acetonitrile solution
Lot/Batch no.: 265-157-0597-A-20140325-NTO
Radiochemical Purity: Radiochemical purity >97%
Specific activity: 13.3 MBq/mg.
Storage : <-15°C

Non-labelled material (used in all tests)
Name: Eugenol, 2-Methoxy-4-(2-propenyl)phenol
Description: Liquid
Lot/Batch no.: 121774
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 for the soil used to investigate the route of aerobic degradation of eugenol

Soil parameter	**Value**			
Source	Brierlow, UK	Ingleby, UK	Empingham, UK	Calke, UK
Envigo batch number	020614A	020614B	020614C	020614D
Particle size distribution, UK[1] % Sand (63 µm to 2 mm) % Silt (2 µm to 63 µm) % Clay (< 0.002µm)	 20 60 20	 84 9 7	 20 41 39	 65 21 14
Textural classification, UK	Clay loam/ silty clay loam	Loamy sand	Clay	Sandy loam
USDA classification	Silt loam	Loamy sand	Silt clay loam	Sandy Loam
Soil pH (water)	6.0	5.0	7.7	6.3
Soil pH (0.01M $CaCl_2$)	5.3	4.0	7.4	5.1
Organic carbon content, (%)	3.6	1.6	3.3	2.8
Cation exchange capacity, (meq/100 g soil)	22.6	7.3	26.4	15.4
Water content (% dry weight)	30.6	10.9	21.6	20.1
Water content at pF 2 (% dry weight)	44.1	13.5	35.0	28.2
Microbial biomass (mg C/kg soil)				
Start of incubation	466.0	256.0	2235.6	719.2
End of incubation	187.6	56.0	1302.0	244.0
Untreated	231.6	70.8	1328.4	307.6

B STUDY DESIGN

Experimental conditions
Glass dishes 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 dishes were established for each soil type. Samples established for treatment with [^{14}C]-eugenol were housed in glass chambers and each chamber, which contained dishes from one soil type, were incorporated into separate flow-through systems arranged in a series. At intervals, the dishes 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]-eugenol was radiodiluted for application. The application solution was prepared by combining a portion of non-radiolabelled eugenol (1.1 mg) with a portion (1.5 mg, 19.3 MBq) of [^{14}C]-eugenol and diluting to volume (20 mL) with water (2 mL) and acetonitrile. The concentration of the application solution was 0.130 mg/mL and the radiodiluted specific activity was 445000dpm/µg. Aliquots (0.2 mL) of these application solutions were applied to the surface of the soil samples. Following application each soil dish was weighed and distilled water added as necessary to maintain the moisture content at that at pF 2. Each dish (with the exception of those taken for zero-time analysis) was then reincorporated into its respective flow-through system.
The amount of eugenol applied per sample was 28 µg. This equated to an application rate of 0.56 mg/kg.

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 3, 10, 17, 21, 24, 28, 35, 38, 45, 52, 73, 86 and 104 days of incubation.

Analysis

The soil samples were extracted with acetonitrile (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 (0.5 mL) of each extract solution were taken for liquid scintillation counting (LSC). The procedure was repeated with extraction of samples done with acetonitrile (days 0-7) or acetonitrile: 0.1M Hydrochloric acid (1:1 v/v) (Days 14-120), and a third extraction was performed with acetonitrile: 0.1M Hydrochloric acid (1:1 v/v).

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 LSC.

The non-extractable radioactivity in one representative sample 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: Water Eluent B: 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		

Thin Layer Chromatography (TLC)

Normal phase TLC was carried out on pre-layered, glass-backed Merck silica gel 60 F254 plates, of layer thickness 0.25 mm. The developing solvent system was: Butanol/acetic acid/water (4/1/1 v/v) The above system was used for the analysis of isolated polar component A.

II. RESULTS

A MASS BALANCE

Total recoveries of radioactivity generally lay in the range 90 – 116.1% applied radioactivity (AR), with 10 vessel have recoveries between 81.2 and 89.2%. 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 46.2 – 74.4% AR in each soil at zero-time to mean values of 7.6 – 14.2% AR after 3 days, before plateau through the remainder of the incubation period. Non-extractable radioactivity accounted for mean values of 30.3 – 58.3% AR at Day 0 before increasing to maximum mean values of 76.5 – 96.2% AR after 3 – 7 days incubation. Non extractable radioactivity then decreased slightly to 65.6 – 70.4% AR after 120 days incubation as levels of evolved $^{14}CO_2$ increased.

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

Days	Ext 1	Ext 2	Ext 3	Total Extractable	NER	Ethyl Digol trap	KOH traps	Total
0	36.9	3.8	5.2	45.9	59.1	NS	NS	105.0

0	43.0	3.6	4.7	51.3	48.7	NS	NS	100.0
3	5.0	0.9	3.3	9.2	94.3	<0.1	5.8	109.3
3	4.6	0.8	3.3	8.7	98.1	<0.1	5.8	112.6
7	3.4	0.6	2.9	6.9	72.5	<0.1	9.0	88.4
7	3.6	0.6	3.0	7.2	84.5	<0.1	9.0	100.7
14	3.0	3.6	2.2	8.8	78.9	<0.1	7.8	95.5
14	2.8	3.8	2.3	8.9	73.7	<0.1	7.8	90.4
59	2.3	3.2	2.1	7.6	66.4	<0.1	15.0	89.0
59	1.5	3.4	2.3	7.2	67.0	<0.1	15.0	89.2
120	1.2	3.0	1.9	6.1	69.1	<0.1	19.1	94.3
120	0.7	3.0	1.9	5.6	62.0	<0.1	19.1	86.7

NS not sampled; NER non-extractable residues

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

Days	Ext 1	Ext 2	Ext 3	Total Extractable	NER	Ethyl Digol trap	KOH traps	Total
0	37.7	2.5	6.2	46.4	56.8	NS	NS	103.2
0	37.5	2.5	6.0	46.0	59.7	NS	NS	105.7
3	5.1	0.9	5.4	11.4	84.0	ND	4.3	99.7
3	5.0	0.8	5.2	11.0	78.6	ND	4.3	93.9
7	4.6	0.7	4.9	10.2	86.8	ND	6.1	103.1
7	4.3	0.8	4.8	9.9	100.1	ND	6.1	116.1
14	3.7	5.5	2.5	11.7	75.0	ND	7.4	94.1
14	3.6	5.4	2.5	11.5	75.2	ND	7.4	94.1
59	2.3	5.2	2.5	10.0	69.6	<0.1	13.9	93.5
59	2.6	5.1	2.5	10.2	68.4	<0.1	13.9	92.5
120	1.1	4.6	2.2	7.9	65.6	<0.1	20.2	93.7
120	1.2	4.7	2.2	8.1	70.4	<0.1	20.2	98.7

NS not sampled; ND not detected; NER non-extractable residues

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

Days	Ext 1	Ext 2	Ext 3	Total Extractable	NER	Ethyl Digol trap	KOH traps	Total
0	53.4	1.8	8.0	63.2	35.5	NS	NS	98.7
0	51.6	2.5	8.9	63.0	41.2	NS	NS	104.2
3	4.2	0.6	9.3	14.1	70.6	<0.1	4.1	88.8
3	4.0	0.5	9.7	14.2	82.4	<0.1	4.1	100.7
7	3.0	0.4	9.7	13.1	74.8	<0.1	6.1	94.0
7	2.9	0.4	10.0	13.3	71.2	<0.1	6.1	90.6
14	2.5	10.1	3.3	15.9	75.9	<0.1	6.4	98.2
14	2.5	9.6	3.3	15.4	67.4	<0.1	6.4	89.2
59	2.1	8.0	3.8	13.9	73.2	<0.1	14.7	101.8
59	2.3	8.7	3.4	14.4	72.2	<0.1	14.7	101.3
120	1.4	7.0	3.2	11.6	68.2	<0.1	19.2	99.0
120	1.4	7.1	3.1	11.6	65.0	<0.1	19.2	95.8

NS not sampled; ND not detected; NER non-extractable residues

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

Days	Ext 1	Ext 2	Ext 3	Total Extractable	NER	Ethyl Digol trap	KOH traps	Total
0	62.8	2.9	4.9	70.6	37.3	NS	NS	107.9
0	71.9	2.8	3.4	78.1	23.3	NS	NS	101.4
3	3.9	0.6	3.0	7.5	76.2	ND	4.9	88.6
3	4.0	0.7	3.0	7.7	82.5	ND	4.9	95.1
7	3.2	0.6	3.4	7.2	74.8	ND	8.4	90.4
7	3.4	0.6	3.4	7.4	76.8	ND	8.4	92.6
14	2.7	2.9	1.4	7.0	75.0	<0.1	9.4	91.4

14	2.7	3.0	1.4	7.1	76.6	<0.1	9.4	93.1
59	1.2	2.2	1.0	4.4	67.5	<0.1	15.9	87.8
59	1.2	2.1	1.1	4.4	60.9	<0.1	15.9	81.2
120	0.9	1.9	1.0	3.8	66.1	<0.1	19.4	89.3
120	0.8	1.8	1.1	3.7	74.7	<0.1	19.4	94.1

NS not sampled; ND not detected; 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
59	C9	Calke	69.6	20.1	32.4	17.1
59	IN9	Ingleby	73.2	19.0	38.1	13.1
59	B9	Brierlow	66.4	19.9	28.1	18.3
59	E9	Empingham	67.5	10.8	14.9	40.3

NER non-extractable residues

Volatile radioactivity, mostly associated with the potassium hydroxide traps increased to a maximum at 120 days incubation accounting for mean values of 19.1 – 20.2% AR. Radioactivity recovered in the potassium hydroxide trapping solution was confirmed as $^{14}CO_2$.

Eugenol accounted for mean values of 18.8 – 53.6% AR at Day 0. Soil extract from Brierlow and Empingham soils post Day 0 were not analysed due to low extractability. In the Calke and Ingleby soils eugenol declined rapidly to mean values of 1.9 – 2.7% AR after 3 days and remained at a similar level throughout the remainder of the incubation period. In addition to eugenol, up to four minor unidentified degradates were detected. Polar component unknown A, detected by HPLC accounted for > 10% AR in a single replicate of the Ingleby soil at time. This component was further resolved into at least seven minor components by TLC each accounting for a maximum of ≤ 3.3% AR. Other unidentified components did not individually exceed 10% AR at any timepoint. Methyleugenol was not detected in any of the soil extracts.

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

Compound	**Sampling time (days)**					
	0	**0**	**3**	**3**	**7**	**7**
Eugenol	22.7	29.5	ns	ns	ns	ns
Unknown A (4 – 5 mins)	5.6	5.0	ns	ns	ns	ns
Unknown B (32 – 33 mins)	7.4	7.4	ns	ns	ns	ns
Unknown C (33 – 34 mins)	3.3	3.0	ns	ns	ns	ns
Unknown D (34 – 35 mins)	1.2	1.2	ns	ns	ns	ns
Others§	5.6	5.1	ns	ns	ns	ns
Total extractable residues	45.9	51.3	9.2	8.7	6.9	7.2
CO_2	ns	ns	5.8	5.8	9.0	9.0
Volatile organic	ns	ns	<0.1	<0.1	<0.1	<0.1
Total volatile organics	ns	ns	5.8	5.8	9.0	9.0
Unextracted residues	59.1	48.7	94.3	98.1	72.5	84.5
Total % recovery	105.0	100.0	109.3	112.6	88.4	100.7
Compound	**Sampling time (days)**					
	14	**14**	**59**	**59**	**120**	**120**
Eugenol	ns	ns	ns	ns	ns	ns
Unknown A (4 – 5 mins)	ns	ns	ns	ns	ns	ns

Unknown B (32 – 33 mins)	ns	ns	ns	ns	ns	ns
Unknown C (33 – 34 mins)	ns	ns	ns	ns	ns	ns
Unknown D (34 – 35 mins)	ns	ns	ns	ns	ns	ns
Others§	ns	ns	ns	ns	ns	ns
Total extractable residues	8.8	8.9	7.6	7.2	6.1	5.6
CO_2	7.8	7.8	15.0	15.0	19.1	19.1
Volatile organic	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Total volatile organics	7.8	7.8	15.0	15.0	19.1	19.1
Unextracted residues	78.9	73.7	66.4	67.0	69.1	62.0
Total % recovery	95.5	90.4	89.0	89.2	94.3	86.7

§ multiple minor components, none of which individually exceed 10 % AR
ns not sampled

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

Compound	**Sampling time (days)**					
	0	**0**	**3**	**3**	**7**	**7**
Eugenol	19.3	18.2	1.8	1.9	2.1	1.8
Unknown A (4 – 5 mins)	7.2	6.5	3.6	2.3	2.4	2.4
Unknown B (32 – 33 mins)	6.0	5.8	1.5	1.5	1.5	1.4
Unknown C (33 – 34 mins)	3.8	4.5	1.7	1.7	1.5	1.5
Unknown D (34 – 35 mins)	2.0	2.3	0.5	1.2	0.9	1.0
Others§	8.0	8.7	2.3	2.4	1.8	1.8
Total extractable residues	46.4	46.0	11.4	11.0	10.2	9.9
CO_2	ns	ns	4.3	4.3	6.1	6.1
Volatile organic	ns	ns	nd	nd	nd	nd
Total volatile organics	ns	ns	4.3	4.3	6.1	6.1
Unextracted residues	56.8	59.7	84.0	78.6	86.8	100.1
Total % recovery	103.2	105.7	99.7	93.9	103.1	116.1
Compound	**Sampling time (days)**					
	14	**14**	**59**	**59**	**120**	**120**
Eugenol	2.3	2.1	1.9	2.2	1.9	1.8
Unknown A (4 – 5 mins)	3.7	4.3	4.4	3.8	3.2	3.4
Unknown B (32 – 33 mins)	1.1	1.2	0.8	0.9	0.6	0.6
Unknown C (33 – 34 mins)	1.3	1.3	0.8	0.9	0.4	0.6
Unknown D (34 – 35 mins)	0.7	0.7	0.5	0.6	0.2	0.4
Others§	2.4	1.9	1.6	1.8	1.6	1.4
Total extractable residues	11.7	11.5	10.0	10.2	7.9	8.1
CO_2	7.4	7.4	13.9	13.9	20.2	20.2
Volatile organic	nd	nd	<0.1	<0.1	<0.1	<0.1
Total volatile organics	7.4	7.4	13.9	13.9	20.2	20.2
Unextracted residues	75.0	75.2	69.6	68.4	65.6	70.4

Total % recovery	94.1	94.1	93.5	92.5	93.7	98.7

§ multiple minor components, none of which individually exceed 10 % AR
ns not sampled
nd not detected

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

Compound	**Sampling time (days)**					
	0	**0**	**3**	**3**	**7**	**7**
Eugenol	34.6	33.1	3.0	2.4	2.6	3.0
Unknown A (4 – 5 mins)	11.0[(a)]	8.9[(a)]	4.7	5.1	5.0	5.2
Unknown B (32 – 33 mins)	3.2	6.0	1.5	1.3	1.1	1.0
Unknown C (33 – 34 mins)	1.3	5.8	1.0	1.2	1.2	0.6
Unknown D (34 – 35 mins)	0.9	1.3	0.5	0.9	0.7	0.3
Others[§]	12.3	7.8	3.3	3.2	2.5	3.2
Total extractable residues	63.2	63.0	14.1	14.2	13.1	13.3
CO_2	ns	ns	4.1	4.1	6.1	6.1
Volatile organic	ns	ns	<0.1	<0.1	<0.1	<0.1
Total volatile organics	ns	ns	4.1	4.1	6.1	6.1
Unextracted residues	35.5	41.2	70.6	82.4	74.8	71.2
Total % recovery	98.7	104.2	88.8	100.7	94.0	90.6
Compound	**Sampling time (days)**					
	14	**14**	**59**	**59**	**120**	**120**
Eugenol	2.6	3.2	2.3	3.0	2.5	2.2
Unknown A (4 – 5 mins)	7.2	6.2	7.0	6.9	6.1	6.3
Unknown B (32 – 33 mins)	1.1	1.3	0.7	0.9	0.6	0.6
Unknown C (33 – 34 mins)	1.1	1.6	0.7	0.6	0.6	0.7
Unknown D (34 – 35 mins)	0.8	0.6	0.5	0.4	0.3	0.3
Others[§]	3.2	2.5	2.7	2.6	1.5	1.5
Total extractable residues	15.9	15.4	13.9	14.4	11.6	11.6
CO_2	6.4	6.4	14.7	14.7	19.2	19.2
Volatile organic	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Total volatile organics	6.4	6.4	14.7	14.7	19.2	19.2
Unextracted residues	75.9	67.4	73.2	72.2	68.2	65.0
Total % recovery	98.2	89.2	101.8	101.3	99.0	95.8

§ multiple minor components, none of which individually exceed 10 % AR
ns not sampled
(a) Isolation and subsequent TLC analysis of polar fraction unknown A from Day 0 (Rep 2) further separated this fraction into at least seven minor unknown components each accounting for ≤ 3.3 %

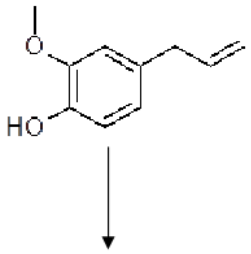
Table 8.1.1.1-11: Proportions of radioactivity in Empingham soil treated with [^{14}C]-eugenol

Compound	Sampling time (days)					
	0	**0**	**3**	**3**	**7**	**7**
Eugenol	47.4	59.7	ns	ns	ns	ns
Unknown A (4 – 5 mins)	4.7	2.9	ns	ns	ns	ns
Unknown B (32 – 33 mins)	9.2	8.1	ns	ns	ns	ns
Unknown C (33 – 34 mins)	2.4	2.0	ns	ns	ns	ns
Unknown D (34 – 35 mins)	1.1	0.9	ns	ns	ns	ns
Others§	5.9	4.5	ns	ns	ns	ns
Total extractable residues	70.6	78.1	7.5	7.7	7.2	7.4
CO_2	ns	ns	4.9	4.9	8.4	8.4
Volatile organic	ns	ns	nd	nd	nd	nd
Total volatile organics	ns	ns	4.9	4.9	8.4	8.4
Unextracted residues	37.3	23.3	76.2	82.5	74.8	76.8
Total % recovery	107.9	101.4	88.6	95.1	90.4	92.6
Compound	**Sampling time (days)**					
	14	**14**	**59**	**59**	**120**	**120**
Eugenol	ns	ns	ns	ns	ns	ns
Unknown A (4 – 5 mins)	ns	ns	ns	ns	ns	ns
Unknown B (32 – 33 mins)	ns	ns	ns	ns	ns	ns
Unknown C (33 – 34 mins)	ns	ns	ns	ns	ns	ns
Unknown D (34 – 35 mins)	ns	ns	ns	ns	ns	ns
Others§	ns	ns	ns	ns	ns	ns
Total extractable residues	7.0	7.1	4.4	4.4	3.8	3.7
CO_2	9.4	9.4	15.9	15.9	19.4	19.4
Volatile organic	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Total volatile organics	9.4	9.4	15.9	15.9	19.4	19.4
Unextracted residues	75.0	76.6	67.5	60.9	66.1	74.7
Total % recovery	91.4	93.1	87.8	81.2	89.3	94.1

§ multiple minor components, none of which individually exceed 10% AR
ns not sampled

C TRANSFORMATION PATHWAY

Eugenol degraded rapidly to multiple minor unidentified degradates, bound residues and carbon dioxide.
The proposed degradation pathway is shown below in Figure 7.1.1.1 1.



Minor metabolites,
bound residues
and carbon dioxide.

Figure 8.1.1.1 1: Transformation pathway

D DEGRADATION KINETICS

The decline of eugenol in soil was modelled in Ingleby and Calke soils using the single first order model (SFO). Modelled DT_{50} and DT_{90} values ranged from 0.5 – 0.6 days and 1.8 – 1.9 days, respectively for Ingleby and Calke soils. Observed DT_{50} and DT_{90} values for Brierlow and Empingham soils were < 3 days. There was insufficient data to determine a DT_{50} in the Brierlow and Empingham soils.

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

Soil	Kinetic model	DT_{50} (days)	DT_{90} (days)	Visual goodness-of-fit
Brierlow	NA	<1[a]	<3[a]	-
Calke	SFO	0.5	1.8	Good
Ingleby	SFO	0.6	1.9	Good
Empingham	NA	<3[a]	<3[a]	-

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

E DEVIATIONS

Five vessels for microbiology analysis were not prepared for all soil types. For Brierlow soil only three vessels were prepared and for Calke and Empingham only four vessels were prepared for each soil type. Only three vessels were required for analysis. The identity of the pH indicator added to the potassium hydroxide trapping solutions was not recorded in the study data. These deviations were not considered to have impacted on the outcome or integrity of the study.

III. CONCLUSION

Eugenol degraded in aerobic soils at 20°C and pF2 with a DT_{50} of less than one day. Eugenol degraded to multiple 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 sterilized before treatment, which precludes characterization by abiotic processes.
- Two soils were not analyzed after 0 day samples as degradation/dissipation was very rapid.
- 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 DT_{90}s will be less than 3 days and there were no metabolites reported at >10%, >5% at 2 consecutive time points, or increasing at the study end.
- This study was requested to identify methyleugenol, a compound of concern. Methyleugenol was not identified in this study and the RMS concluded that the information was sufficient to address the concerns related to the formation of methyleugenol in soil.

Assessment and conclusion by RMS:

The route and rate of degradation of eugenol were studied in four soils in the laboratory under aerobic conditions. Soil samples were treated with [^{14}C]-eugenol at a nominal concentration of 0.52 mg/kg, approximately equivalent to a use rate of 520 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.

Jones, 2015a was submitted to address the confirmatory data requirement on the groundwater exposure assessment for potential metabolites of eugenol, in particular for methyl eugenol. After the evaluation, since methyl eugenol was not identified in this study, it was not considered relevant with regards its groundwater exposure. Member States' and EFSA experts considered the previous assessment by RMS valid.

Comments from this previous assessment has been included in the applicant´s box. That is, samples were not sterilized before treatment and only two soils were analyzed after 0-day samples as degradation/dissipation was very rapid. This issue was commented by EFSA in the outcome of the consultation of confirmatory data (doi:10.2903/sp.efsa.2017.EN-1165), which stated that the data requirement to stablish the degradation rate of eugenol in four soils was not formally fulfilled. However, it was thought that the information provided was sufficient to conclude that eugenol degraded very rapidly in soil and that the use of a conservative modelling soil DT50 of 1 day was acceptable.

It was also commented whether extraction methods were sufficiently robust to yield higher extractable recoveries. The overall recoveries of applied radioactivity (AR) were in the range 81.2 – 116.1 % AR, which falls outside of the recommended range of 90 – 110 % for this kind of study. This issue was justified in the study report with the difficult quantification of bound radioactivity since the solid residue was not dried and the homogenization was poor before the combustion. Additionally, test solutions were not mixed into the soil samples (to minimise volatilisation losses, as stated in the preliminary study).

Regarding to the identification of unknown components, the unknown A, accounted for > 10% AR in a single replicate of the Ingleby soil at time. The unknown A was further investigated by isolating it from the HPLC. The column elute corresponding to the retention time of this component was collected. The isolated fraction was concentrated under nitrogen to a small volume and then subsequently analysed by TLC. As a result, this component was resolved into at least seven minor components each accounting for a maximum of ≤ 3.3% AR. Other unidentified components did not individually exceed 10% AR at any timepoint.

Finally, some deviations were observed with respect to the soil selection. The RMS checked that the organic carbon content exceeded the limit value of 2.5% in three of the four soils, which is the maximum recommended value according to OECD 307. In addition, for three soils, the microbial biomass at the end of the experiment was lower that the limit value of 1% OC (Brierlow, Ingleby and Calke). However, it was considered that due to the rapid dissipation of eugenol in the four soils, the organic carbon and microbial biomass content did not have a significant impact in the dissipation rate values.

DT50 and DT90 values ranged from 0.5 – 0.6 days and 1.8 – 1.9 days, respectively for Ingleby and Calke soils. Observed DT50 and DT90 values for Brierlow and Empingham soils were < 3 days. There was insufficient data to determine a DT50 in the Brierlow and Empingham soils, since the percentage of extractable radioactivity was lower than 10% at 3 days.

Outcome and conclusion of the study:

Despite the reported deviations, the study is considered acceptable.

1. **Information on the study**

Data point	-
Report author	Tadasa, K.
Report year	1977
Report title	Degradation of Eugenol by a Microorganism.
Report No.	*Agric. Biol. Chem.*, **41** (6), 925-929
Document No.	-
Guidelines followed in study	-
Deviations from current test guideline	-
Previous evaluation	Yes, DAR 2011
GLP/Officially recognized testing facilities	No
Acceptability/Reliability	Additional information

2. **Full summary (Abstract)**

Degradation of eugenol as the sole carbon and energy source was studied with a newly isolated microorganism. Ferulic acid(4-hydroxy-3-methoxycinnamic acid) and vanillin(4- hydroxy-3-methoxybenzaldehyde) were obtained as products. These are produced by oxidation and β-oxidation of the aromatic side-chain and metabolized further to form vanillic acid and protocatechuic acid. Protocatechuic acid was cleaved by "ortho" fission to form β-ketoadipate. From these results, the degradation pathway was proposed. The strain was tentatively identified as *Corynebacterium* sp. from the results of physiological and morphological tests.

3. **Assessment and Conclusion**

Assessment and conclusion by applicant:
Two papers were mentioned briefly in the DAR (2011) but were discounted and, apart from the information below, no other information is provided or required.
Tadasa, K. (1977) Degradation of Eugenol by a Microorganism. *Agric. Biol. Chem.*, **41** (6), 925-929 – demonstration of degradation of eugenol by a culture of *Cornebacterium* sp isolated from soil. Metabolic pathway in this organism proposed. The RMS considers this study to be of very limited value to the evaluation as there are no details of rate of degradation and the study appears to be limited to a single bacterium species rather than in natural soil. However, it demonstrates that eugenol may be degraded in the environment by biotic processes.

Assessment and conclusion by RMS:
This study has been included for completeness.

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

1. **Information on the study**

Data point	-
Report author	Tadasa, K.
Report year	1983
Report title	Initial Steps of Eugenol Degradation Pathway of a Microorganism
Report No.	*Agric. Biol. Chem.*, **47** (11), 2639-2640
Document No.	-
Guidelines followed in study	-
Deviations from current test guideline	-
Previous evaluation	Yes, DAR 2011
GLP/Officially recognized testing facilities	No

Acceptability/Reliability	Additional information

2. **Full summary (Abstract)**

Lignin and related compounds have been investigated and reported by many workers. The authors have reported the study of the degradation of eugenol. Eugenol is biosynthesized in plants via ferulic acid and coniferylalcohol and it is a strongly antibacterial substance. It was confirmed that eugenol was converted to ferulic acid, vanillin, vanillic acid and protocatechuic acid, and subsequently ring fission occurred by a Corynebacterium sp. which was isolated from soil. It is expected that there are a few steps before the formation of ferulic acid in the degradation pathway of eugenol. The culture of a Corynebacterim sp., however, did not include such metabolites in the initial steps before the formation of ferulic acid. The crude extract of that strain did not show action towards eugenol. The authors newly isolated a bacterium from soil which could assimilate eugenol very well as the sole carbon and energy source. The bacterium was tentatively identified as a Pseudomonas sp. by means of morphological and physiological tests: Aerobic, one to a few polar flagella, gram negative, and water soluble pigment. This strain accumulated ferulic acid as in the case of Corynebacterium sp., but the crude extract of this newly isolated strain acted towards eugenol. Moreover, a few metabolites besides those produced by Corynebacterium sp., were detected both in the broth with intact cells and in the reaction mixture of the cell-free extract with eugenol as the substrate.

3. **Assessment and Conclusion**

Assessment and conclusion by applicant:
Two papers were mentioned briefly in the DAR (2011) but were discounted and, apart from the information below, no other information is provided or required.
Tadasa, K. Kayahara, H. (1983) Initial Steps of Eugenol Degradation Pathway of a Microorganism. *Agric. Biol. Chem.*, **47** (11), 2639-2640 - breakdown of eugenol with a culture of *Pseudomonas* species. The RMS considers this study to be of very limited value to the evaluation as there are no details of rate of degradation and the study appears to be limited to a single bacterium species rather than in natural soil. However, it demonstrates that eugenol may be degraded in the environment by biotic processes.

Assessment and conclusion by RMS:
This study has been included for completeness.

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

B.8.1.1.2. Anaerobic degradation

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

B.8.1.1.3. Soil photolysis

1. **Information on the study**

Data point	CA 7.1.1.3/01
Report author	D. Kelly
Report year	2021a
Report title	Eugenol - Soil Photolysis of [^{14}C]-Eugenol
Report No.	Smithers ERS Ltd No. 3203050
Document No.	-
Guidelines followed in study	OECD draft document for Phototransformation of Chemicals on Soil Surfaces, January 2002
Deviations from current test guideline	None
Previous evaluation	No
GLP/Officially recognised testing	Yes, conducted under GLP/Officially recognised testing facilities

facilities	
Acceptability/Reliability	Yes

2. Full summary

The photodegradation of [^{14}C]-eugenol was studied on Speyer 5M soil, a sandy loam (USDA) from Rheinland-Pfalz, Germany at 132 g ai/ha soil for 30 days at 20 ± 2°C and under dry conditions. Soil samples (*ca* 2 g dry weight equivalent) were dispensed into metal trays and left to air-dry at room temperature. Samples were treated with 16.55 µg of [^{14}C]-eugenol and were continuously irradiated using a Suntest accelerated exposure machine (xenon lamp fitted with a filter to remove radiation below 290 nm). Test vessels were connected to two 2M NaOH traps for the collection of liberated CO_2.

Samples were taken for analysis at 0 days, 2 hours, 6 hours, 24 hours, 7 days, 14 days and 30 days after treatment for the determination of the parent compound. The soil samples were extracted twice with acetonitrile (25 mL) followed by twice with acetonitrile:water (1:1 v/v, 25 mL). Soil extracts were analysed directly by reverse phase High Performance Liquid Chromatography (HPLC).

The mean recovery of radioactivity ranged from 88.9 to 93.8% applied radioactivity (AR) in both irradiated and dark control samples throughout the incubation period. Levels of extractable radioactivity dropped sharply following 0 DAT analysis (86.6% AR) to between 54.5 and 72.5% AR. Radioactivity remaining unextracted from soil increased sharply following 0 DAT analysis with maximum levels of 28.0 and 30.8% AR observed in 30 DAT samples for irradiated and dark control samples, respectively. In volatile traps, up to 8.7% AR was recovered from irradiated samples and 0.5% from dark control samples and was confirmed to be carbon dioxide by barium chloride precipitation.

Degradation of eugenol was rapid in both irradiated and dark control conditions, with a DT_{50} of < 1 hour. There were no significant differences in the rate of degradation of eugenol between irradiated and dark control samples. No conversion to equivalent summer sunlight days was therefore calculated.

In irradiated samples, volatile radioactivity reached a maximum of 8.7% AR at the end of the test, compared to 0.5% AR from dark control samples, and was confirmed to be carbon dioxide. The increased carbon dioxide in irradiated samples suggests that the route of degradation for irradiated samples differs from dark controls.

Eugenol degraded to multiple unknowns, the largest of which reached 22.8% AR in irradiated samples (6 hours) and 27.6% AR in dark control samples (2 hours). Non-extractable radioactivity increased throughout the test with maximum levels of 28.0 and 30.8% AR observed in irradiated and dark control samples, respectively, at the end of the test.

I. MATERIALS AND METHODS

A MATERIALS

Test material 1 (used in standard rate tests)

Name:	[ring-^{14}C(U)]-Eugenol
Description:	Clear solution in ethanol
Lot/Batch no.:	CFQ44613
Radiochemical Purity:	Radiochemical purity 96.7%
Specific activity:	9.27 MBq/mg.
Storage:	Frozen (nominally -20°C)

Non-labelled material (used in all tests)

Name:	Eugenol
Description:	Colourless to pale yellow
Lot/Batch no.:	ESTS 167/20 40002011619
Purity:	99.7%
CAS #:	97-53-0
Storage/stability:	Room temperature

The soil used in this study was Speyer 5M supplied by LUFA Speyer. Prior to use, the soil was stored in an incubator routinely maintained at 4 ± 2°C, in loosely tied plastic bags in accordance with the ISO international standard.

Table 8.1.1.3-1: Characterisation details for the soil used to investigate the soil photolysis of eugenol

Soil parameter	Value
Source	Speyer 5M
Test system code	CS74/20
Particle size distribution, UK[1] % Sand (63 µm to 2 mm) % Silt (2 µm to 63 µm) % Clay (< 0.002µm)	 53 36 11
Textural classification, UK	Sandy loam
Soil pH (water)	8.4
Soil pH (0.01M $CaCl_2$)	7.4
Organic carbon content, (%)	0.9
Cation exchange capacity, (meq/100 g soil)	12.7
Water content (% dry weight)	30.6
Water content at pF 2 (%)	25.5

B STUDY DESIGN

Experimental conditions

Soil samples (2 g dry weight equivalent) were dispensed and using RO water mixed into a slurry. Using a spatula, the soil slurry was transferred into individual metal trays (*ca* 12.57 cm^2 surface area), and then spread evenly to give a smooth surface and a soil depth of *ca* 2 mm. The vessels were left to air dry at room temperature. Each metal tray was labelled with the study number and a unique sample code before transferring the metal tray into glass containers with a lid (quartz glass for irradiated vessels).

0 days after treatment (DAT) samples were analysed immediately following application.

All remaining samples were maintained at 20 ± 2°C. In order to maintain the temperature of the irradiated samples, they were placed in an aluminium-cooling block designed to enable cooling water to circulate through its base and containing a layer of polyethylene glycol (PEG) to facilitate transfer of heat. A thermocouple was placed in an untreated sample and positioned in the block alongside the treated samples. Temperature measurements were taken daily throughout the photolysis period.

Air was pulled through a drierite trap (to dry the air) and over the treated vessels and, after test substance application, through a series of traps. The first trap was an empty vessel used as a security trap. The second and third traps contained sodium hydroxide solution (2M) to collect liberated carbon dioxide.

Atlas Suntest accelerated exposure machines (Atlas Materials Testing Technology, Bicester, UK) were used as the light source. These instruments filter radiation from a xenon burner to remove light below 290 nm to give ultra-violet and visible light with a spectral distribution close to that of natural sunlight.

The application rate of 16.59 µg per unit was calculated from the field application rate of 132 g a.i./ha, and the soil surface area of 12.57 cm^2 (diameter 4 cm). This is equivalent to a surface area application to the soil of 1.3 µg/cm^2.

In the definitive test, vessels were treated with 88 µL of application solution 1, equivalent to 16.55 µg (153.5 kBq) of [^{14}C]-eugenol, dropwise over the soil surface.

Duplicate samples were removed for analysis at 0 DAT. Duplicate samples were removed for analysis under irradiated and dark conditions at 2 hours, 6 hours, 24 hours, 7 days, 14 days and 30 days after treatment.

The traps were quantified for radioactivity when the associated incubation vessels were removed for analysis.

Sample Analysis

The soil was transferred to a pre-weighed plastic vessel, by washing across with acetonitrile (25 mL). The vessel was shaken for 10 minutes at room temperature and centrifuged (2500 *g*, 10 minutes). The supernatant was transferred into a separate container labelled "Extract 1". The soil was extracted in the same way again with acetonitrile (25 mL), combining the supernatants with the first extract in the Extract 1 vessel. The soil was then extracted in the same way with acetonitrile:water (1:1 v/v, 25 mL) and the supernatant added to Extract 1. The soil then had a final extraction with acetonitrile:water (1:1 v/v, 25 mL) and the supernatant added to Extract 1. Extract 1 was then quantified by LSC. A sub-sample of Extract 1 was analysed directly by HPLC.

Following extraction, the post-extracted soil samples were placed in a fume cupboard and left to air-dry prior to grinding and combustion, to determine the amount of radioactivity unextracted.

Radioactivity in the trapping solutions were quantified by LSC when the samples to which they were attached were removed for analysis. The radioactivity in sodium hydroxide traps was confirmed as $^{14}CO_2$ by pooling collected traps from selected samples, followed by precipitation using barium chloride.

High performance Liquid chromatography (HPLC)
The following HPLC method was used for the determination of the radiochemical purity of the application solutions and for sample analysis.

Table 8.1.1.3-2: HPLC method for soil samples

HPLC System	Agilent 1260		
Column	YMC Triat C18, 5µ (250 mm x 4.6 mm)		
Column temperature	25°C		
Mobile phases	Eluent A: water / Eluent B: acetonitrile		
	Time (min)	% Eluent A	% Eluent B
	0	70	30
	30	50	50
	35	0	100
	40	0	100
	41	70	30
	45	70	30
Flow rate (mL/min)	1		

Samples were co-chromatographed with non-radiolabelled eugenol reference standard and chromatograms were evaluated using Laura (version 6.0.4.92 and 6.1.5.69) software (Lablogic).

II. RESULTS

Light intensity measurements were taken at the beginning and end of the irradiation period. Measurements were taken at the positions of the soil trays and at the same height as the soil layers. An overall average intensity was calculated from the measurements, between 300 and 400 nm, and was 25.3 Watt/m^2

A Distribution and Recovery of radioactivity

The amount of radioactivity extracted decreased immediately after application from 86.6% AR at 0 DAT to 67.1 – 70.3% AR after 2 hours. Extractable radioactivity further decreased to 54.5 – 61.2% AR at the end of the incubation. The amount of radioactivity remaining in the residue increased from 6.3% AR at 0 DAT to 28.0 – 30.8% AR at the end of the incubation.

Volatile radioactivity steadily increased to a mean maximum of 8.7% AR in the irradiated samples and 0.5% AR in dark control samples, at the end of incubation. The volatile radioactivity was confirmed as $^{14}CO_2$ by barium chloride precipitation.

Table 8.1.1.3-3: Mean Distribution and recovery of radioactivity from irradiated Speyer 5M soil following application of [^{14}C]-Eugenol

Sampling Interval	% Applied Radioactivity			
	Soil Extract	Traps	Residue	Total
0	86.6	NA	6.3	92.9
2 hour	67.1	0.2	24.2	91.4
6 hour	72.5	0.8	20.4	93.7
24 hour	63.7	2.0	23.2	88.9
7 day	63.4	3.2	23.2	89.8
14 day	63.9	4.9	20.6	89.3
30 day	54.5	8.7	28.0	91.1

NA = Not Applicable

Table 8.1.1.3-4: Mean Distribution and recovery of radioactivity from Speyer 5M soil incubated in the dark following application of [^{14}C]-Eugenol

Sampling Interval	% Applied Radioactivity			
	Soil Extract	Traps	Residue	Total

0	86.6	NA	6.3	92.9
2 hour	70.3	ND	21.3	91.6
6 hour	64.2	0.2	25.6	89.9
24 hour	69.7	0.1	24.0	93.8
7 day	62.4	0.2	26.4	88.9
14 day	65.6	0.1	26.7	92.4
30 day	61.2	0.5	30.8	92.4

NA = Not Applicable; ND = Not Detected (or < 0.1% AR)

B Characterisation

Chromatographic profiles were similar between irradiated and dark control samples. Immediately after application, 60.2% AR was recovered as [^{14}C]-eugenol with one major unknown detected at 17.3% AR in 0 DAT samples. Eugenol degraded rapidly under both irradiated and dark control conditions to between 9.6 and 13.9% AR, 2 hours after application. Levels of [^{14}C]-eugenol plateaued from 24 hours to the end of incubation with 3.8 to 6.2% AR recovered. The Unknown at *ca* 38 minutes, reached a maximum of 22.8% AR (24 hours) in irradiated samples and 27.6% AR (2 hours) in dark control samples and decreased towards the end of incubation, most notably in the irradiated samples. Other unknowns increased sharply up to 24 hours and then reached a plateau. The largest individual unknown observed was 11.8% AR (14 DAT) in irradiated samples and 11.1% AR (2 hours) in dark control samples.

Table 8.1.1.3-5: Mean Characterisation of applied radioactivity from irradiated Speyer 5M soil following application of [^{14}C]-Eugenol

Sampling Interval	% Applied Radioactivity				
	Eugenol	Unknown 38 min	Total unknowns	Largest unknown	Unresolved background
2 hour	9.6	22.4	33.9	7.2	1.2
6 hour	17.3	22.8	31.4	6.6	1.0
24 hour	5.0	16.8	41.6	8.1	0.4
7 DAT	5.1	12.5	45.2	4.7	0.6
14 DAT	4.7	12.4	45.9	11.8	0.8
30 DAT	4.0	7.0	42.5	10.5	1.0

NA = Not Applicable
DAT = days after treatment

Table 8.1.1.3-6: Mean Characterisation of applied radioactivity from Speyer 5M soil incubated in the dark following application of [^{14}C]-Eugenol

Sampling Interval	% Applied Radioactivity				
	Eugenol	Unknown 38 min	Total unknowns	Largest unknown	Unresolved background
0 DAT	60.2	17.3	7.7	2.7	1.4
2 hour	13.9	27.6	28.2	11.1	0.6
6 hour	3.0	24.8	35.6	8.6	0.7
24 hour	6.2	17.4	45.4	9.2	0.6
7 DAT	3.8	19.1	38.9	7.3	0.6
14 DAT	5.4	20.2	39.5	8.7	0.4
30 DAT	4.0	16.8	40.1	9.8	0.3

NA = Not Applicable
DAT = days after treatment

C Kinetics

SFO kinetics of all sampling intervals in both irradiated and dark control samples were similar, with a DT_{50} and DT_{90} of < 1 day. A high chi^2 error percentage (> 15%) was observed for SFO kinetics indicating a poor fit. Eugenol was therefore modelled with DFOP, HS and FOMC kinetic models.

DFOP kinetics also gave a DT_{50} of < 1 day in both irradiated and dark control conditions. A high chi^2 error percentage was observed for irradiated samples indicating a poor fit.

First Order Multi-Compartment (FOMC) and Hockey Stick (HS) models were also investigated but found to have unacceptable values in the confidence limits of the model parameters.

As the DT_{90} was reached within the study duration, SFO kinetics were performed using the results from the initial 24 hours. In both irradiated and dark control samples, the DT_{50} was < 1 hour and the DT_{90} was *ca* 3 hours. A high chi^2 error percentage was observed for irradiated samples due to variability in the results.

There was very little difference in the kinetics of the irradiated and dark control samples and therefore photolysis kinetics were not calculated.

III. CONCLUSION

Degradation of eugenol was rapid in both irradiated and dark control conditions, with a DT_{50} of < 1 hour. There was no significant differences in the rate of degradation of eugenol between irradiated and dark control samples. No conversion to equivalent summer sunlight days was therefore calculated.

In irradiated samples, volatile radioactivity reached a maximum of 8.7% AR at the end of the test, compared to 0.5% AR from dark control samples, and was confirmed to be carbon dioxide. The increased carbon dioxide in irradiated samples suggests that the route of degradation for irradiated samples differs from dark controls.

Eugenol degraded to multiple unknowns, the largest of which reached 22.8% AR in irradiated samples (6 hours) and 27.6% AR in dark control samples (2 hours). Non-extractable radioactivity increased throughout the test with maximum levels of 28.0 and 30.8% AR observed in irradiated and dark control samples, respectively, at the end of the test.

3. Assessment and conclusions

Assessment and conclusion by applicant:

This study was performed well and degradation rate in both dark and irradiated samples was <1 hour and the DT_{90} less than 3 hours.

Assessment and conclusion by RMS:

The photodegradation of [^{14}C]-eugenol was studied on Speyer 5M soil, a sandy loam (USDA) from Rheinland-Pfalz, Germany at 132 g ai/ha soil for 30 days at 20 ± 2°C and under dry conditions. Samples were treated with 16.55 µg of [^{14}C]-eugenol and were continuously irradiated using a Suntest accelerated exposure machine (xenon lamp fitted with a filter to remove radiation below 290 nm). Test vessels were connected to two 2M NaOH traps for the collection of liberated CO2.

The study was performed according to OECD draft document for Phototransformation of Chemicals on Soil Surfaces, January 2002. The evaluation of the study by the RMS revealed the following deviations from the draft OECD test guideline:

- The recoveries ranged from 88.9 to 93.7 for irradiated samples and from 88.9 to 93.8 for dark samples, falling slightly below the recommended range for labelled chemicals (90-100%).
- The selected soil was not characterized for texture according to FAO or USDA classification system.
- There was no significant differences in the rate of degradation of eugenol between irradiated and dark control samples. Therefore, photolysis kinetics and the conversion to equivalent summer sunlight days was not calculated.
- Characterisation of applied radioactivity from irradiated soil extracts from Speyer 5M soil at 0 hours was not reported.
- According to the OECD guideline, reference substances should be used for the characterization and/or identification of phototransformation product. In this case, eugenol was confirmed by HPLC co-chromatography with the non-radiolabelled reference standard, however transformation products were not characterized.

The deviations mentioned above are considering minor issues with the exception of the characterization of the degradation products. Chromatographic profiles were similar between irradiated and dark control samples, however, according to the study report, the differences in the CO_2 evolution suggest that the route of degradation for irradiated samples differs from dark controls. The total unknown radioactivity reached a maximum value of 45.9%AR at 14 days in irradiated samples, with largest unknown above 10%AR.
It is thought that the unknown at min 38 is not a specific photoproduct since is present in irradiated and non-irradiated samples. However, it is not possible to stablish a comparison nor the evolutions for the other unknowns without the radiochromatograms profiles. **Applicant is called to include the identification of the observed unknowns.**

Additionally, as it has been commented by co-RMS, it is noted that the component 'Unknown 38 min' was not identified in the aerobic degradation study, or not detected at those high percentages. It is not clear, if the low extracted percentage obtained in Jones, 2015a could led to the omission of any relevant metabolite in soil under dark conditions.

Outcome and conclusion of the study:
Degradation of eugenol was rapid in both irradiated and dark control conditions, with a DT_{50} of < 1 hour. There were no significant differences in the rate of degradation of eugenol between irradiated and dark control samples.

The characterization of the photoproducts was not carried out in the experiment since reference compounds were not included. Therefore, the route of degradation in soil under irradiated conditions is not considered fully addressed.
The following comment was added by applicant during the co-RMS-applicant´s initial revision: *Samples were contained at the CRO and analysis of the photoproducts will be carried out by the laboratory.*

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	Eugenol: Aerobic soil metabolism
Report No.	Envigo Study No. PIF0002
Document No.	
Guidelines followed in study	OECD: Guideline 307, Aerobic and Anaerobic Transformation in Soil, 2002
Deviations from current test guideline	Five vessels for microbiology analysis were not prepared for all soil types. For Brierlow soil only three vessels were prepared and for Calke and Empingham only four vessels were prepared for each soil type. Only three vessels were required for analysis. The identity of the pH indicator added to the potassium hydroxide trapping solutions was not recorded in the study data.
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 eugenol in two of the aerobic soils (Calke and Ingelby) 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 eugenol 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 eugenol 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	1.30732 97.1994	0.229385 1.27645	Bad[a]	7.3	0.5	1.8
Ingleby	K M0	1.18187 97.1996	0.20651 1.70427	Bad[a]	9.4	0.6	1.9

In the Student's t-test, the majority of values were significant at the 0.1% level
[a] Calculated values are lower than actual values from about day 3 onwards
na insufficient data points to calculate Chi^2 value

The visual analysis is shown below:

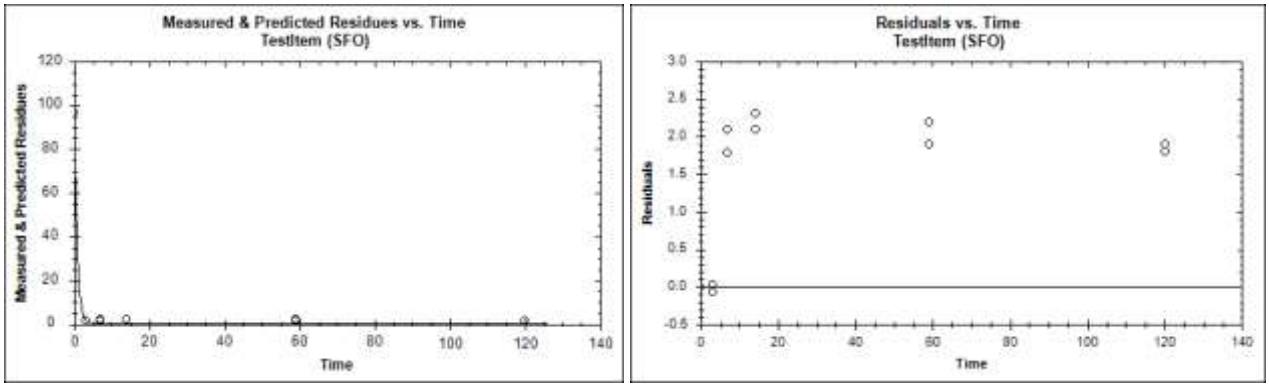
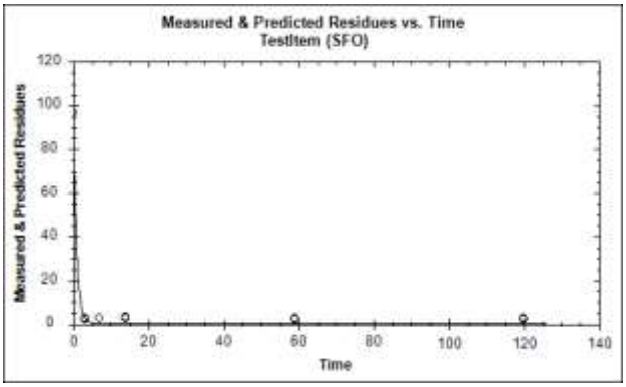


Figure 8.1.2.1.1-1: SFO curve for Calke soil.



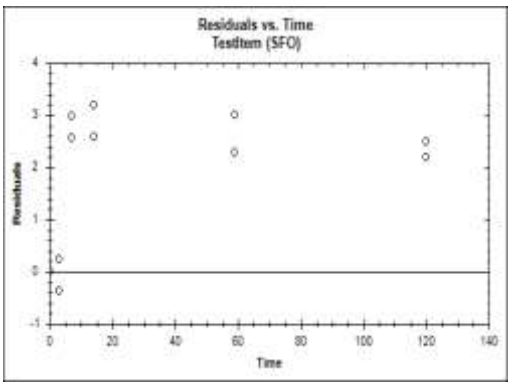


Figure 8.1.2.1.1-2: SFO curve for Ingleby soil.

3. Assessment and conclusions

Assessment and conclusion by RMS:

The aerobic soil degradation rate of eugenol was investigated in four soils. However, DT50 and DT90 was determined only for two of them (Ingleby and Calke soils). For the two remaining soils, Brierlow and Empingham, there were not enough data points to perform the kinetic analysis since the percentage of extractable radioactivity was lower than 10% at 3 days and eugenol was only analysed in the extractable once at 0 days.

Since a significant decrease of eugenol was observed at the first sampling points (mean zero-time measurements were between 18.8 and 33.9 % AR in the Calke and Ingleby soils), the radiochemical purity value (97.2 %) 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.

An attempt was done in the confirmatory data to analysed the data considering the measured zero-time value using CAKE (version 3.1), however the results were similar to the applicant`s proposal and the kinetic fitting was not improved. As it was pointed out, *the study did not take into account the nature of the test substance (very rapid degradation). The selected sampling timepoints missed much of the degradation of eugenol and for this reason the kinetic assessment was only possible on two of the four soils (Calke and Ingleby).*

According to the applicant's calculations, DT50 and DT90 values ranged from 0.5 – 0.6 days and 1.8 – 1.9 days, respectively for Ingleby and Calke soils. Observed DT50 and DT90 values for Brierlow and Empingham soils were < 3 days.

Table 8.1.2.1.1-2: Kinetic data for the decline of eugenol in aerobic soil

Soil	Kinetic model	DT_{50} (days)	DT_{90} (days)	Visual goodness-of-fit
Brierlow	NA	<1[a]	<3[a]	-
Calke	SFO	0.5	1.8	Good
Ingleby	SFO	0.6	1.9	Good
Empingham	NA	<3[a]	<3[a]	-

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

This study confirms the rapid degradation of eugenol. 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.

Outcome and conclusion of the study:

Despite the reported deviations reported under point CA 7.1.1.1/01, the study is considered acceptable.

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 eugenol, 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.
However, for information only, the following paper has been summarised on the environmental fate of methyleugenol.

1. **Information on the study**

Data point	CA 7.1.2.1.2/01
Report author	T.N.Shaver and D.L.Bull
Report year	1980
Report title	Environmental Fate of Methyl Eugenol
Report No.	NA
Document No.	Environ. Contam. Toxicol. 24,619-626 (1980)
Guidelines followed in study	None
Deviations from current test guideline	No guideline followed. Only one soil used. No information provided on biomass or soil properties. No mass balance data reported. Study was not performed in the dark.
Previous evaluation	No
GLP/Officially recognized testing facilities	No
Acceptability/Reliability	No, supplementary data only

2. **Full summary**

The environmental fate of methyleugenol (purity >99%) was examined in one fine sandy loam soil. 15 mL of water were added to the soil that was then treated with 2 mL of hexane containing 10 mg of radiolabelled methyleugenol. The samples were stored in an environmental chamber at 32°C or 22°C, with 14 h light and 10 h dark cycle. Three samples were taken at 0, 24, 48, 72 and 96 hours.
Soils were extracted 5 times with *ca* 20 ml portions of dichloromethane. Combined extracts were concentrated under vacuum and adjusted to 3 mL with hexane. Analyses was performed with gas chromatography (GC). 2m x 2 mm ID glass column packed with 1.5% SP2250 + 1.95% SP2401 on 10-Supelcoport at a column temperature of 140°C.
Unextracted residues in soil were determined by combustion.
Methyleugenol dissipated rapidly from soil with 98% of the material being lost within 96 hours at 32°C, and 81% being lost after 96 hours at 22°C. TLC analyses revealed methyleugenol as the only radiolabelled compound.
The half-life of methyleugenol was calculated to be 6 and 16 hours at 32 and 22°C, respectively.

3. **Assessment and conclusions**

Assessment and conclusion by applicant:
This data is shown for information only. Methyleugenol was not seen in the soil degradation study previously submitted and this data is shown for information only and indicates that any methyleugenol that may be formed in soil will dissipate rapidly with a half-life of <1 day.

Assessment and conclusion by RMS:
This study has been included for completeness.

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

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 > 3 days, field studies are not triggered for eugenol 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 in soil

B.8.1.3.1.1. 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	Eugenol: Adsorption/Desorption in Five Soils
Report No.	Envigo Study No. PIF0003
Document No.	
Guidelines followed in study	OECD: Guideline 106, Adsorption-Desorption Using a Batch Equilibrium Method (January 2000)
Deviations from current test guideline	Replicate samples from the preliminary experiment at the selected soil-to-solution ratio at equilibration were not analysed to obtain a mass balance and to confirm stability at the chosen equilibration time
Previous evaluation	Yes, Confirmatory Data 2016
GLP/Officially recognized testing facilities	Yes
Acceptability/Reliability	No

2. **Full summary**

The sorption properties of eugenol 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 7.2 and organic carbon contents between 1.1 and 3.9%. Eugenol 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 eugenol in solution and that adsorbed to soil and (3) the extent of any binding of eugenol to the glass test vessels.

Nominal initial solution concentrations of eugenol in the range 0.05 – 5 mg/L in aqueous 0.01 M calcium chloride were studied. Soil-to-solution ratios of 1:50 w/v (Bromsgrove, Warsop and Evesham 3) or 1:20 w/v (Elmton 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 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 eugenol 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	4.9	1.5	1.51	101	0.63	8.24	549	0.69
Evesham 3	Clay loam	6.4	2.0	2.31	116	0.83	13.0	650	0.84
Elmton	Sandy Clay Loam	7.2	3.9	7.18	184	0.94	50.2	1290	1.05
Warsop	Sand	3.9	1.1	3.49	317	0.73	9.78	889	0.78
Calke	Sandy loam	5.2	3.5	27.8	794	1.14	113	3230	1.11

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 eugenol. Using the McCall scale to assess a chemical's potential mobility, the coefficient values indicate a slight to medium potential for mobility of eugenol in the soils tested.

I. MATERIALS AND METHODS

A MATERIALS

Test material 1 (used in standard rate tests)

Name:	[Phenyl-U-^{14}C]-eugenol
Description:	In acetonitrile solution
Lot/Batch no.:	265-157-0597-A-20140325-NTO
Radiochemical Purity:	Radiochemical purity >97%
Specific activity:	13.3 MBq/mg.
Storage :	<-15°C

Non-labelled material (used in all tests)

Name:	Eugenol, 2-Methoxy-4-(2-propenyl)phenol
Description:	Liquid
Lot/Batch no.:	121774
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 eugenol

Soil parameter	**Value**				
Source	Calke	Elmton	Evesham 3	Warsop	Bromsgrove
Envigo batch number	100412C	070114A	H101104C	190713C	220909B
Particle size distribution, UK[1] % Sand (63 µm-2 mm) % Silt (2 µm-63 µm) % Clay (< 0.002µm)	 69 16 15	 55 21 24	 37.5 30.8 31.8	 90 6 4	 66 19 15
Textural classification, UK	Sandy loam	Sandy clay loam	Clay loam	Sand	Sandy loam
Soil pH (water)	5.8	7.9	6.8	4.9	5.7
Soil pH (0.01M $CaCl_2$)	5.2	7.2	6.4	3.9	4.9
Organic carbon (%)	3.5	3.9	2.0	1.1	1.5
Organic matter (%)	6.0	6.7	1.2	1.9	2.6
Cation exchange capacity,(meq/100 g soil)	10.9	21.3	18.1	2.8	9.1
Nitrogen (%)	0.29	0.45	0.17	0.07	0.14
Water content (% dry weight)	26.04	25.62	4.34	4.28	8.2

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 1:50 w/v was selected for Bromsgrove, Evesham 3 and Warsop soils and a soil-to-solution ratio of 1:20 w/v was selected for Elmton and Calke soils. 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 and Calke) or 0.4 g (Bromsgrove, Warsop and Evesham 3) dry weight equivalent) of soil were weighed into each tube. A volume of 0.01 M calcium chloride solution was added to achieve soil-to-solution ratios of 1:20 or 1:50 w/v. The tubes were pre-equilibrated by shaking in the dark at approximately 25ºC for approximately 18 hours.

Following pre-equilibration, an aliquot (20 µL) of the appropriate eugenol treatment solution was added to duplicate vessels of each soil type. This gave initial nominal eugenol 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 eugenol 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 (1.0 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 eugenol. Soil samples were extracted once with acetonitrile (1 x ca 5 mL) by sonicating for 15 minutes then shaking for 15 minutes. The extracts and soil were separated by centrifugation. The procedure was repeated using acetonitrile:0.1 M hydrochloric acid (1:1 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 eugenol 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: Water Eluent B: Acetonitrile		
	Time (min)	% Eluent A	% Eluent B
	0	90	10
	10	90	10
	30	0	100
	40	0	100
	41	90	10
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 eugenol concentration following the adsorption phase. Recoveries of radioactivity were in the range 93.1 – 98.1% of the amounts applied.

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

Parameter/Soil	Bromsgrove	Evesham 3	Elmton	Warsop	Calke
Adsorption solution	74.6	73.0	5.9	86.2	7.8
Soil extracts	12.5	9.6	14.5	10.5	24.6
Non-extractable	9.4	13.3	72.7	1.4	61.8
Total Recovery	96.5	95.9	93.1	98.1	94.2

Eugenol represented 21.1–92.5% sample radioactivity in the aqueous solution and 7.8–38.5% sample radioactivity in soil extracts.

Eugenol represented 21.1–92.5% sample radioactivity in the aqueous solution and 7.8–38.5% 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 eugenol to form degradates which bound to the soil. Eugenol was readily extractable in the extraction solvents used therefore it is unlikely that the remaining radioactivity on the soil residues was eugenol. The proportion of Eugenol in total soil (soil extracts and soil non-extractable residue) was calculated based on the amount of eugenol extracted and quantified by HPLC. Eugenol was therefore considered to represent 1.3 – 34.0% of total radioactivity in the soil.

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

Parameter/Soil	Bromsgrove	Evesham 3	Elmton	Warsop	Calke
Aqueous solution	54.1	92.5	21.1	86.4	24.8
Soil extracts	12.0	29.2	7.8	38.5	12.8
% total soil radioactivity	6.8	12.2	1.3	34.0	3.6

The concentrations of eugenol (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 eugenol 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	2.349	2.565	1.10
1.500	0.6882	1.441	2.11
0.500	0.24435	0.462	1.96
0.150	0.04105	0.2379	5.83
0.050	0.01145	0.08652	8.20

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

Table 8.1.3.1.1-6: Adsorption of eugenol 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	3.433	8.344	2.45
1.500	1.181	2.532	2.15
0.500	0.4234	0.7819	1.85
0.150	0.08885	0.2996	3.42
0.050	0.02372	0.1263	5.34

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

Table 8.1.3.1.1-7: Adsorption of eugenol 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	0.1381	1.155	9.73
1.500	0.05055	0.3787	10.4
0.500	0.01242[b]	0.1357	10.9
0.150	0.003688	0.03321	9.17
0.050	0.001011	0.01087	10.8

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

Table 8.1.3.1.1-8: Adsorption of eugenol 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	3.786	12.15	3.21
1.500	1.276	3.694	2.90
0.500	0.4326	1.434	3.33
0.150	0.09636	0.5836	6.06
0.050	0.02547	0.2879	11.3

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

Table 8.1.3.1.1-9: Adsorption of eugenol 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	0.1151	3.336	30.0
1.500	0.06773	1.02	16.9
0.500	0.02648	0.345	13.1
0.150	0.005878	0.08795	15.0
0.050	0.002179	0.02723	12.6

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

The Freundlich adsorption coefficients (K_F^{ads}) were in the range 2.67 – 98.9 indicating some adsorption of eugenol to the test soils. Adsorption coefficients corrected for organic carbon content (K_{Foc}^{ads}) were in the range 178 – 2830. Values of the exponent 1/n were in the range 0.63 – 1.14. There was no correlation between soil pH and the degree of adsorption.

Table 8.1.3.1.1-10: Adsorption characteristics of eugenol

Soil	Organic carbon content (%)	K_F	K_{foc}	1/n
Bromsgrove	1.5	1.51	101	0.63
Evesham 3	2.0	2.31	116	0.83
Elmton	3.9	7.18	184	0.94
Warsop	1.1	3.49	317	0.73
Calke	3.5	27.8	794	1.14

B DESORPTION

The mass balance of radioactivity was measured in the two remaining vessels of each soil type treated at the highest eugenol concentration following the desorption phase. Mean recoveries were in the range 88.4 – 98.6% of applied radioactivity.

Eugenol represented 21.1–71.2% sample radioactivity in the aqueous solution and 7.7–14.8% total radioactivity in soil extracts.

Table 8.1.3.1.1-11: Proportions of eugenol 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	62.6	70.6	21.1	71.2	22.7
Soil extracts	8.8	14.8	7.7	9.0	10.7
%Total soil radioactivity	4.4	3.9	1.0	7.1	2.3

For each soil the Freundlich desorption coefficient (K_F^{des}, in the range 12.4 – 525) was higher than the adsorption coefficient, indicating some degree of irreversibility to the adsorption of eugenol.

Table 8.1.3.1.1-12: Desorption characteristics of eugenol

Soil	Organic carbon content (%)	K_F	K_{foc}	1/n
Bromsgrove	1.5	8.24	549	0.69
Evesham 3	2.0	13.0	650	0.84
Elmton	3.9	50.2	1290	1.05
Warsop	1.1	9.78	889	0.78
Calke	3.5	113	3220	1.11

III. CONCLUSION

Freundlich adsorption coefficients (K_F^{ads}) for eugenol were in the range 1.51 – 27.8 and adsorption coefficients corrected for organic carbon content (K_{Foc}^{ads}) were in the range 101 - 794. Using the McCall scale to assess a chemical's potential mobility, these values indicate a slight to medium potential for mobility of eugenol 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 eugenol.

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 eugenol. The RMS suggested that due to the rapid degradation of eugenol, it's stability during a batch equilibrium adsorption/desorption study cannot be relied upon.
This study has been repeated and this study is reported here as supplementary data.

Assessment and conclusion by RMS:

A batch equilibrium adsorption/desorption study for eugenol 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:

- Replicate samples at the selected soil-to-solution ratio at equilibration were not analysed to obtain a mass balance and to confirm stability of the test substance.
- The study states that equilibrium was generally not reached during the preliminary studies. On this basis, an adsorption equilibrium time of 24 hours was selected for all soils. However, it was noted that the percentage radioactivity in the solution generally decreased after initially displaying stability. Therefore, it was 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 Annex I inclusion RMS (UK) 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 eugenol.
- The sorption of eugenol was determined by the indirect method, which OECD106 specifically recommends as not appropriate for this type of substance (instability of the test substance in the time scale of the experiment).
- The study only reports the mass balance for samples with an initial eugenol concentration of 5.0 mg/L. The total recoveries ranged from 93.1 – 98.1 % AR that is an acceptable result according to the guidelines. However, a significant proportion of this overall recovery was classed as unextracted radioactivity.
- For Warsop and Bromsgrove soil, the chosen ratios correspond to an adsorption after 24 hours of 8.4 and 17.7 %, respectively. These values are below the recommended adsorption levels outlined in OECD 106 (above 20 % and preferably above 50 %).
- 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 for the five selected soils. Additionally, the soil characterisation was not conducted according to GLP.

The adsorption and desorption isotherms for each concentration were used to calculate Freundlich coefficients (Kf) and Kfoc values for each soil. However, Freundlich isotherms cannot be reliably used in this case because a robust assessment of the test substance stability was not produced. In addition, the radiochromatograms presented for Bromsgrove soil showed that a significant amount of the measured radioactivity was not associated with eugenol. Given this and that the soil radioactivity was calculated indirectly (by analysis of the supernatant), the Annex I RMS suggested that the adsorption calculated in this study was not representative of eugenol alone. Instead, the values calculated were mean characteristics for the range of compounds shown on the radiochromatograms.

According to the Outcome of the consultation on confirmatory data used in risk assessment for eugenol (EFSA Supporting publication 2017:EN-1165), these major deviations identified in the study leading to results not robust enough to be used to derive the sorption endpoints for eugenol. Default values (KFoc of 10 mg/L and 1/n of 0.9) were used in the assessment of the confirmatory data; therefore, this study is not valid anymore. For the renewal process, a new batch equilibrium study based on the direct method has been submitted (Kelly, 2021).

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	1:50	1:50	1:20	1:50	1:20
Mass balance of (at highest tested conc.)*	%	n.r	n.r	n.r	n.r	n.r
Adsorbed percentage	%	51.13-77.10	15.32-52.56	96.63-97.98	13.48-49.06	94.70-97.70

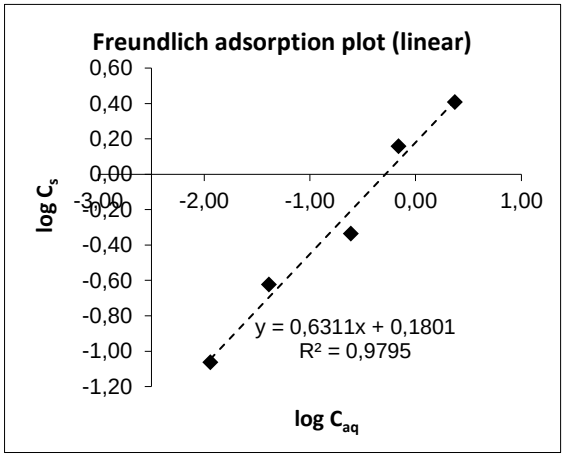
K_d × (soil: solution ratio)	-	0.02-0.15	0.04-0.11	0.37-0.55	0.06-0.23	0.62-1.45
$^{ads}K_F$ (95% confidence interval)	L/kg dw	1.514	2.311	7.185	3.491	27.831
$^{ads}1/n$ (95% confidence interval)	L/kg dw	0.631	0.829	0.943	0.734	1.139
$^{ads}R^2$	-	0.98	0.978	0.996	0.976	0.983
$^{ads}K_{F, OC}$	L/kg OC	100.9	115.6	184.2	317.3	795.2
K_{fE} /K_f	-	-	-	-	-	-

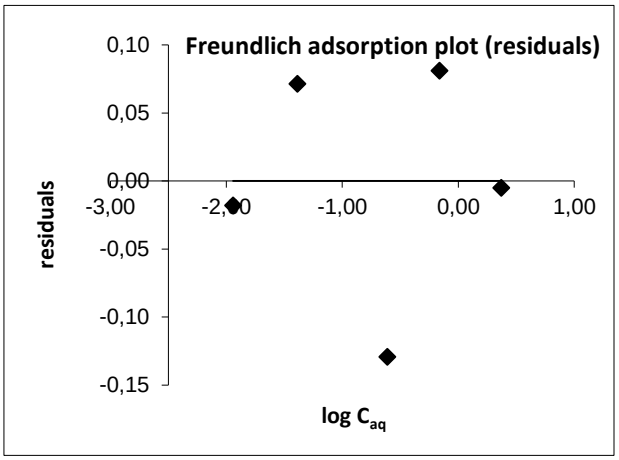
**n.r*: not reported

The checklist has been completed using the concentration values in aqueous solution and soil extracts corrected for the degradation of eugenol 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 (Bromsgrove soil at 5 mg/L).

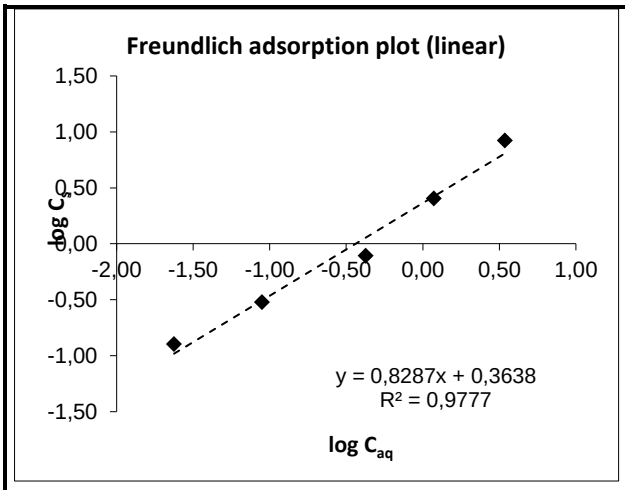
Parental mass balance was not reported. HPLC was carried out for all soils at the highest concentration; however, the chromatograms were not included with the exception of the chromatogram for Bromsgrove soil. Theoretically, the proportions of eugenol in aqueous and soil extracts after adsorption found (Table 8.1.3.1.1-5) were used for the correction at all the tested concentration.

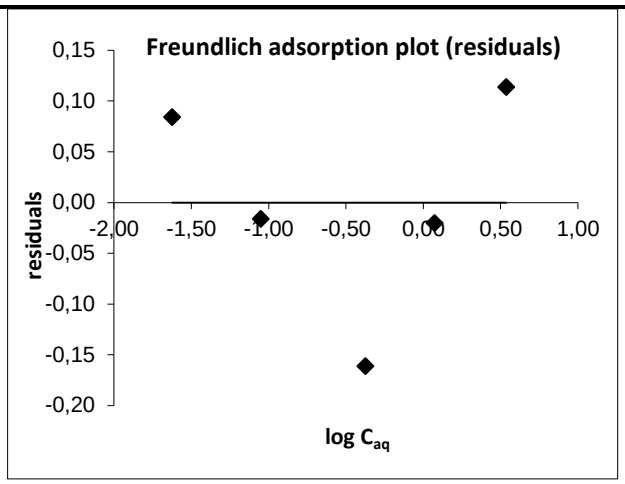
Bromsgrove



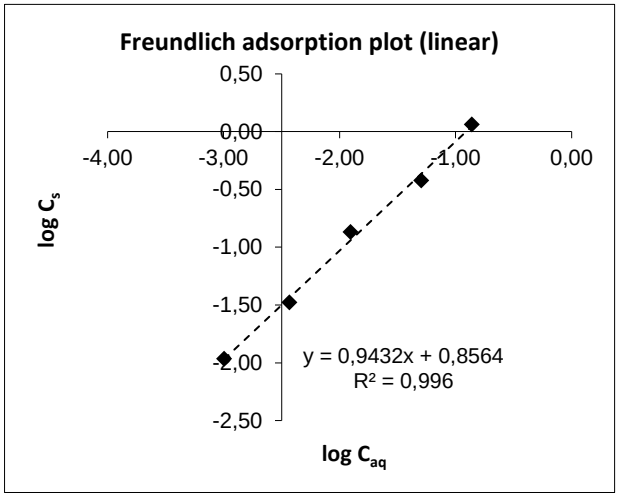


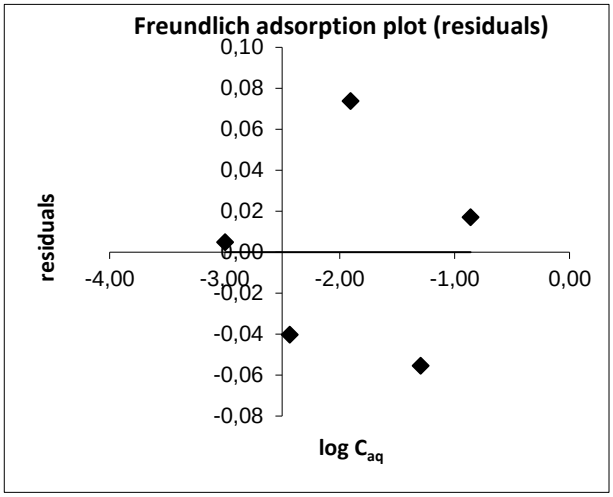
Evesham 3



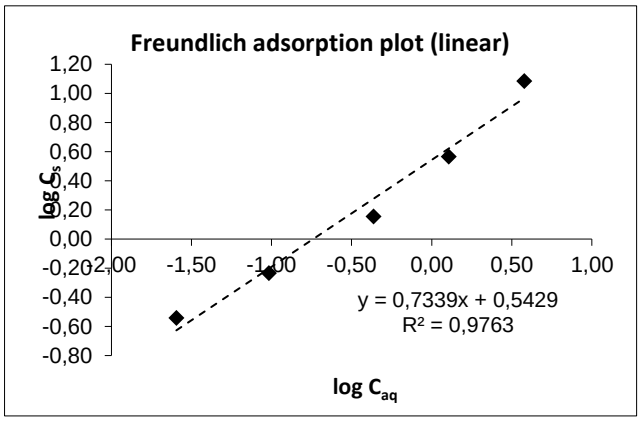


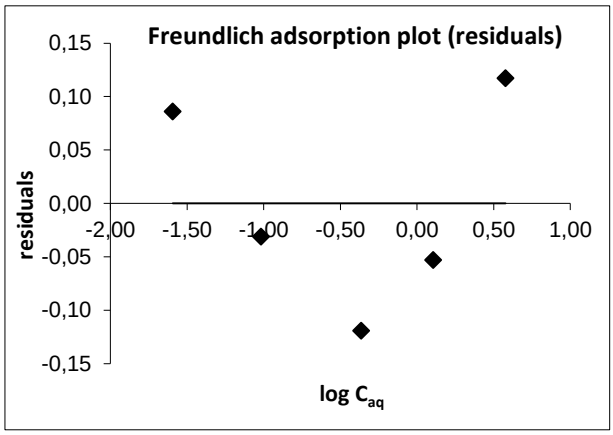
Elmton



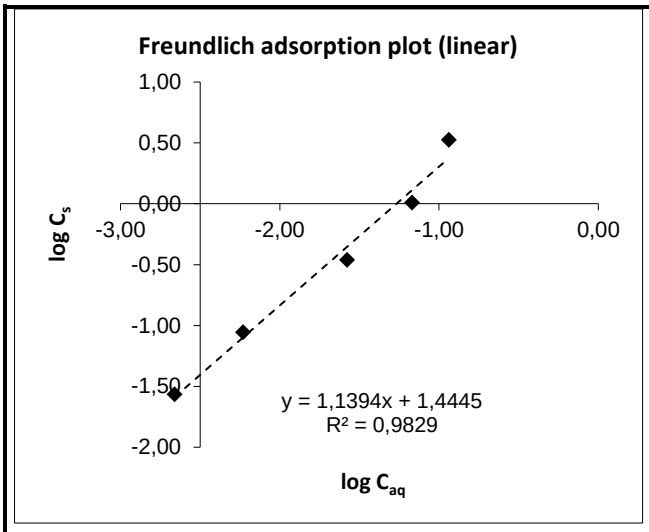


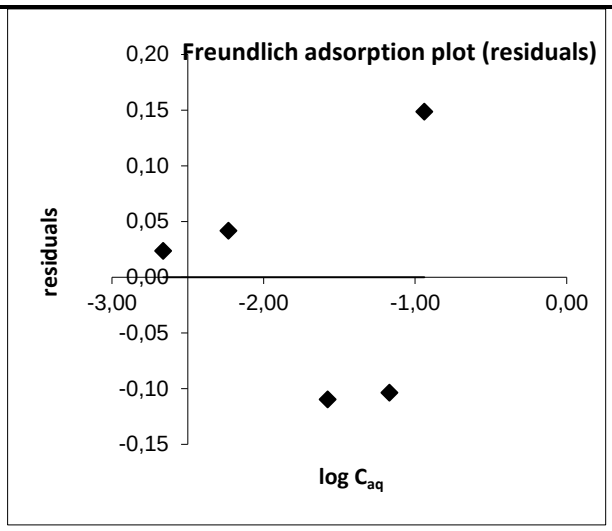
Warsop





Calke





Outcome and conclusion of the study:

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

1. Information on the study

Data point	CA 7.1.3.1.1/02
Report author	Kelly, D
Report year	2021
Report title	[^{14}C]-Eugenol: Adsorption/Desorption in Soil
Report No.	Smithers ERS Ltd No. 3202786
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/desorption characteristics of [^{14}C]-eugenol were determined in four soils using a batch equilibrium method (direct method), in the dark at 20 ± 2°C. One soil was from the UK (Kenslow, clay loam), two from Germany (RefeSol 01-A, sandy loam and RefeSol 03-G, Silty clay loam) and one from Spain (La Reina, clay).
The test was performed in Teflon® vessels. A soil: solution ratio of 1:50 w/v (0.5 g dry weight equivalent and 25 mL of solution) was used for all soils tested. The adsorption and desorption times were 6 hours for RefeSol 01-A and Kenslow soils, 3 hours for RefeSol 03-G soil and 12 hours for La Reina soil. The concentration of [^{14}C]-eugenol was determined by HPLC in supernatants and soil extracts after extraction of the test item from soil. Constants were therefore determined by the direct method.
The definitive isotherm test was conducted in the dark at 20 ± 2°C. Soil samples were pre-equilibrated with 0.01M calcium chloride solution overnight. The equilibrated soils were then treated with solutions of [^{14}C]-eugenol (25 µL) in ethanol to produce duplicate samples for analysis. Concentrations of test item in the aqueous phase were initially 5.0, 1.5, 0.5, 0.15 and 0.05 µg/mL.
Adsorption and desorption partition coefficients K_d, K_{OC}, K_{OM} and corresponding Freundlich coefficients K_F, K_{FOC} and K_{FOM} were determined for each soil.

A summary of the key adsorption and desorption values of [^{14}C]-eugenol 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	5.3	0.9	1.11	123.3	0.999	9.89	1099	0.873
RefeSol 03-G	Silty clay loam	6.0	3.0	3.59	119.8	0.945	12.30	410	0.938
Kenslow	Clay loam	5.1	3.7	1.78	48.2	0.980	3.04	82.2	0.649
La Reina	Clay	7.7	1.1	1.32	120.1	1.014	1.40	126.8	0.478

Freundlich adsorption coefficients, K_{FOC}, were in the range 48.2 to 123.3 L/kg. Using the McCall Classification scale to assess the potential mobility of a chemical in soil (based on K_{OC}), eugenol can be classified as having high mobility in soil (K_{OC} 50-150 L/kg) for each of the soils tested.
Freundlich desorption coefficients, K_{FOC}, were in the range 82.2 to 1099 L/kg. Desorption values were generally higher than corresponding adsorption values indicating that the adsorption was not fully reversible.
In all four soils, low adsorption to the soil was noted. Significant degradation of [^{14}C]-eugenol was seen in soil extracts, indicating chemical instability in the sterile soils tested.

I. MATERIALS AND METHODS

A MATERIALS

Test material 1

Name: [ring-^{14}C(U)]-Eugenol
Description: Clear solution in ethanol
Lot/Batch no.: 200925-RP1 and CFQ44613
Radiochemical Purity: Radiochemical purity 96.3% and 96.7%
Specific activity: 0.399 and 3.049 MBq/mg.
Storage: Frozen (nominally -20°C)

Non-labelled material (used in all tests)

Name: Eugenol
Description: Colourless to pale yellow
Lot/Batch no.: ESTS 167/20
Purity: 99.7%
CAS #: 97-53-0
Storage/stability: 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 eugenol

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	6.7	5.7	8.5
Soil pH (0.01M $CaCl_2$)	5.3	6.0	5.1	7.7
Organic carbon (%)	0.9	3.0	3.7	1.1
Organic matter (%)	1.6	5.2	6.4	1.9
Cation exchange capacity, (meq/100 g soil)	5.3	28.4	18.8	20.7
Moisture at pF2.0 (w/w %)	13.0	39.1	33.5	34.2

B STUDY DESIGN

Experimental conditions

In the preliminary investigations, experiments were conducted to determine the conditions to be used in the definitive isotherm test.

A summary of the tests performed are detailed below:

- A test was performed to determine the solubility of [^{14}C]-eugenol in 0.01M calcium chloride solution at a nominal concentration of 5.0 µg/mL, using ethanol as a co-solvent for application.
- A test was performed to determine whether [^{14}C]-eugenol adsorbed to Teflon® or plastic vessels from a solution of 0.05 µg/mL eugenol in 0.01M calcium chloride.
- Investigation of appropriate soil: aqueous ratios were conducted using all soils at a nominal concentration of 5.0 µg/mL. Ratios of 1:5, 1:25 and 1:50 w/v were tested. Results of the test were used to decide on the soil: aqueous ratios to be used for the remaining tests.
- The time to adsorption equilibrium in soil was determined using all soils, at a 1:50 w/v soil: solution ratio and a nominal concentration of 5.0 µg/mL. Following incubation for 1, 3, 6 and 12 hours samples were analysed to determine the levels adsorbed.

Stability was determined in the soil: solution and adsorption equilibrium tests.

Definitive Test

Parameter		Description	
Test system condition		Sterilised, air-dried soil passed through 2 mm sieve prior to use	
Test system sample weight		0.5 g (dry weight) per replicate	
Equilibrium solution		0.01M Calcium chloride solution, sterile (25 mL)	
Number of replicates		2	
Test apparatus		Teflon® tubes	
Test item application	Identity of solvent	Ethanol	
	Volume of test solution used/treatment	≤ 25 µL	
Test item concentration	Nominal application rates (µg/mL)	5.0, 1.5, 0.5, 0.15 and 0.05	
		Adsorption	Desorption

	Actual application rates (µg/mL)	4.994 1.493 0.492 0.147 0.049	5.412 1.590 0.518 0.150 0.050
Test system : solution ratio		1:50 (w:v) (soil)	
Indication of test item adsorbing to walls of test apparatus		No	
Adsorption equilibrium conditions	Temperature (°C)	20 ± 2°C	
	Time	6 hours: RefeSol 01-A and Kenslow 3 hours: RefeSol 03-G 12 hours: La Reina	
	Application method	Calibrated positive displacement pipette	
	Continuous darkness (Yes/No)	Yes	
	Shaking method	Reciprocating shaker	
Desorption equilibrium conditions	Temperature (°C)	20 ± 2°C	
	Time	6 hours: RefeSol 01-A and Kenslow 3 hours: RefeSol 03-G 12 hours: La Reina	
	Application method	Glass syringe	
	Continuous darkness (Yes/No)	Yes	
	Shaking method	Reciprocating shaker	
Method of separation of supernatant		Centrifugation	
Centrifugation	Speed	5000 *g*	
	Duration	34 minutes	
	Method of separating supernatant	Decanting	

Following removal of the terminal supernatant, soil samples were extracted twice with acetonitrile (25 mL), followed by twice with acetonitrile: water (1:1 v/v, 25 mL) combining the extracts. Soil samples were placed on a shaker for *ca* 10 minutes and centrifuged set at 2500 *g* (10 minutes) before removing the supernatant. The radioactivity in the soil extracts was quantified directly by LSC.
A sub-sample (50 mL) of each soil extract was concentrated by rotary evaporation (5 mL) and re-constituted in acetonitrile (2 mL). Concentrated soil extracts were quantified by LSC and analysed for [^{14}C]-eugenol by HPLC.
The soil residue, post-extraction, was air dried and ground. The radioactivity was quantified following combustion with LSC.

High performance Liquid chromatography (HPLC)
The following HPLC method was used for the determination of the radiochemical purity of the application solutions and for sample analysis.

Table 8.1.3.1.1-14: HPLC method for soil samples

HPLC System	Agilent 1260		
Column	YMC Triat C18, 5µm (250 mm x 4.6 mm)		
Column temperature	25°C		
Mobile phases	Eluent A: water Eluent B: acetonitrile		
	Time (min)	% Eluent A	% Eluent B
	0	70	30
	30	50	50
	35	0	100
	40	0	100
	41	70	30
	45	70	30
Flow rate (mL/min)	1		

Samples were co-chromatographed with non-radiolabelled eugenol reference standard and chromatograms were evaluated using Laura (version 6.0.4.92 and 6.1.5.69) software (Lablogic).

II RESULTS

A Preliminary investigations

Test details	Result
Solubility test Solubility was determined at 5 µg/mL in 0.01M calcium chloride solution.	The compound was soluble at this concentration.
Adsorption to container test Adsorption to Teflon® and plastic containers from 0.01M calcium chloride solution at a concentration of 0.05 µg/mL [^{14}C]-eugenol was tested.	Some adsorption (*ca* 5%) was seen in plastic vessels. No significant adsorption (1%) was seen in Teflon® vessels.
Ratio of soil to aqueous phase test Performed at 5 µg/mL [^{14}C]-eugenol concentration, in Teflon® tubes and the following ratios: 1:5 ratio 5 g soil : 10 mL 0.01M $CaCl_2$ solution 1:25 ratio 1 g soil : 25 mL 0.01M $CaCl_2$ solution 1:50 ratio 0.5 g soil : 25 mL 0.01M $CaCl_2$ solution Four soils were tested. The stability of [^{14}C]-eugenol was investigated in each sample.	Significant degradation of [^{14}C]-eugenol in the soil extracts was observed and was more significant where more soil was present. A ratio of 1:50 w/v was therefore chosen and the isotherms test to be performed by the direct method.
Equilibrium time test Performed at 5 µg/mL [^{14}C]-eugenol concentration and a 1:50 w:v soil ratio. Four soils were tested. Samples were shaken for 1, 3, 6 and 12 hours. Results from the 24 hour soil ratio test were also included in the equilibrium modelling. The stability of [^{14}C]-eugenol was investigated in selected samples.	The following equilibrium times were selected: 6 hrs – RefeSol 01-A and Kenslow 3 hrs – RefeSol 03-G 12 hrs – La Reina

Ratio of soil to aqueous phase test

Table 8.1.3.1.1-15: Percent of applied [^{14}C]-eugenol absorbed to soil following 24 hours mixing at soil to aqueous phase ratios of 1:5, 1:25 and 1:50 w/v.

Vessel Code	Ratio (w/v)	Recovery of Radioactivity as Parent (%)				
		Supernatant	Soil Extract			Total
			Neutral	Acid	Base	
SSR A1	1:5	46.7	11.5	0.2	ND	58.3
SSR A2		39.3	8.0	ND	NA	47.2
SSR B1		16.8	14.9	0.1	NA	31.8
SSR B2		13.6	12.8	ND	ND	26.4
SSR C1		ND	0.5	NA	NA	0.5
SSR C2		ND	0.5	NA	ND	0.5
SSR D1		5.5	3.2	0.1	NA	8.8
SSR D2		4.1	4.3	0.2	NA	8.6
SSR A3	1:25	69.0	6.7	ND	ND	75.7
SSR A4		74.2	6.7	0.1	ND	81.0
SSR B3		56.0	9.6	ND	ND	65.6
SSR B4		59.0	10.9	0.1	ND	70.0
SSR C3		6.4	0.7	ND	NA	7.1
SSR C4		9.5	0.7	NA	ND	10.2
SSR D3		45.7	3.3	NA	ND	49.0
SSR D4		42.5	4.3	ND	0.2	47.1
SSR A5	1:50	76.7	3.9	ND	ND	80.6
SSR A6		80.9	2.8	NA	ND	83.7

SSR B5 SSR B6		69.4 72.0	7.2 7.9	ND ND	ND ND	76.6 79.9
SSR C5 SSR C6		28.8 23.8	0.9 0.9	ND NA	ND ND	29.6 24.7
SSR D5 SSR D6		63.2 58.7	2.4 3.2	0.1 0.1	ND NA	65.6 62.0

ND = Not Detected (or < 0.1% AR), NA = Not Analysed
A: RefeSol 01-A soil; B: RefeSol 03-G soil; C: Kenslow soil; D: La Reina.

Equilibrium time test

Table 8.1.3.1.1-16: Total extractables as [^{14}C]-eugenol following HPLC analysis

	Recovery of Radioactivity as Parent (%)		
Vessel Code	**AdsorptionSupernatant**	**Soil Extract**	**Total**
AEQ A1 AEQ A3 AEQ A5 AEQ A7	88.0 87.3 86.2 84.2	2.3 3.8 4.6 3.9	90.3 91.1 90.8 88.1
AEQ B1 AEQ B3 AEQ B5 AEQ B7	80.4 80.1 83.3 79.3	5.7 8.0 6.7 7.7	86.1 88.1 90.0 87.0
AEQ C1 AEQ C3 AEQ C5 AEQ C7	83.5 76.0 66.7 64.9	1.7 4.1 4.4 2.9	85.2 80.1 71.1 67.8
AEQ D1 AEQ D3 AEQ D5 AEQ D7	85.0 83.3 80.2 72.2	1.9 2.6 3.5 4.5	86.9 85.9 83.7 76.7

A: RefeSol 01-A soil; B: RefeSol 03-G soil; C: Kenslow soil; D: La Reina. 1: 1 hour; 3 : 3 hours; 5: 6 hours; 7: 12 hours.

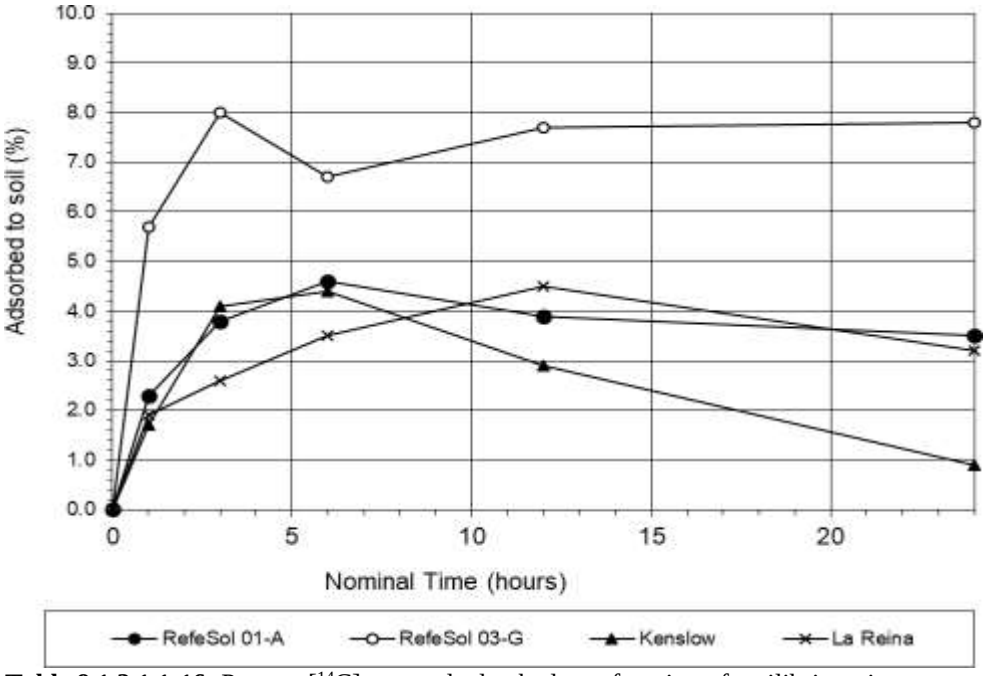


Table 8.1.3.1.1-16: Percent [^{14}C]-eugenol adsorbed as a function ofequilibrium time

B Definitive Isotherms

Adsorption Isotherms

The mean radioactive mass balance was 101.3% for RefeSol 01-A, 103.5% for RefeSol 03-G, 101.3% for Kenslow and 102.0% for La Reina soils. Following HPLC analysis, the mean recovery of [^{14}C]-eugenol was 94.1% for RefeSol 01-A, 93.0% for RefeSol 03-G, 65.1% for Kenslow and 76.4% for La Reina soils.

The percent adsorbed ranged from 2.1 to 6.7%. Values for adsorption partition coefficients (K_d) per soil were in the range 1.12 to 3.79 L/kg and K_{OC} values were in the range 51.2 to 126.4 L/kg. The range of 1/n values was 0.945 to 1.014. The Freundlich coefficients (K_{FOC}) were in the range 48.2 to 123.3 L/kg. Eugenol can be classified as highly mobile on the McCall classification scale.

Radioactive mass balance following adsorption

Table 8.1.3.1.1-17: Radioactive mass balance following adsorption in RefeSol 01-A soil

Nominal application rate (mg/L)	Vessel Code	Recovery of Radioactivity (%)			
		Adsorption Supernatant	Soil Extract	Unextracted from Soil	Total
5	ISO A1	97.3	5.0	1.2	103.5
	ISO A2	103.6	5.1	1.4	110.1
	Mean	**100.5**	**5.1**	**1.3**	**106.8**
1.5	ISO A3	100.3	4.9	1.0	106.2
	ISO A4	95.8	6.1	0.7	102.6
	Mean	**98.1**	**5.5**	**0.9**	**104.4**
0.5	ISO A5	96.0	5.3	1.4	102.7
	ISO A6	93.8	5.2	1.9	100.9
	Mean	**94.9**	**5.3**	**1.7**	**101.8**
0.15	ISO A7	92.3	5.3	2.1	99.7
	ISO A8	90.4	5.2	1.8	97.4
	Mean	**91.4**	**5.3**	**2.0**	**98.6**

	ISO A9	85.5	4.2	2.2	91.9
0.05	ISO A10	90.1	5.2	2.6	97.9
	Mean	**87.8**	**4.7**	**2.4**	**94.9**
				Overall Mean	**101.3**

Table 8.1.3.1.1-18: Radioactive mass balance following adsorption in RefeSol 03-G soil.

Nominal application rate (mg/L)	Vessel Code	Recovery of Radioactivity (%)			
		Adsorption Supernatant	Soil Extract	Unextracted from Soil	Total
	ISO B1	96.5	10.6	2.2	109.3
5	ISO B2	92.0	9.7	2.1	103.8
	Mean	**94.3**	**10.2**	**2.2**	**106.6**
	ISO B3	89.9	10.0	3.0	102.9
1.5	ISO B4	90.4	10.9	3.1	104.4
	Mean	**90.2**	**10.5**	**3.1**	**103.7**
	ISO B5	90.6	11.4	4.3	106.3
0.5	ISO B6	88.5	10.8	4.4	103.7
	Mean	**89.6**	**11.1**	**4.4**	**105.0**
	ISO B7	83.0	10.6	6.3	99.9
0.15	ISO B8	84.1	11.9	4.7	100.7
	Mean	**83.6**	**11.3**	**5.5**	**100.3**
	ISO B9	81.3	10.4	9.1	100.8
0.05	ISO B10	80.8	10.6	11.3	102.7
	Mean	**81.1**	**10.5**	**10.2**	**101.8**
				Overall Mean	**103.5**

Table 8.1.3.1.1-19: Radioactive mass balance following adsorption in Kenslow soil.

Nominal application rate (mg/L)	Vessel Code	Recovery of Radioactivity (%)			
		Adsorption Supernatant	Soil Extract	Unextracted from Soil	Total
	ISO C1	81.1	10.4	15.9	107.4
5	ISO C2	80.6	9.1	15.5	105.2
	Mean	**80.9**	**9.8**	**15.7**	**106.3**
	ISO C3	79.6	8.7	13.6	101.9
1.5	ISO C4	75.1	10.1	19.7	104.9
	Mean	**77.4**	**9.4**	**16.7**	**103.4**
	ISO C5	66.9	11.5	29.7	108.1
0.5	ISO C6	66.2	12.4	25.8	104.4
	Mean	**66.6**	**12.0**	**27.8**	**106.3**
	ISO C7	64.9	9.0	25.2	99.1
0.15	ISO C8	59.9	9.3	24.8	94.0
	Mean	**62.4**	**9.2**	**25.0**	**96.6**
	ISO C9	58.9	7.4	27.6	93.9
0.05	ISO C10	69.9	6.3	18.1	94.3
	Mean	**64.4**	**6.9**	**22.9**	**94.1**
				Overall Mean	**101.3**

Table 8.1.3.1.1-20: Radioactive mass balance following adsorption in La Reina.

Nominal application rate (mg/L)	Vessel Code	Recovery of Radioactivity (%)			
		Adsorption Supernatant	Soil Extract	Unextracted from Soil	Total
5	ISO D1	87.3	14.4	4.2	105.9
	ISO D2	84.4	10.9	3.4	98.7
	Mean	**85.9**	**12.7**	**3.8**	**102.3**
1.5	ISO D3	84.6	15.1	6.5	106.2
	ISO D4	89.3	15.6	4.7	109.6
	Mean	**87.0**	**15.4**	**5.6**	**107.9**
0.5	ISO D5	84.8	11.2	8.0	104.0
	ISO D6	81.0	10.2	6.9	98.1
	Mean	**82.9**	**10.7**	**7.5**	**101.1**
0.15	ISO D7	83.3	9.0	10.6	102.9
	ISO D8	79.9	9.7	9.5	99.1
	Mean	**81.6**	**9.4**	**10.1**	**101.0**
0.05	ISO D9	74.2	10.0	14.1	98.3
	ISO D10	78.4	7.5	10.8	96.7
	Mean	**76.3**	**8.8**	**12.5**	**97.5**
				Overall Mean	**102.0**

Analysis of [14C]-eugenol in soils following adsorption

Table 8.1.3.1.1-21: Total extractables as [^{14}C]-eugenol from HPLC chromatograms. RefeSol 01-A soil

Nominal application rate (mg/L)	Vessel code	Applied Radioactivity (AR) from HPLC Chromatogram (%)		
		Adsorption supernatant	Soil extract	Total
5	ISO A1	95.0	3.6	98.6
	ISO A2	100.7	3.8	104.5
	Mean	**97.9**	**3.7**	**101.6**
1.5	ISO A3	96.5	4.0	100.5
	ISO A4	91.7	2.7	94.4
	Mean	**94.1**	**3.4**	**97.4**
0.5	ISO A5	91.3	3.2	94.4
	ISO A6	87.9	2.8	90.6
	Mean	**89.6**	**3.0**	**92.5**
0.15	ISO A7	89.4	3.5	92.9
	ISO A8	86.8	3.6	90.3
	Mean	**88.1**	**3.6**	**91.6**
0.05	ISO A9	82.0	2.7	84.8
	ISO A10	86.3	3.7	90.1
	Mean	**84.2**	**3.2**	**87.4**
			Overall Mean	**94.1**

Table 8.1.3.1.1-22: Total extractables as [^{14}C]-eugenol from HPLC chromatograms. RefeSol 03-G soil

Nominal application rate (mg/L)	Vessel code	Applied Radioactivity (AR) from HPLC Chromatogram (%)		
		Adsorption supernatant	Soil extract	Total

5	ISO B1	93.2	8.8	101.9
	ISO B2	88.2	8.4	96.5
	Mean	**90.7**	**8.6**	**99.2**
1.5	ISO B3	87.0	8.7	95.7
	ISO B4	88.1	9.1	97.2
	Mean	**87.6**	**8.9**	**96.5**
0.5	ISO B5	88.1	10.1	98.2
	ISO B6	86.0	8.1	94.1
	Mean	**87.0**	**9.1**	**96.1**
0.15	ISO B7	75.5	9.2	84.6
	ISO B8	77.0	9.6	86.6
	Mean	**76.2**	**9.4**	**85.6**
0.05	ISO B9	79.3	9.3	88.5
	ISO B10	76.5	9.8	86.3
	Mean	**77.9**	**9.5**	**87.4**
			Overall Mean	**93.0**

Table 8.1.3.1.1-23: Total extractables as [^{14}C]-eugenol from HPLC chromatograms.
Kenslow soil.

Nominal application rate (mg/L)	**Vessel code**	**Applied Radioactivity (AR) from HPLC Chromatogram (%)**		
		Adsorption supernatant	**Soil extract**	**Total**
5	ISO C1	72.4	3.6	76.0
	ISO C2	73.0	3.7	76.6
	Mean	**72.7**	**3.7**	**76.3**
1.5	ISO C3	72.0	2.4	74.4
	ISO C4	65.9	5.0	70.8
	Mean	**68.9**	**3.7**	**72.6**
0.5	ISO C5	59.0	2.2	61.2
	ISO C6	57.9	3.3	61.2
	Mean	58.4	2.8	61.2
0.15	ISO C7	54.2	2.8	56.9
	ISO C8	50.9	3.4	54.3
	Mean	**52.5**	**3.1**	**55.6**
0.05	ISO C9	50.7	3.7	54.4
	ISO C10	62.5	2.4	64.9
	Mean	**56.6**	**3.0**	**59.6**
			Overall Mean	**65.1**

Table 8.1.3.1.1-24: Total extractables as [^{14}C]-eugenol from HPLC chromatograms.
La Reina soil

Nominal application rate (mg/L)	Vessel code	Applied Radioactivity (AR) from HPLC Chromatogram (%)		
		Adsorption supernatant	Soil extract	Total
5	ISO D1	81.1	2.6	83.7
	ISO D2	80.2	3.8	84.0
	Mean	**80.7**	**3.2**	**83.9**
1.5	ISO D3	77.1	2.0	79.1
	ISO D4	80.6	3.6	84.2
	Mean	**78.9**	**2.8**	**81.7**
	ISO D5	74.0	2.2	76.2

0.5	ISO D6	69.4	3.9	73.3
	Mean	**71.7**	**3.0**	**74.8**
0.15	ISO D7	72.2	2.3	74.5
	ISO D8	68.2	2.4	70.6
	Mean	**70.2**	**2.4**	**72.5**
0.05	ISO D9	63.6	3.0	66.6
	ISO D10	68.9	3.2	72.2
	Mean	**66.3**	**3.1**	**69.4**
			Overall Mean	**76.4**

Concentrations of [14C]-eugenol in supernatants following adsorption
Ce = Concentration of test substance in the supernatant at the end of adsorption X/m = Weight absorbed / weight of dry soil (adsorption step)

Table 8.1.3.1.1-25: Concentrations of [14C]-eugenol in the supernatant and soil at the end of adsorption equilibration period in RefeSol 01-A soil.

Nominal application rate (mg/L)	Vessel Code	Adsorption	
		Ce (mg/L)	X/m (mg/kg)
5	ISO A1	4.8655	5.5234
	ISO A2	5.1905	5.9363
	Mean	**5.0280**	**5.7299**
1.5	ISO A3	1.4815	1.7744
	ISO A4	1.4164	1.2090
	Mean	**1.4489**	**1.4917**
0.5	ISO A5	0.4631	0.5325
	ISO A6	0.4451	0.4875
	Mean	**0.4541**	**0.5100**
0.15	ISO A7	0.1355	0.1640
	ISO A8	0.1315	0.1783
	Mean	**0.1335**	**0.1711**
0.05	ISO A9	0.0416	0.0367
	ISO A10	0.0442	0.0522
	Mean	**0.0429**	**0.0444**

Table 8.1.3.1.1-26: Concentrations of [14C]-eugenol in the supernatant and soil at the end of adsorption equilibration period in RefeSol 03-G soil.

Nominal application rate (mg/L)	Vessel Code	Adsorption	
		Ce (mg/L)	X/m (mg/kg)
5	ISO B1	4.8250	15.3801
	ISO B2	4.6128	15.2499
	Mean	**4.7189**	**15.3150**
1.5	ISO B3	1.3552	4.6349
	ISO B4	1.3845	4.8066
	Mean	**1.3699**	**4.7207**
0.5	ISO B5	0.4520	1.9470
	ISO B6	0.4393	1.5490
	Mean	**0.4457**	**1.7480**
0.15	ISO B7	0.1154	0.4956
	ISO B8	0.1200	0.5262
	Mean	**0.1177**	**0.5109**
0.05	ISO B9	0.0410	0.1537
	ISO B10	0.0397	0.1698
	Mean	**0.0404**	**0.1617**

Table 8.1.3.1.1 27: Concentrations of [14C]-eugenol in the supernatant and soil at the end of adsorption equilibration period in Kenslow soil.

Nominal application rate (mg/L)	Vessel Code	Adsorption	
		Ce (mg/L)	X/m (mg/kg)
5	ISO C1	3.7863	7.0208
	ISO C2	3.8101	7.0306
	Mean	**3.7982**	**7.0257**
1.5	ISO C3	1.1313	1.3131
	ISO C4	1.0261	2.2387
	Mean	**1.0787**	**1.7759**
0.5	ISO C5	0.3052	0.4436
	ISO C6	0.2972	0.6587
	Mean	**0.3012**	**0.5511**
0.15	ISO C7	0.0832	0.1627
	ISO C8	0.0778	0.2041
	Mean	**0.0805**	**0.1834**
0.05	ISO C9	0.0262	0.0660
	ISO C10	0.0319	0.0360
	Mean	**0.0290**	**0.0510**

Table 8.1.3.1.1 28: Concentrations of [14C]-eugenol in the supernatant and soil at the end of adsorption equilibration period in La Reina soil.

Nominal application rate (mg/L)	Vessel Code	Adsorption	
		Ce (mg/L)	X/m (mg/kg)
5	ISO D1	4.2455	5.1768
	ISO D2	4.2034	6.2525
	Mean	**4.2245**	**5.7147**
1.5	ISO D3	1.2481	1.1221
	ISO D4	1.2552	2.1552
	Mean	**1.2516**	**1.6387**
0.5	ISO D5	0.3789	0.4309
	ISO D6	0.3539	0.7681
	Mean	**0.3664**	**0.5995**
0.15	ISO D7	0.1110	0.1171
	ISO D8	0.1015	0.1136
	Mean	**0.1062**	**0.1154**
0.05	ISO D9	0.0328	0.0524
	ISO D10	0.0357	0.0388
	Mean	**0.0343**	**0.0456**

Desorption Isotherms

The recovery of [^{14}C]-eugenol in The mean radioactive mass balance was 95.3% for RefeSol 01-A, 99.0% for RefeSol 03-G, 96.2% for Kenslow and 88.4% for La Reina soils. Following HPLC analysis, the mean recovery of [^{14}C]-eugenol was 90.2% for RefeSol 01-A, 89.9% for RefeSol 03-G, 55.9% for Kenslow and 62.8% for La Reina soils.

The percent desorbed ranged from 43.3 to 75.1%. Values for the mean desorption partition coefficients (K_d) per soil were in the range 15.31 to 30.96 L/kg and K_{OC} values were in the range 510 to 2814 L/kg. The range of 1/n values was 0.478 to 0.938. The Freundlich desorption coefficients (K_{FOC}) were in the range 82.2 to 1099 L/kg.

Parameter	RefeSol 01-A (CS 18/18)	RefeSol 03-G (CS19/18)	Kenslow (CS 20/18)	La Reina (CS 08/19)
Soil Texture (UK)	sandy loam	silty clay loam	clay loam	clay
pH (0.01 M $CaCl_2$)	5.3	6.0	5.1	7.7
%OC	0.9	3.0	3.7	1.1
Adsorption				
K_F (95% confidence intervals)	1.11 (0.986-1.253)	3.59 (3.360-3.842)	1.78 (1.353-2.346)	1.32 (1.039-1.680)
K_{FOC}	123.3	119.8	48.2	120.1
1/n (95% confidence intervals)	0.999 (0.934-1.064)	0.945 (0.910-0.981)	0.980 (0.849-1.111)	1.014 (0.892-1.135)
r^2	0.994	0.998	0.974	0.979
K_d*	1.12	3.79	1.89	1.35
K_{OC}*	124.51	126.40	51.20	122.63
% adsorbed	2.1	6.7	2.4	2.1
Radioactive mass balance (% AR)*	101.3	103.5	101.3	102.0
Mass balance of [^{14}C]-eugenol (% AR)*	94.1	93.0	65.1	76.4
K_d × (soil/solution ratio)	0.022	0.076	0.038	0.027
Desorption				
K_F	9.89	12.30	3.04	1.40
K_{FOC}	1099	410	82.2	126.8
1/n	0.873	0.938	0.649	0.478
r^2	0.982	0.999	0.869	0.808
K_d*	17.54	15.31	26.32	30.96
K_{OC}*	1949.30	510.23	711.33	2814.40

K_F, K_{FOC}, K_d and K_{OC} all have the units mL/g, NA = Not applicable
* Average values

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 8.1.3.1.1-29: Summary of OECD quality checks for eugenol

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	1:50	1:50	1:50	1:50
Mass balance of test item (at highest tested conc.)*	%	94.1	93.0	65.1	76.4
Adsorbed percentage**	%	2.1	6.7	2.4	2.1
K_d × (soil: solution ratio)	-	0.022	0.076	0.038	0.027
$^{ads}K_F$ (95% confidence interval)	L/kg dw	1.11	3.59	1.78	1.32
^{ads}1/n (95% confidence interval)	L/kg dw	0.999	0.945	0.980	1.014
$^{ads}R^2$	-	0.994	0.998	0.974	0.979
$^{ads}K_{F,\,OC}$	L/kg OC	123.3	119.8	48.2	120.1
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

Freundlich adsorption coefficients, K_{FOC}, were in the range 48.2 to 123.3 L/kg. Using the McCall Classification scale to assess the potential mobility of a chemical in soil (based on K_{OC}), eugenol can be classified as having high mobility in soil (K_{OC} 50-150 L/kg) for each of the soils tested.
Freundlich desorption coefficients, K_{FOC}, were in the range 82.2 to 1099 L/kg. Desorption values were generally higher than corresponding adsorption values indicating that the adsorption was not fully reversible.
In all four soils, low adsorption to the soil was noted. Significant degradation of [^{14}C]-eugenol was seen in soil extracts, indicating chemical instability in sterile soils tested.

3. Assessment and conclusions

Assessment and conclusion by applicant:
This study was performed well and passes the OECD quality checks. The K_{FOC} ranges from 48.2 to 123.3 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.

For all soils, low adsorption to soil was noted. Significant degradation of [14C]-eugenol was seen in soil extracts, indicating chemical instability in the sterile soils tested, therefore the direct method was employed.

Regarding to the preliminary test, the instability of eugenol was confirmed. The selected soil:solution ratio was 1:50 in all cases. The reason for this selection is that at this ratio, the stability of eugenol was greater than the other two tested ratios (see Table 8.1.3.1.1-15). However, what it is observed is that at 24 h the adsorbed percentages were low or extremely low. It is thought that this preliminary test is designed to select the soil: solution ratios where the adsorbed percentage is between 20-80%, and preferably greater than 50%, therefore the criteria considering the stability of the substance would not be entirely suitable. In fact, the adsorbed percentages for the soils RefeSol 01-A and RefeSol 03-G were greater at 1:5.

The selection of equilibrium times for the definitive tests were considered appropriate with 3 hours for RefeSol 03-G, 12 hours for La Reina soil and 6 hours for RefeSol 01-A and Kenslow soil (see Table 8.1.3.1.1-16).

Regarding to the definitive test, the mean radioactive mass balance was 101.3% for RefeSol 01-A, 103.5% for RefeSol 03-G, 101.3% for Kenslow and 102.0% for La Reina soils. It is noted that for Kenslow soil, the unextracted radioactivity reached values up to 27.8% (see Table 8.1.3.1.1-19). Note that the OECD guideline requires that a suitable extraction solvent (extraction coefficient of at least 95% related to the amount of test item in soil after removal of supernatant).

Following HPLC analysis, the mean recovery of [^{14}C]-eugenol was 94.1% for RefeSol 01-A, 93.0% for RefeSol 03-G, 65.1% for Kenslow and 76.4% for La Reina soils. These percentages are within the normal range considering that the direct method was followed.

The limit of detection and quantification, determined at the lowest concentration, by LSC was 0.18% (0.0001 mg/L). This is two orders of magnitude below the lowest concentration, demonstrating that the analytical method was acceptable over the entire range of concentrations measured.

The K_d × (soil: solution ratio) were far below the recommended limit of 0.3. However, it is thought that the soil: solution ratio was not correctly reported. According to OECD 106 evaluator's checklist guideline, for the indirect method, the soil/solution ratio is simply calculated from the soil mass and the total liquid volume present in the original soil suspension. However, for the direct method, the ratio has to be derived *after* centrifugation, and is therefore calculated as the ratio between the soil mass divided by the residual moisture volume in the soil pellet.

The percent adsorbed ranged from 2.1 to 6.7. It was calculated as 'calculated as % Concentration on soil/Initial concentration x 100', since parameter *delta* which is the decrease in concentration in aqueous phase, would not be equivalent to the percentage adsorbed in the direct method. The adsorbed percentages are considered too low outside the acceptable range being <20%. According to the OECD 106 guideline where low adsorption occurs, a 1:1 soil/solution ratio is recommended, although for some very organic soil types smaller ratios may be necessary to obtain a slurry. Additionally, it is pointed out that care must be taken with the analytical methodology to measure small changes in solution concentration; otherwise, the adsorption measurement will be inaccurate. For eugenol, the instability of the test substance must be added to the low adsorbed percentages. Therefore, the reliability of the calculated parameters should be questioned.

According to the guideline, in the absence of reliable endpoints needed to perform an exposure assessment the use of conservative default values may be appropriate, especially when adsorption was too low to reliably measure.

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 0.974 to 0.998 and the visual fit of both the standard regression and the residual plots were good for RefeSol 01-A and RefeSol 03-G and were considered acceptable for Kenslow (CS 20/18) and La Reina.

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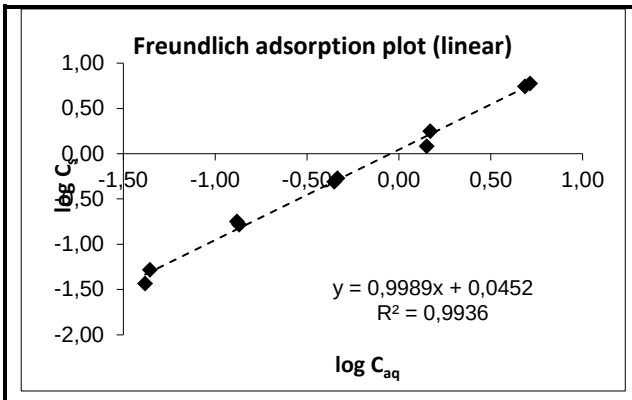
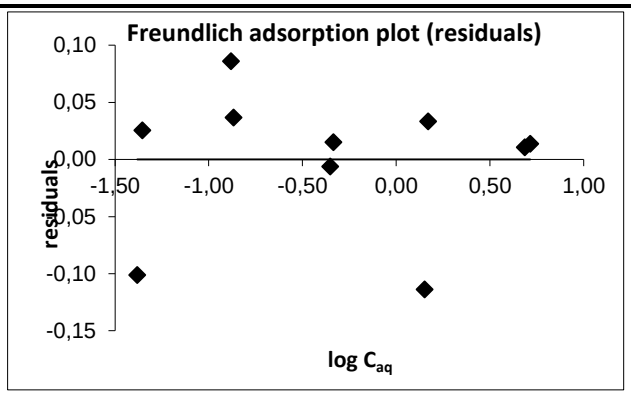
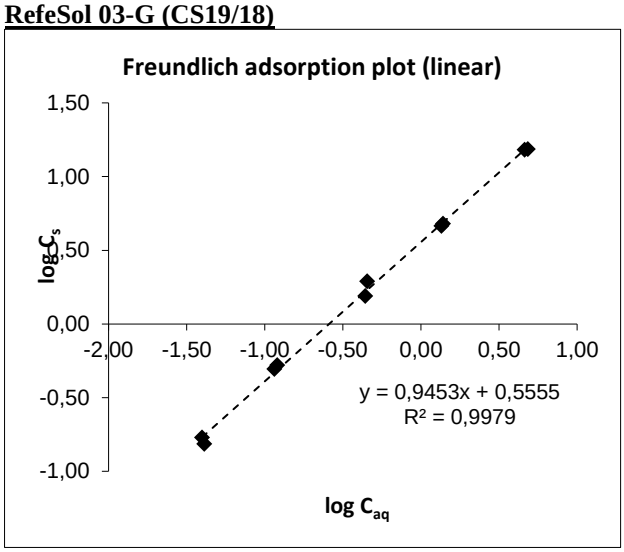
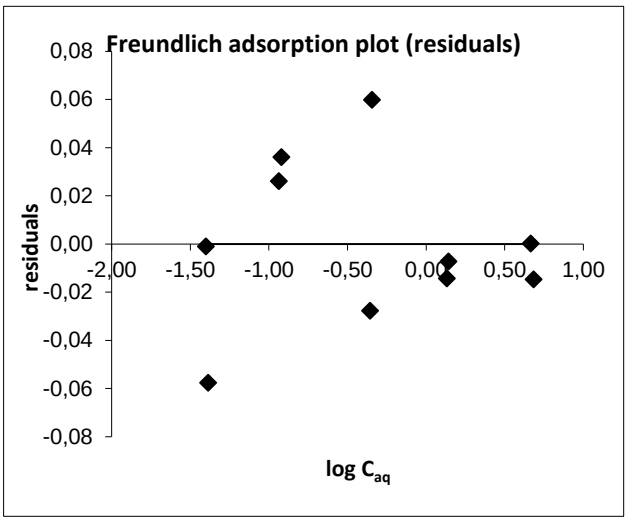


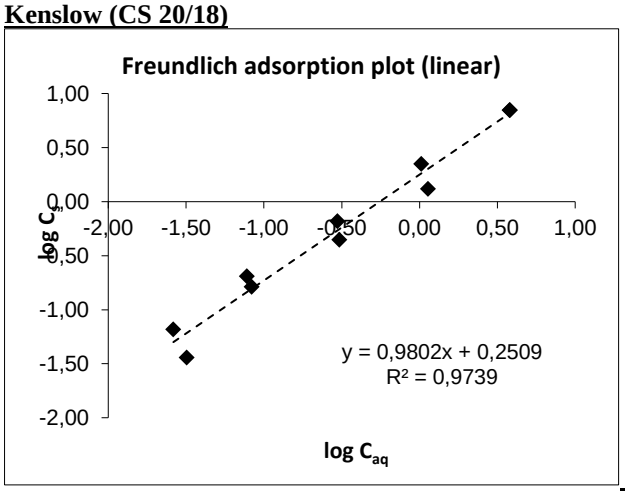


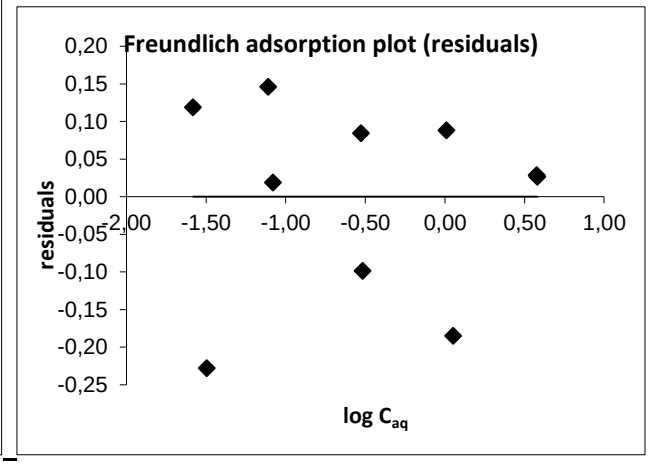
RefeSol 03-G (CS19/18)



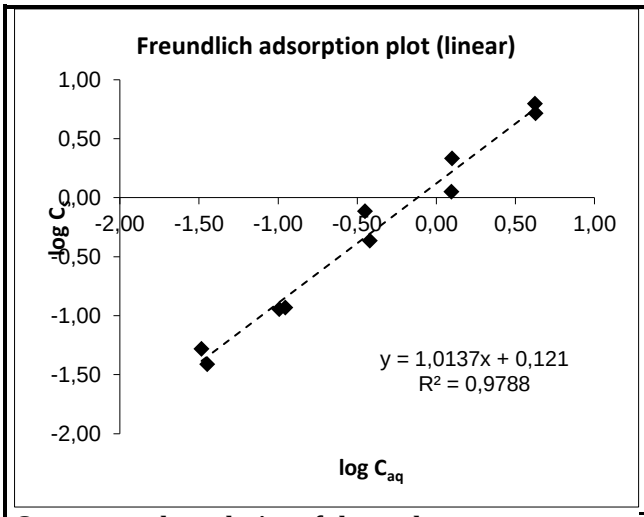


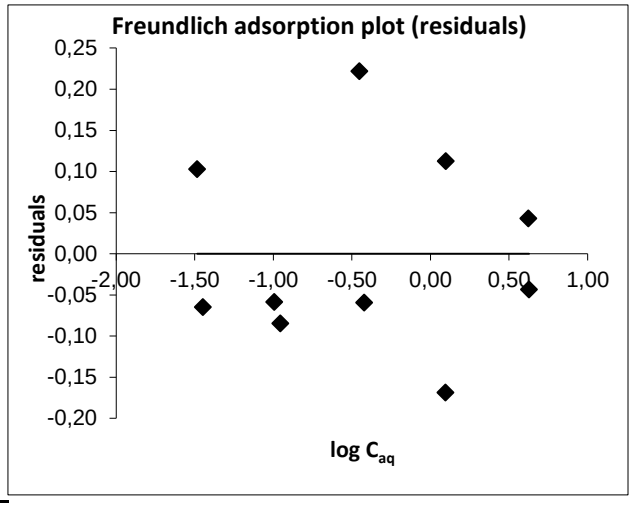
Kenslow (CS 20/18)





La Reina (CS 08/19)





Outcome and conclusion of the study:
Supplementary information
Considering the difficulties to achieve a precise adsorption endpoint for eugenol due to the low adsorption and the instability of this substance, default values will be used for risk assessment purposes as it was previously proposed in the confirmatory data: KFoc of 10 mg/L and 1/n of 0.9.

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 eugenol, 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
No data submitted

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 eugenol, 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

No data submitted.

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
Report year	2021
Report title	Eugenol – Hydrolysis of [^{14}C]-Eugenol in Sterile Buffer at pH 4,7 and 9
Report No.	Smithers ERS Ltd No. 3202784
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

EXECUTIVE SUMMARY

The hydrolysis of [^{14}C]-eugenol was studied in the dark in sterile aqueous buffered solutions at pH 4 (acetate buffer), pH 7 (phosphate buffer) and pH 9 (borate buffer).

Tier I Test
Solutions of aqueous buffers were prepared at pH 4, 7 and 9 at 50°C then sterilised by autoclaving. [^{14}C]-Eugenol (in ethanol, 25 µL), was applied to glass vials containing buffer (3 mL) to achieve a final concentration of *ca* 1.0 mg eugenol/L. Duplicate incubation vessels were analysed at the start and completion of the 5-day incubation period.

Radioactivity present in test solutions and vial washes was determined by liquid scintillation counting (LSC). All buffer samples were analysed for [^{14}C]-eugenol by high performance liquid chromatography (HPLC).

Additional vials, treated with non-radiolabelled eugenol, 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 25, 35 and 50°C. [^{14}C]-Eugenol (in ethanol, 25 µL), was applied to glass vials containing buffer (3 mL) to achieve a final concentration of *ca* 1.0 mg eugenol/L. Duplicate incubation vessels were analysed at regular intervals up to 30 days.

Radioactivity present in test solutions and vial washes was determined by liquid scintillation counting (LSC). All buffer samples were analysed for [^{14}C]-eugenol by HPLC.

Degradation rates were determined by single first order (SFO) kinetics according to FOCUS kinetic modelling.

Findings
Tier I Test
In the Tier I test, [^{14}C]-eugenol was shown to be stable at 50°C in pH 4 and 7 buffers, and unstable in pH 9 buffer.

In pH 4 and 7 buffers, eugenol accounted for 92.1-92.9% AR initially and at the end of the 5-day incubation accounted for 88.7-89.2% AR.

In pH 9 buffer, the level of eugenol dropped from 92.5% AR initially to 63.7% AR at the end of the 5-day incubation. At pH 9, several unknowns were observed, the largest reaching a maximum of 15.9% AR, after the 5-day incubation period.

Tier II Test
In the Tier II test, the mean recovery for all samples was 94.6 to 100.7% AR. The majority of radioactivity was present in the test solution and less than 6% AR remaining in the vessel rinse.

The temperature generally remained constant throughout 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.

[^{14}C]-Eugenol decreased from mean values of 94.1 – 95.0% AR at 0 DAT to between 24.2% AR at 50°C and 81.1% AR at 25°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:

SFO					
Temperature (°C)	$DegT_{50}$ (days)	$DegT_{90}$ (days)	k	Chi^2	r^2
pH4					
25	150	499	0.0046	1.3	0.8789
35	31	103	0.0225	3.1	0.8781
50	14	47	0.0495	4.8	0.9425

Using the Arrhenius equation, the half-life value was estimated at 25°C and was 120 days at pH 9, showing agreement with the measured value.

[^{14}C]-Eugenol was hydrolytically stable in buffered aqueous solutions at 50°C, under sterile conditions, at pH values of 4 and 7.

Tier II hydrolysis at pH 9 and incubated in the dark at 25, 35 and 50 ± 0.5°C for up to 30 days, showed eugenol was not hydrolytically stable at environmentally relevant temperatures under basic conditions.

I. MATERIALS AND METHODS

A MATERIALS

Test material 1

Name:	[ring-^{14}C(U)]-Eugenol
Description:	Clear solution in ethanol
Lot/Batch no.:	200925-RP1 and CFQ44613
Radiochemical Purity:	Radiochemical purity 96.3% and 96.7%
Specific activity:	27.99 and 9.27 MBq/mg.
Storage:	Frozen (nominally -20°C)

Non-labelled material (used in all tests)

Name:	Eugenol
Description:	Colourless to pale yellow
Lot/Batch no.:	ESTS 167/20
Purity:	99.7%
CAS #:	97-53-0
Storage/stability:	Room temperature

B STUDY DESIGN

Experimental conditions

Preparation of Buffers

Acetate *buffer, pH 4*
0.01 M Acetate buffer solution was prepared by mixing 0.1 M acetic acid solution with 0.1 M sodium acetate solution and diluting with Milli-Q water. The pH was confirmed to be pH 4 ± 0.2.

Phosphate buffer, pH 7
0.01 M Phosphate buffer was prepared by diluting potassium dihydrogen orthophosphate in Milli-Q water. The pH was confirmed to be pH 7 ± 0.2.

Borate buffer, pH 9
0.01 M Borate buffer was prepared by addition of boric acid and sodium hydroxide solution (2 M), and diluted in Milli-Q water. The pH was confirmed to be pH 9 ± 0.2.

Buffers were deoxygenated by sonication then by bubbling nitrogen through them. The pH of the buffer solutions was adjusted (as required) at the desired temperature with a calibrated pH meter (precision at least 0.1 pH units) to pH values of 4.0 ± 0.2, 7.0 ± 0.2 and 9.0 ± 0.2.

The buffers, glass vials and caps (PTFE lined septum seals) were sterilised by autoclaving (set at 121°C, 25 minutes).

Tier I Test

The incubation vessels were prepared by dispensing 3 mL of the appropriate 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, all prepared samples were placed in a water bath set at 50°C. The incubation was performed in the dark.

With the exception of samples analysed at 0 DAT, samples were removed from the 50°C incubation after 5 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.

Tier II Test
Samples were incubated in a water bath at the required temperature for up to 30 days under dark conditions.

Duplicate samples at each temperature were removed for analysis immediately after treatment and after incubation at 2, 7, 14, 21 and 30 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.

Sample Analysis

The vials were removed from the water bath and allowed to reach room temperature. The contents were removed from the vials and transferred to a pre-weighed glass vial labelled Extract 1 and analysed by HPLC. Duplicate aliquots of each sample were taken for LSC.
The incubation vessel was then rinsed with acetonitrile (3 mL) and transferred to a pre-weighed glass vial labelled Extract 2. Duplicate aliquots were taken for LSC.

High performance Liquid chromatography (HPLC)

The following HPLC method was used for the determination of the radiochemical purity of the application solutions and for sample analysis.

Table 8.2.1.1-1: HPLC method for soil samples

HPLC System	Agilent 1260		
Column	YMC Triat C18, 5µm (250 mm x 4.6 mm)		
Column temperature	40°C		
Mobile phases	Eluent A: water Eluent B: methanol		
	Time (min)	% Eluent A	% Eluent B
	0	70	30
	30	50	50
	35	0	100
	40	0	100
	41	70	30
	45	70	30
Flow rate (mL/min)	1		

Samples were co-chromatographed with non-radiolabelled eugenol reference standard and chromatograms were evaluated using Laura (version 6.0.4.92) software (Lablogic).

II. RESULTS

A Tier I

Mean recovery of applied radioactivity (AR) in buffer solutions was 97.3 to 99.3% AR at 0 DAT and 96.7 to 98.7% at 5 DAT. In the vessel washes, < 3% AR was detected. The mean total recovery was in the range 98.9 to 101.3% AR.

[^{14}C]-Eugenol comprised mean values of 92.1 to 92.9% AR at 0 day in buffer samples.

In pH 4 and 7 buffer samples, only minor degradation (< 10%) was observed during the 5-day incubation period and therefore no Tier II testing was required. [^{14}C]-Eugenol comprised mean values of 89.2 and 88.7% AR in pH 4 and pH 7 buffers, respectively, after 5-day incubation at 50°C.

In pH 9 buffer samples, [^{14}C]-eugenol decreased to 63.7% AR after 5-day incubation at 50°C. There were multiple unknown components, the largest of which was 15.9% AR. A Tier II test was performed to determine the rate of hydrolysis at pH 9.

Table 8.2.1.1-2: Percent recovery of applied radioactivity from buffers and Percent of applied radioactivity present as [^{14}C]-Eugenol and transformation products in buffers incubated at 50°C (Tier I Test) (pH4)

*** pH 4**

	% Applied Radioactivity Time after application (days)					
		0			5	
Vessel Code	A1	A2	Mean	A3	A4	Mean
Aqueous Phase	97.7	99.0	98.4	97.0	100.3	98.7
Vessel Wash	2.0	2.2	2.1	2.2	2.2	2.2
Total	**99.7**	**101.2**	**100.5**	**99.2**	**102.5**	**100.9**

	% Applied Radioactivity Time after application (days)	
	0	5

Component	A1	A2	Mean	A3	A4	Mean
Eugenol	92.3	93.5	92.9	86.9	91.5	89.2
Total Unknowns	5.3	5.4	5.4	9.6	8.0	8.8
Unresolved Background	0.1	ND	0.1	0.6	0.8	0.7
Total	**97.7**	**99.0**	**98.4**	**97.0**	**100.3**	**98.7**
No. of Unknowns	*2*	*2*		*4*	*3*	
Largest Unknown	*4.8*	*5.0*		*6.3*	*6.4*	

ND = Not Detected (or < 0.1%)

Table 8.2.1.1-3: Percent recovery of applied radioactivity from buffers and Percent of applied radioactivity present as [^{14}C]-Eugenol and transformation products in buffers incubated at 50°C (Tier I Test) (pH7)

*** pH 7**

	% Applied Radioactivity Time after application (days)					
Vessel Code		**0**			**5**	
	B1	**B2**	**Mean**	**B3**	**B4**	**Mean**
Aqueous Phase	96.5	98.0	97.3	96.5	96.9	96.7
Vessel Wash	2.5	2.4	2.5	2.4	1.9	2.2
Total	**99.0**	**100.4**	**99.7**	**98.9**	**98.8**	**98.9**

	% Applied Radioactivity Time after application (days)					
Component		0			5	
	B1	B2	Mean	B3	B4	Mean
Eugenol	91.4	92.7	92.1	88.8	88.6	88.7
Total Unknowns	4.8	5.3	5.1	7.2	8.2	7.7
Unresolved Background	0.3	ND	0.1	0.5	0.1	0.3
Total	**96.5**	**98.0**	**97.3**	**96.5**	**96.9**	**96.7**
No. of Unknowns	*2*	*2*		*3*	*3*	
Largest Unknown	*4.3*	*4.7*		*5.0*	*6.3*	

ND = Not Detected (or < 0.1%)

Table 8.2.1.1-4: Percent recovery of applied radioactivity from buffers and Percent of applied radioactivity present as [^{14}C]-Eugenol and transformation products in buffers incubated at 50°C (Tier I Test) (pH9).

*** pH 9**

	% Applied Radioactivity Time after application (days)					
		0			5	
Vessel Code	C1	C2	Mean	C3	C4	Mean
Aqueous Phase	99.7	98.8	99.3	97.9	99.4	98.7
Vessel Wash	1.9	2.1	2.0	1.6	1.3	1.5
Total	**101.6**	**100.9**	**101.3**	**99.5**	**100.7**	**100.1**

	% Applied Radioactivity Time after application (days)					
Component		0			5	
	C1	C2	Mean	C3	C4	Mean
Eugenol	92.8	92.2	92.5	63.7	63.6	63.7

Total Unknowns	6.7	6.3	6.5	33.5	35.6	34.5
Unresolved Background	0.2	0.2	0.2	0.7	0.2	0.5
Total	**99.7**	**98.8**	**99.3**	**97.9**	**99.4**	**98.6**
No. of Unknowns	*2*	*2*		*6*	*7*	
Largest Unknown	*6.0*	*5.9*		*15.8*	*15.9*	

B Tier 2

Mean recovery of applied radioactivity (AR) in buffer solutions ranged from 96.3 to 99.7% AR at 25°C, 92.7 to 98.5% AR at 35°C and 89.6 to 97.7% AR at 50°C.

In the vessel washes, < 6% AR was detected. The mean total recovery was in the range 94.6 to 100.7% AR.

There were two major (> 10% AR) regions of interest observed across all three temperatures. Unknown 1 was a polar peak observed at ca 3 minutes in the HPLC run and continued to increase throughout each of the incubations, reaching a mean maximum of 18.6% AR (50°C, 30 DAT). Unknown 2 was observed after the elution of eugenol, at ca 39 minutes and reached a mean maximum of 22.3% AR (50°C, 21 DAT). Unknown 2 increased throughout the incubations at 25 and 35°C, and at 50°C reached a maximum at 21 days before decreasing at the end of the incubation. There were many other minor degradation products formed (<10% AR), where the largest individual peak reached a maximum of 9.3% AR (50°C, 21 DAT).

Table 8.2.1.1-5: Percent recovery of applied radioactivity from pH 9 buffer solutions (Tier II Test) and Percent of applied radioactivity present as [^{14}C]-Eugenol and degradants from pH 9 buffer solutions (Tier II Test) (25ºC).

Incubated at 25°C

	Time after application (days)																	
	0			2			7			14			21			30		
Vessel Code	C7	C8	Mean	C9	C10	Mean	C11	C12	Mean	C13	C14	Mean	C15	C16	Mean	C17	C18	Mean
Aqueous Phase	97.3	97.0	97.2	97.3	95.9	96.6	97.5	98.2	97.9	99.2	100.1	99.7	98.2	97.8	98.0	96.0	96.5	96.3
Vessel Wash	2.7	1.4	2.1	1.9	2.4	2.2	1.9	1.5	1.7	0.8	1.3	1.1	2.0	2.4	2.2	2.6	1.6	2.1
Total	**100.0**	**98.4**	**99.2**	**99.2**	**98.3**	**98.8**	**99.4**	**99.7**	**99.6**	**100.0**	**101.4**	**100.7**	**100.2**	**100.2**	**100.2**	**98.6**	**98.1**	**98.4**

	Percent Applied Radioactivity (% AR) Time after application (days)																	
	0			2			7			14			21			30		
Vessel Code	C7	C8	Mean	C9	C10	Mean	C11	C12	Mean	C13	C14	Mean	C15	C16	Mean	C17	C18	Mean
Eugenol	94.1	94.1	94.1	91.7	91.7	91.7	92.2	92.8	92.5	90.6	91.6	91.1	86.7	83.7	85.2	80.7	81.5	81.1
Unknown 1	ND	ND	ND	0.3	0.4	0.3	0.3	0.2	0.3	0.8	0.9	0.9	1.7	1.9	1.8	2.1	1.9	2.0
Unknown 2	1.2	1.4	1.3	1.7	1.6	1.7	1.7	1.9	1.8	3.1	3.2	3.2	4.3	5.5	4.9	6.8	6.6	6.7
Other Unknowns	1.1	1.3	1.2	2.6	2.1	2.4	2.8	2.6	2.7	3.9	3.5	3.7	5.2	6.6	5.9	5.6	5.5	5.5
Unresolved Background	0.8	0.3	0.6	1.0	0.1	0.5	0.6	0.7	0.6	0.7	0.9	0.8	0.2	0.0	0.1	0.7	1.0	0.9
Total	**97.3**	**97.0**	**97.2**	**97.3**	**95.9**	**96.6**	**97.5**	**98.2**	**97.9**	**99.2**	**100.1**	**99.7**	**98.2**	**97.8**	**98.0**	**96.0**	**96.5**	**96.2**

ND = Not Detected (or < 0.1%)

Table 8.2.1.1-6: Percent recovery of applied radioactivity from pH 9 buffer solutions (Tier II Test) and Percent of applied radioactivity present as [^{14}C]-Eugenol and degradants from pH 9 buffer solutions (Tier II Test) (35ºC).

Incubated at 35°C

	Time after application (days)																	
	0			2			7			14			21			30		
Vessel Code	C21	C22	Mean	C23	C24	Mean	C25	C26	Mean	C27	C28	Mean	C29	C30	Mean	C31	C32	Mean
Aqueous Phase	97.4	97.7	97.6	97.6	97.0	97.3	97.9	99.1	98.5	93.1	97.9	95.5	94.8	95.6	95.2	90.1	95.3	92.7
Vessel Wash	2.2	1.9	2.1	2.4	2.1	2.3	2.3	1.0	1.7	2.8	1.5	2.2	2.4	2.6	2.5	4.9	3.2	4.1
Total	**99.6**	**99.6**	**99.6**	**100.0**	**99.1**	**99.6**	**100.2**	**100.1**	**100.2**	**95.9**	**99.4**	**97.7**	**97.2**	**98.2**	**97.7**	**95.0**	**98.5**	**96.8**

	Percent Applied Radioactivity (% AR) Time after application (days)																	
Vessel Code	0			2			7			14			21			30		
	C21	C22	Mean	C23	C24	Mean	C25	C26	Mean	C27	C28	Mean	C29	C30	Mean	C31	C32	Mean
Eugenol	95.2	94.8	95.0	92.7	93.2	92.9	87.8	89.8	88.8	62.2	81.5	71.9	66.0	60.0	63.0	36.4	56.3	46.3
Unknown 1	ND	ND	ND	ND	ND	ND	1.4	0.9	1.2	2.7	2.4	2.6	4.8	5.4	5.1	7.8	6.4	7.1
Unknown 2	0.6	0.7	0.6	3.0	1.5	2.2	4.8	3.8	4.3	17.0	6.8	11.9	12.4	15.8	14.1	26.6	17.0	21.8
Other Unknowns	1.5	1.7	1.6	1.8	2.1	2.0	3.0	4.1	3.5	10.1	6.2	8.2	11.1	13.2	12.2	19.2	14.6	16.9
Unresolved Background	0.1	0.6	0.3	0.1	0.3	0.2	1.0	0.5	0.7	1.0	1.0	1.0	0.6	1.2	0.9	0.1	1.1	0.6
Total	**97.4**	**97.7**	**97.6**	**97.6**	**97.0**	**97.3**	**97.9**	**99.1**	**98.5**	**93.1**	**97.9**	**95.5**	**94.8**	**95.6**	**95.2**	**90.1**	**95.3**	**92.7**

ND = Not Detected (or < 0.1%)

Table 8.2.1.1-7: Percent recovery of applied radioactivity from pH 9 buffer solutions (Tier II Test) and Percent of applied radioactivity present as [^{14}C]-Eugenol and degradants from pH 9 buffer solutions (Tier II Test) (50ºC).

Incubated at 50°C

	Time after application (days)																	
	0			2			7			14			21			30		
Vessel Code	C35	C36	Mean	C37	C38	Mean	C39	C40	Mean	C41	C42	Mean	C43	C44	Mean	C45	C46	Mean
Aqueous Phase	97.9	97.5	97.7	96.7	96.4	96.6	94.0	96.4	95.2	93.9	92.3	93.1	90.4	88.7	89.6	88.5	92.1	90.3
Vessel Wash	1.9	2.1	2.0	2.0	2.1	2.1	3.3	1.9	2.6	3.7	4.3	4.0	4.2	5.9	5.1	4.8	3.8	4.3
Total	**99.8**	**99.6**	**99.7**	**98.7**	**98.5**	**98.6**	**97.3**	**98.3**	**97.8**	**97.6**	**96.6**	**97.1**	**94.6**	**94.6**	**94.6**	**93.3**	**95.9**	**94.6**

	Percent Applied Radioactivity (% AR) Time after application (days)																	
Vessel Code	0			2			7			14			21			30		
	C35	C36	Mean	C37	C38	Mean	C39	C40	Mean	C41	C42	Mean	C43	C44	Mean	C45	C46	Mean
Eugenol	94.8	94.4	94.6	82.9	78.5	80.7	56.0	69.3	62.6	48.1	51.8	49.9	32.2	23.3	27.7	14.3	34.1	24.2
Unknown 1	ND	ND	ND	1.2	1.7	1.5	3.8	3.2	3.5	10.1	7.9	9.0	8.3	9.0	8.6	19.2	18.0	18.6
Unknown 2	1.1	1.1	1.1	6.0	8.3	7.2	17.4	10.4	13.9	17.2	12.9	15.1	21.2	23.4	22.3	19.0	16.4	17.7
Other Unknowns	1.7	1.5	1.6	5.9	7.0	6.5	15.9	12.7	14.3	17.1	18.2	17.7	28.0	32.5	30.2	34.7	23.2	29.0
Unresolved Background	0.4	0.5	0.4	0.7	0.9	0.8	0.9	0.8	0.9	1.4	1.6	1.5	0.7	0.5	0.6	1.3	0.4	0.8
Total	**97.9**	**97.5**	**97.7**	**96.7**	**96.4**	**96.5**	**94.0**	**96.4**	**95.2**	**93.9**	**92.3**	**93.1**	**90.4**	**88.7**	**89.6**	**88.5**	**92.1**	**90.3**

ND = Not Detected (or < 0.1%)

C Degradation rate

The degradation rate ($DegT_{50}$) of eugenol was determined using non-linear regression and single first order kinetics model (CAKE, Version 2.0).

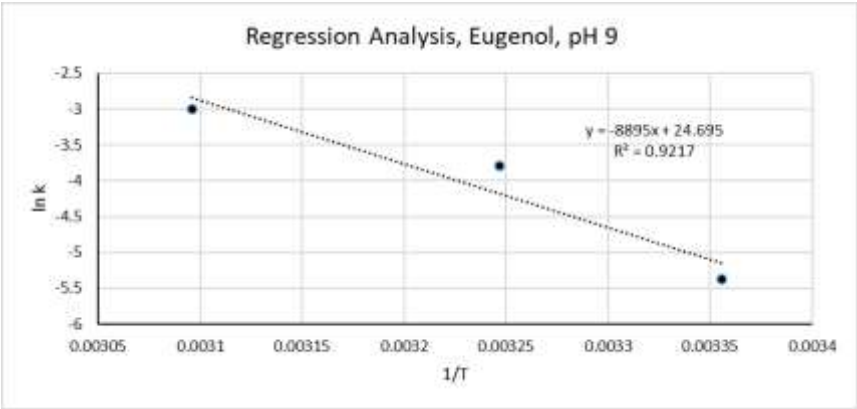
The $DegT_{50}$ values at pH 9, calculated by CAKE were 150, 31, and 14 days at 25, 35, and 50°C respectively.

For eugenol, the $DegT_{50}$ at 25°C was estimated as 120 days, at pH 9. The calculated $DegT_{50}$ value was in good accordance with the measured value calculated by CAKE 2.0.

The Arrhenius plot was generated with the following data:

Temperature (T) °C	K	Rate Constant, k (per day)
25	298	0.004611
35	308	0.02247
50	323	0.04947

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



III. CONCLUSION

[^{14}C]-Eugenol was hydrolytically stable in buffered aqueous solutions at 50°C, under sterile conditions, at pH values of 4 and 7.

Tier II hydrolysis at pH 9 and incubated in the dark at 25, 35 and 50 ± 0.5°C for up to 30 days, showed eugenol was not hydrolytically stable at environmentally relevant temperatures under basic conditions ($DegT_{50}$ < 1 year).

3. Assessment and conclusions

Assessment and conclusion by applicant:
This study shows eugenol is stable at pH 4 and pH 7 but shows hydrolysis at pH 9 with a DT50 of 120 days.

Assessment and conclusion by RMS: This study by Kelly D, 2021 has not been previously evaluated.

The hydrolysis of [14C]-Eugenol was studied in sterile aqueous buffered solutions at pH 4, pH 7 and pH 9 according to OECD guideline 111.
The preliminary test, conducted at 50ºC during 5 days, showed that approximately less than 10 per cent of hydrolysis was produced at pH 4 and 7. Eugenol can be considered stable and no additional testing was required.
However, at pH 9, eugenol degraded up to 63.7% at Tier 1 and therefore higher tier tests were carried out considering three temperatures.
For Tier 2, eugenol was incubated in the dark at pH 9 and at 25, 35 and 50 ± 0.5°C for up to 30 days. It is noted that OECD 111 recommends that one of the three temperatures be below 25ºC.
The mean recovery for all samples was 94.6 to 100.7% AR. [14C]-Eugenol decreased from mean values of 94.1 – 95.0% AR at 0 DAT to between 24.2% AR at 50°C and 81.1% AR at 25°C, at the end of incubation.

There were two major regions which accounted percentages of applied radioactivity above 10%. The Unknown 1 was described as a polar peak observed at ca 3 minutes in the HPLC. The Unknown 2 was observed after the elution of eugenol, at ca 39 minutes with maximum of 22.3% AR (50°C, 21 DAT). Unknown 2 increased throughout the incubations at 25 and 35°C, and at 50°C reached a maximum at 21 days before decreasing at the end of the incubation at 50ºC. This decrease could not be observed at the other two lower temperatures.
According to OECD 111, any mayor hydrolysis products at least those representing ≥ 10% of the applied dose should be identify by appropriate analytical methods. In the study, no reference standards other than eugenol were used and therefore the identification of any degradation product was not possible. Therefore, applicant is called to complete the identification of the unknowns 1 and 2. Furthermore, it should be noted that according to Regulation 283/2013, additional studies on hydrolytic degradation shall also be performed for degradation and reaction products which account at any time for more than 10 % (unless sufficient information on their degradation is available from the test performed).

Finally, there were many other minor degradation products formed (<10% AR), where, according to the study report, the largest individual peak reached a maximum of 9.3% AR (50°C, 21 DAT). This could not be checked by RMS, since the percentages reached by other any individual compound were not submitted.

Outcome and conclusion of the study:
The study is considered acceptable but more information is needed.
For eugenol, the $DegT_{50}$ at 25°C was estimated as 120 days, at pH 9. At pH 4 and pH 7, eugenol is considered stable.
The identity of the hydrolysis products ‘Unknown 1’ and ‘Unknown 2’ should be resolved.
More information is needed regarding to the percentages of AR reached by ‘other unknowns.
The following comment was added by applicant during the co-RMS-applicant´s initial revision: *Samples were contained at the CRO and analysis of the hydrolysis unknowns will be carried out by the laboratory.*
More information from the CRO on the other percentages of unknowns will be requested.

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	2021
Report title	Eugenol – Photolysis of [^{14}C]-Eugenol in Sterile Aqueous Solution
Report No.	Smithers ERS Ltd No. 3202785
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 photolysis of Eugenol was investigated in sterile aqueous buffered solutions. [Ring-^{14}C(U)]-Eugenol was applied, at a rate of 0.9 µg/mL, to sterile pH 7 buffer solutions in individual photolysis vessels. The treated irradiated buffer solutions were continuously[1] 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, vessels were treated and 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 six sampling intervals during irradiation (2, 4, 8, 14, 21 and 30 days after treatment (DAT)).

At each sampling interval, irradiated vessels were removed and chilled. Each vessel was connected to a system including a trap containing 2 M sodium hydroxide. Chilling was maintained using an ice bath. Acetonitrile was added to the buffer via the injection port and the vessel was shaken for *ca* 1 minute. After 5 minutes, the vacuum system was switched on and run for *ca* 30 minutes, drawing an air flow through the vessel and the 2 M sodium hydroxide trap. Tubing and foam bung washes were taken and quantified by LSC. The contents of the test vessel (Extract 1) were transferred to a pre-weighed vessel and the test vessels rinsed with Milli-Q water and combined with Extract 1.

The dark vessels were chilled and acetonitrile was added to the buffer. The vessel was shaken for *ca* 1 minute and the contents of the vessel (Extract 1) were transferred to a pre-weighed vessel and the test vessels were rinsed with Milli-Q water and combined with Extract 1.

Recovery of radioactivity in individual vessels was > 79%.
Volatile radioactivity recovered in sodium hydroxide traps (carbon dioxide) was low (≤ 0.2% AR). In the preliminary test, up to 10.5 %AR was recovered in sodium hydroxide traps after 29 days of irradiation. Despite attempts being made in the definitive test to recover carbon dioxide, *ca* 10% AR is suggested to have been lost during incubation. Low levels (< 2% AR) of radioactivity were also present in the foam bung traps. Radioactivity present in the vessel and tubing washes was ≤ 1% AR.

Eugenol degraded extensively under irradiated conditions to numerous, mostly polar, unidentified components. Only one component (retained for *ca* 25 minutes on HPLC) showed a clear maximum (8.1% AR at 14 DAT) followed by a decrease to the end of the incubation (30 days, 4.2% AR). After 30 days incubation under dark conditions, 89.5% AR (mean) was present as Eugenol.

Two unknown degradation products with HPLC retention times of 2.5 and 6 minutes exceeded 10% AR.

Degradation in non-irradiated samples was minor and primarily produced a non-polar product present at the start of the incubation. This hydrolysis product was not seen in irradiated samples after 8 days.

Single first-order kinetics provided a good fit for the data. The DT_{50} value for Eugenol applicable to Europe and North America (latitudes 30°, 40° and 50°) for irradiated samples was 9.2 days. In Japanese spring sunlight the DT_{50} value for Eugenol in irradiated samples was the equivalent of 29.8 days.

The major degradation products did not decline during the incubation and were not, therefore, kinetically modelled.

Photodegradation of eugenol occurred extensively in pH 7 aqueous buffer solutions exposed to simulated sunlight and produced numerous unknown degradation products. Degradation in non-irradiated samples was minor.

Single first-order kinetics provided a good fit for the data. The corrected DT_{50} value for photolysis of eugenol applicable to Europe and North America (latitudes 30°, 40° and 50°) was 9.2 days. In Japanese spring sunlight the DT_{50} value for eugenol in irradiated samples was the equivalent of 29.8 days.

[1] Irradiation was interrupted for almost 16 hours due to a fuse requiring replacement, see Appendix 12 for details.

I. MATERIALS AND METHODS

A MATERIALS

Test material 1

Name:	[ring-^{14}C(U)]-Eugenol
Description:	Clear solution in ethanol
Lot/Batch no.:	200925-RPI and CFQ44613
Radiochemical Purity:	Radiochemical purity 96.3% and 96.7%
Specific activity:	27.99 and 9.27 MBq/mg.
Storage:	Frozen (nominally -20°C)

Non-labelled material (used in all tests)

Name:	Eugenol
Description:	Colourless to pale yellow
Lot/Batch no.:	ESTS 167/20 40002011619
Purity:	99.7%
CAS #:	97-53-0
Storage/stability:	Room temperature

B STUDY DESIGN

Experimental conditions
Test System

Preparation of Buffer
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. The buffer was sterilised by autoclaving prior to use in the study.

Experimental Procedures
The application rate of *ca* 0.9 µg/mL, equivalent to 22.5 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. Solutions of [ring-^{14}C(U)]-Eugenol (79 µL) were dispensed dropwise over the surface of the aqueous solution and were treated with 22.8 µg (211.2 kBq).

The exact concentrations of radioactivity in the [ring-^{14}C(U)]-Eugenol application solutions were determined pre- and post-application, diluting an accurate volume of application solution in triplicate (50 µL) to 10 mL with acetonitrile and taking triplicate 100 µL aliquots for LSC.

To ensure homogeneity of the test substance, after application, the test vessels were swirled gently, and then incubated at *ca* 25°C in the dark for up to 30 days.

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 and an injection port. The side arms were connected by Marprene tubing and they had no air flow and were not connected to flow through traps.

Non-irradiated and zero time samples were contained in glass 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 similar to irradiated or non-irradiated vessels.

Irradiation Source

Atlas Suntest accelerated exposure machines (Atlas Materials Testing Technology, Bicester, UK) were used as the light source. These instruments filter radiation from a xenon burner to remove light below 290 nm to give ultra-violet and visible light with a spectral distribution close to that of natural sunlight.

The spectral properties and intensity of the lamp were measured at the height of the water surface at the position of each of the irradiated vessels. Measurements were made at 1 nm intervals before the irradiation period and after the final sampling interval using a Bentham spectroradiometer and Benwin+ software. The average intensity over the 300 to 400 nm range for the definitive tests was in the range 26.2 Watts/m^2, approximately equivalent to one US or UK summer day. This mean intensity was used to compare the artificial light intensity (Ia) to sunlight intensity (Is) at summer sunlight latitudes of 30, 40 and 50° N.

Sampling Intervals
At intervals of 0 (after treatment), 2, 4, 8, 14, 21 and 30 days incubation, duplicate vessels treated with radiolabelled test substance were removed for analysis.

Sample Analysis
At each sampling interval, irradiated vessels were removed and chilled. Each vessel was connected to a system including a trap containing 2 M sodium hydroxide (*ca* 25 mL). Chilling was maintained using an ice bath. Acetonitrile (*ca* 10 mL) was added to the buffer via the injection port and the vessel was shaken for *ca* 1 minute. After 5 minutes, the vacuum system was switched on and run for *ca* 30 minutes, drawing an air flow through the vessel and the 2 M sodium hydroxide trap. The vessel was removed from the system, the tubing was removed and added to acetonitrile (*ca* 40 mL) and the foam bungs were removed and added to acetonitrile (*ca* 2 mL). The tubing and foam bung washes were shaken, weighed and aliquotted for LSC. The contents of the test vessel were transferred to a pre-weighed vessel labelled Extract 1 and the vessels were rinsed with Milli-Q water (*ca*. 2 mL) and combined with Extract 1, to account for any radioactivity remaining in the vessel.

The dark vessels were chilled and acetonitrile (*ca* 10 mL) was added to the buffer. The vessel was shaken for *ca* 1 minute and the contents of the vessel were transferred to a pre-weighed vessel labelled Extract 1. The vessels were rinsed with Milli-Q water (*ca*. 2 mL) and combined with Extract 1.

<u>High performance Liquid chromatography (HPLC)</u>
The following HPLC method was used for the determination of the radiochemical purity of the application solutions and for sample analysis.

Table 8.2.1.2-1: HPLC method for soil samples

HPLC System	Agilent 1260		
Column	YMC Triat C18, 5 µm (250 mm x 4.6 mm)		
Column temperature	25°C		
Mobile phases	Eluent A: water Eluent B: acetonitrile		
	Time (min)	% Eluent A	% Eluent B
	0	70	30
	30	50	50
	35	0	100
	40	0	100
	41	70	30
	45	70	30
Flow rate (mL/min)	1		

Samples were co-chromatographed with non-radiolabelled eugenol reference standard and chromatograms were evaluated using Laura (versions 6.0.4.92 and 6.1.5.69) software (Lablogic).

II. RESULTS

The photodegradation of [ring-^{14}C(U)]-Eugenol, has been studied in sterile aqueous buffered solutions maintained at 25 ± 2°C over a period of 30 days.

The pH values were within 7 ± 0.2 during the test period. All analysed samples were found to be sterile.

A DISTRIBUTION AND RECOVERY

Total recovery of applied radioactivity was 84 to 100% (mean values) over all sampling intervals and test conditions.

Carbon dioxide recovery by trapping during sample analysis was low (≤ 0.2% AR) during the definitive test. In the preliminary test, up to 10.5 %AR was recovered in sodium hydroxide traps after 29 days of irradiation. Attempts were made, in the definitive test, to recover carbon dioxide by sealing the vessels during incubation and evacuating the headspace via a sodium hydroxide trap at sampling. As only ≤ 0.2% AR was recovered from the sodium hydroxide trap, *ca* 10% AR is suggested to have been lost during incubation. Small amounts (< 2% AR) of radioactivity were still present in the foam bung traps despite shaking with the buffer acetonitrile mixture. Radioactivity present in the vessel wash and tubing wash was both ≤ 1% AR.

Table 8.2.1.2-1: Mean Distribution and recovery of radioactivity f following application of [ring-^{14}C(U)]-Eugenol to buffered solution – irradiated samples

Sampling Interval	% Applied Radioactivity					
	2	4	8	14	21	30
Buffer extract (Ext 1)	97.5	95.2	89.2	90.4	83.9	82.6
Vessel Wash (Extract 2)	ND	ND	0.2	ND	ND	ND
Sodium hydroxide Traps	0.1	0.1	0.1	0.2	0.2	0.2
Foam Bung	0.6	1.1	0.9	1.1	1.1	0.7
Mass Balance	98.3	96.6	90.6	92.1	85.4	83.7

NA Not applicable
ND Not detected (< 0.1% AR)

Table 8.2.1.2-2: Mean Distribution and recovery of radioactivity following application of [ring-^{14}C(U)]-Eugenol to buffered solution – non irradiated samples

Sampling Interval	% Applied Radioactivity						
	0	2	4	8	14	21	30
Buffer extract (Ext 1)	99.2	99.6	100.1	99.8	99.7	98.9	99.5
Mass Balance	99.2	99.6	100.1	99.8	99.7	98.9	99.5

B CHARACTERISATION

Eugenol degraded extensively under irradiated conditions to a number of metabolites. A small amount of degradation occurred in dark conditions, at 30 days, 90% AR (mean) was present as Eugenol.

Eugenol degraded extensively under irradiated conditions to numerous, mostly polar, unidentified components. Only one component (retained for *ca* 25 minutes on HPLC) showed a clear maximum (8.1% AR at 14 DAT) followed by a decrease to the end of the incubation (30 days, 4.2% AR). After 30 days incubation under dark conditions, 89.5% AR (mean) was present as Eugenol. Two unknown degradation products with HPLC retention times of 2.5 and 6 minutes exceeded 10% AR. The unknown degradation products standards did not co-chromatograph with reference standards used in HPLC. Degradation in non-irradiated samples was minor and primarily produced a non-polar product present at the start of the incubation. This hydrolysis product was not seen in irradiated samples after 8 days.

Table 8.2.1.2-3: Mean Percent of applied radioactivity present as [ring-^{14}C(U)]-Eugenol and metabolites – irradiated samples

Sampling Interval	% Applied Radioactivity					
	2	4	8	14	21	30
Eugenol	84.4	76.1	46.4	29.8	18.9	10.8
Unknown ca 2.5 min	2.3	4.1	14.3	15.2	24.4	30.3
Unknown ca 4 min	2.1	2.3	5.4	8.7	9.6	9.5
Unknown ca 6 min	3.5	4.7	10.2	19.5	17.7	18.5
Unknown ca 7 min	0.6	0.8	1.8	3.6	3.0	3.8
Unknown ca 25 min	1.8	2.7	4.1	8.1	5.3	4.2
Unknown ca 31 min	1.5	1.3	1.1	0.8	0.5	0.4
Unknown ca 40 min	ND	0.2	0.3	ND	ND	ND
Minor unknowns	0.9	2.8	5.4	4.5	3.2	4.2
Unresolved background	0.3	0.3	0.2	0.1	1.1	1.0
Total	97.5	95.2	89.2	90.4	83.9	82.6

Table 8.2.1.2-4: Mean Percent of applied radioactivity present as [ring-^{14}C(U)]-Eugenol and metabolites – non-irradiated samples

Sampling Interval	% Applied Radioactivity						
	0	2	4	8	14	21	30
Eugenol	94.7	95.2	95.2	93.9	93.1	89.2	89.5
Unknown ca 31 min	1.9	1.8	1.6	1.8	1.6	1.9	1.3
Unknown ca 40 min	2.4	2.4	2.9	4.0	4.5	6.2	8.0
Minor unknowns	NA	NA	0.3	NA	NA	NA	NA
Unresolved background	0.1	0.1	0.1	0.0	0.5	1.5	0.7
Total	99.2	99.5	100.1	99.7	99.6	98.9	99.5

C KINETICS

SFO modelling of parent degradation in irradiated and non-irradiated samples displays a good visual fit to the data and low χ^2 values.

Test system	**DegT50 (days)**	**DegT90 (days)**	**χ^2 %**	**r^2**
Irradiated (experimental result)	8.6	28.6	4.81	0.9589
Non-irradiated	289	959	1.01*	0.8394
Photolysis	8.9	29.4	-	-
Corrected DegT50 and DegT90 for different latitudes				
Summer Sunlight 30-50°N (OECD)	9.2	30.7	-	-
Tokyo Spring Sunlight	29.8	99.1	-	-

*Limit of quantification

III. CONCLUSION

Photodegradation of eugenol occurred extensively in pH 7 aqueous buffer solutions exposed to simulated sunlight and produced numerous unknown degradation products. Degradation in non-irradiated samples was minor.
Single first-order kinetics provided a good fit for the data. The corrected DT_{50} value for photolysis of eugenol applicable to Europe and North America (latitudes 30°, 40° and 50°) was 9.2 days. In Japanese spring sunlight the DT_{50} value for eugenol in irradiated samples was the equivalent of 29.8 days

3. Assessment and conclusions

Assessment and conclusion by applicant:
This study was performed well and the DT_{50} of eugenol in sunlight is 9.2 days.

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 photolysis of [Ring-^{14}C(U)]-Eugenol was investigated in a sterile aqueous buffered solutions at pH 7 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 mean recovery of applied radioactivity (mass balance) was 83.7-98.3% for irradiated samples and 98.9-100.1 for non-irradiated samples. Regarding to the quality criteria, it is noted that the recoveries should range from 90% to 110% for labelled compounds. The recoveries for irradiated samples at 21 and 30 days were below this recommended range. The explanation provided in the study report was that an amount of c.a 10% AR of CO_2 was captured in the sodium hydroxide traps in the preliminary tests ((irradiated; 29 DAT), but this amount was not observed in the definitive test. Therefore, since transformation is observed, it is suggested that CO_2 was lost during incubation.

The repeatability and sensitivity of analytical method together with the accuracy of the transformation data are considered in line with the OECD guideline.

Eugenol degraded under irradiated conditions to a number of metabolites (7 peaks), 3 of them chromatographed in the first 6 minutes. These metabolites were above the 10 % of AR with the exception of the unknown ca. 4 min which reached a value of 9.6. Considering that the mass balance was low at the time when this maximum was reached, RMS considers that this metabolite can be included as relevant photoproduct metabolite.
The photodegradation pathway was not shown as the major degradation products were not identified. According to the study report eugenol degraded extensively under irradiated conditions to numerous, mostly polar, unidentified components, however it was not specified which metabolites were considered polar. Two potential degradation product reference standards, isoeugenol (mixed isomers) and isoeugenol (predominantly trans), were purchased to investigate a degradation product. These standards did not co-chromatograph with radio peaks when used in HPLC.
As the major degradation products were not identified, the phototransformation pathway was not included. Therefore this study would be valid only to determine the photodegradation rate but not the route.

Additionally, the major degradation products did not decline during the incubation and were not kinetically modelled. Only one component (retained for *ca* 25 minutes on HPLC) showed a clear maximum (8.1% AR at 14 DAT) followed by a decrease to the end of the incubation (30 days, 4.2% AR).

Under dark conditions, small amount of degradation occurred, at 30 days, 90% AR (mean) was present as Eugenol.
There were two metabolites, unknown ca 31 min and unknown ca 40 min, which were present under irradiated and non-irradiated conditions and therefore, it was assumed that these metabolites resulted from the hydrolysis process.

The rate constant (k) for non-irradiated degradation was subtracted from the rate constant for irradiated degradation to produce a rate constant for photolysis only. Times were then converted to the equivalent times for OECD summer sunlight at 30 - 50°N and Tokyo spring sunlight, by multiplying the time by the intensity of the irradiation light and dividing by the intensity of the sunlight calculated for the required regions.

Outcome and conclusion of the study:
This study is valid to stablish the rate of degradation under irradiated conditions but not the route.
The DT_{50} of eugenol in sunlight was 9.2 days.
The identity of the unknown peaks should be resolved to identify any possible photoproduct susceptible to be included in the residue definition for risk assessment.
The following comment was added by applicant during the co-RMS-applicant´s initial revision: *Samples were contained at the CRO and analysis of the photolysis unknowns will be carried out by the laboratory.*

1. **Information on the study**

Data point	CA 7.2.1.2/02
Report author	Z. Ye, Y. Zhuang, Y. Chen, Z. Zhao, S. Ma, H. Huang, Y. Chen and X. Ge
Report year	2020
Report title	Aqueous-phase oxidation of three phenolic compound by hydroxyl

	radical: Insight into secondary organic aerosol formation yields, mechanisms, products and optical properties
Report No.	-
Document No.	Atmospheric Environment 223 (2020) 117240
Guidelines followed in study	NA
Deviations from current test guideline	Not performed to current guidelines, no pH given for solution.
Previous evaluation	No
GLP/Officially recognised testing facilities	No
Acceptability/Reliability	Supplementary information only

2. Full summary

ABSTRACT (copied from original literature)
This work performed a systematic investigation on the aqueous hydroxyl radical (OH) - induced photochemical oxidation of three modestly-soluble precursors from biomass combustion including 4-methylsyringol (DMP), eugenol (Eug), and 2,4,6-trimethylphenol (TRMP) under both simulated sunlight and ultraviolet (UV) light irradiations. An Aerodyne soot particle aerosol mass spectrometer (SP-AMS) was used to monitor the bulk chemical and elemental compositions of aqueous secondary organic aerosol (aqSOA) formed. AqSOA mass yields varied in ranges of 80–190% and 0–200% under sunlight and UV light conditions, respectively. AqSOA oxygento-carbon (O/C) ratio and carbon oxidation state increased steadily under sunlight + OH condition, but increased then decreased under UV + OH condition. Organic acids including malic acid, glycolic acid, formic acid and oxalic acid were formed, and their total accounted for ~12% of SOA mass. The UV–vis spectral change suggested formation of light-absorbing organics. Reaction pathways were proposed by combining gas chromatographymass spectrometry (GC-MS) and SP-AMS results. Under sunlight + OH condition, oligomerization, functionalization, and fragmentation processes all involved in aqSOA evolution, with more contribution from functionalization via hydroxylation and oxygenation reactions. Reaction mechanism of UV + OH oxidation was initially dominated by functionalization then by fragmentation, indicating by the decrease of total organic carbon (TOC) contents, formation of small organic acids and low-molecular-weight products. Our work highlights that combination of SP-AMS with GC-MS is a powerful method for laboratory investigation of aqueous-phase reactions.

I. MATERIALS AND METHODS

Test material 1

Name:	Eugenol
CAS number:	NS
Purity:	>99%

The aqueous-phase photo-oxidation reaction was carried out inside a photo-reactor equipped with a 500 W Xe/Hg lamp (sunlight/UV lamp) located in the centre. Wavelengths <290 nm were cut off by a Pyrex glass filter to mimic tropospheric sunlit conditions. Hg lamp provides UVB radiation. The light intensity of Hg lamp at the surface of the reaction solution was 6257.1 μW/cm^2 in the range of 290–320 nm (UVB), significantly higher than that of Xe lamp (290.6 μW/cm^2).
A total volume of 600 mL aqueous solution containing 0.3 mM precursor was divided into 6 x 100 mL aliquots, filled in six 150 mL quartz tubes. The six tubes were arranged equidistantly around the lamp in a circular manner, in a water bath maintained at a temperature of 23–25°C, and with a magnetic agitator in each tube to ensure the homogeneity. All solutions were prepared with Milli-Q water. The following initial conditions were applied: H_2O_2 = 300 μM, precursor = 300 μM and pH = Eug:4.57. These conditions led to a steady-state concentration of OH radical of about 6 x 10^{-12} M throughout the course of sunlight + OH experiment, but a higher concentration of 1.3 x 10^{-11} M at first 50 min for UV x OH experiment. Concentration of OH radical was determined by Micro EPR spectrometer. The OH concentration in this study was one order of magnitude higher than that in cloud environment, but close to that in wet aerosol microenvironment. Aqueous-phase samples were collected at the first 1 h, then taken each 2 h with a total reaction time of 23 h. In addition, prior to photo-oxidation experiment, 10 mg/L $(NH_4)2SO_4$ was added to solution as an internal standard to quantify aqSOA measured by the SP-AMS.
Upon irradiation, 2 mL sample solution was drawn periodically to analyze the concentrations of precursors via HPLC analysis. HPLC equipped with the InertSustain AQ-C18 reverse phase column (4.6 x 250 mm, 5.0 μm, Shimadzu) and a UV–vis detector (wavelength of 280 nm was selected). The mobile phase was a mixture of acetonitrile and H_2O (60:40, v/v) with a flow rate of 0.6 mL/min. TOC concentration was determined by a TOC analyzer. Aqueous solution (5 mL) was taken from the photochemical reactor for quantification of organic acids by an ion chromatography (IC) equipped with an Dionex IonPac AS11-HC column (250 mm x 4 mm, ThermoFisher Scientific) coupled to a Dionex AG11-HC

guard column (50 mm x 4 mm, ThermoFisher Scientific). Solution (10 µL) was injected into the IC system for analysis. The analysis was performed with a flow rate of 1.2 mL/min of 15 mM KOH as eluent. All photolysis experiments were conducted at least three times. Reaction solution (30 mL) was extracted by 10 mL dichloromethane, concentrated to 0.3 mL using dry N_2, transferred to a GC vial, and analyzed with a gas chromatography coupled to mass spectrometry (GC MS), using a DB-5ms capillary column (30 m x 0.25 mm x 0.5 µm). Two internal standards of 100 µL hydroquinone and methoxyphenol (c0 = 30 µM) were tested to evaluate the extraction efficiency. Results showed that extraction efficiencies of these standards were 90.5 and 92.5% respectively.

II. RESULTS

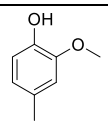
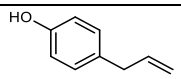
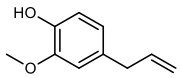
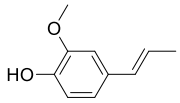
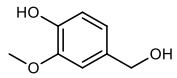
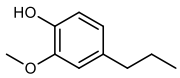
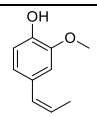
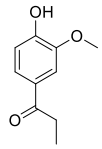
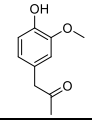
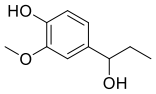
The decay kinetics of eugenol under both sunlight x OH conditions were 5–7 h. Conversely, >95% eugenol was decomposed within the first 1 h under UV x OH condition

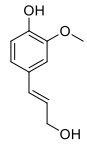
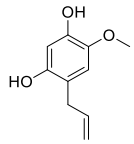
The rate constant under sunlight x OH was 0.26524 h^{-1}. The reactions under UV x OH system were too fast to calculate rate constants.

TOC removal was low (<10%) for sunlight + OH conditions. But reached 95.6% at 23 h for eugenol under UV + OH conditions.

A total of 12 intermediates were identified after 23 h photolysis (Sunlight + OH). $C_{10}H_{12}O_2$, isomerized products were abundant in Eug-aqSOA according to GC-MS data. Additionally, the product $C_{10}H_{12}O_3$ with carbonyl group was also abundant. Under UV + OH, the degradation was fast and intermediates were negligible after 11 h photolysis.

Table 8.2.1.2-6: Products identified via GC-MS detection for Eugenol degradation under sunlight+OH reaction systems.

	RT (min)	Material name	Chemical structure	Chemical formula	MW (g/mol)
1	10.27	2-methoxy-4-methylphenol		$C_8H_{10}O_2$	138
2	10.68	4-(2-propenyl)- phenol		$C_9H_{10}O$	134
3	11.79	vanillin		$C_8H_8O_3$	152
4	12.06	(E)-2-methoxy-4-propenyl-phenol		$C_{10}H_{12}O_2$	164
5	12.11	4-hydroxy-3-methoxybenzyl alcohol		$C_8H_{10}O_3$	154
6	12.18	2-methoxy-4-propyl-phenol		$C_{10}H_{14}O_2$	166
7	12.29	(Z)-2-methoxy-4-propenyl-phenol		$C_{10}H_{12}O_2$	164
8	12.50	1-(4-hydroxy-3-methoxyphenyl)propan-1-one		$C_{10}H_{12}O_3$	180
9	12.65	1-(4-hydroxy-3-methoxyphenyl)- 2-propanone		$C_{10}H_{12}O_3$	180
10	12.73	4-(1-hydroxypropyl)-2-methoxyphenol		$C_{10}H_{14}O_3$	182

11	12.91	(E)-4-(3-hydroxyprop-1-en-1-yl)-2-methoxyphenol		$C_{10}H_{12}O_3$	180
12	13.20	4-allyl-6-methoxybenzene-1,3-diol		$C_{10}H_{12}O_3$	180

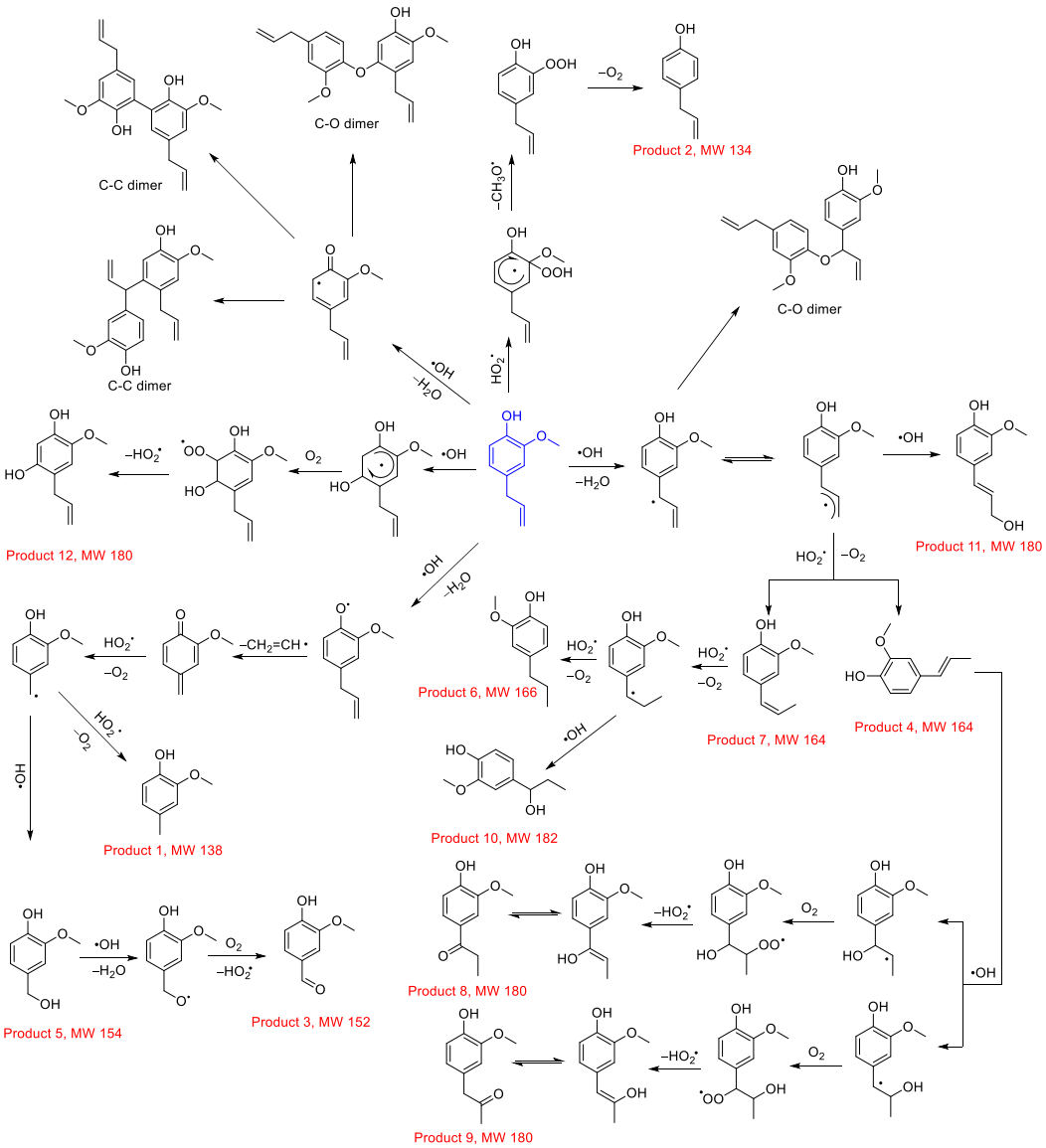


Figure 8.2.1.2-1: Proposed Eugenol aqueous OH oxidation mechanism when exposed to sunlight irradiation. The red text represents the compounds listed in Table 8.2.1.2-6 via GC-MS.

3. Assessment and conclusions

<u>Assessment and conclusion</u> *Reliability assessment*

For full details and justification, please refer to Document K.
Proposed category: 3 not reliable, supporting data only.

Assessment and conclusion by RMS:
This study from the open literature was identified as a relevant study included after detailed assessment of full-text documents for relevance and found to be not reliable.

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

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**

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

B.8.2.2.1. "Ready biodegradability"

1. Information on the study

Data point	CA 7.2.2.1/01
Report author	B. Seyfried
Report year	2008
Report title	Eugenol: Ready Biodegradability in a Manometric Respirometry Test
Report No.	RCC Study number B65160
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 recognized testing facilities	Yes
Acceptability/Reliability	Yes

2. Full summary

EXECUTIVE SUMMARY

The test item, Eugenol was investigated for its ready biodegradable 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 2.34 mg O_2/mg test item.

The biochemical oxygen demand (BOD) of eugenol 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 eugenol amounted to 84%. Consequently, eugenol 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, Eugenol can be classified as readily biodegradable under the test conditions.

In the toxicity control, containing both eugenol and the reference item sodium benzoate, biodegradation was >25% within 14 days of exposure. Thus, eugenol had no inhibitory effect on the activity of activated sludge microorganisms at the concentration of 100 mg/L.

In the procedure controls, the reference item sodium benzoate was degraded by an average of 90% by exposure day 14, and reached an average biodegradation of 93% 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:	Eugenol
Description:	Pale straw coloured oily liquid
Lot/Batch no.:	95217
Purity:	98.8%
Storage :	In a refrigerator at about 4°C, in a desiccator in the dark

Reference Item

Name:	Sodium benzoate
Lot/Batch no.:	1274106
Purity:	100.0%
CAS #:	
Storage/stability:	In tightly closed original container, at room temperature at about 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 2 days 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, stabilized with one drop of concentrated HCl per litre

To obtain the final test water, 10 mL of stock solution No. 1 and 1 mL each of stock solution No's 2, 3 and 4 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 100 mg/L. Activated sludge was then added to each flask with a final test volume of 250 mL per test flask.

¡Error! No se encuentra el origen de la referencia.**: Test concentrations**

Identification	Replicate	Amount of test item		Amount of reference item	
		mg/L	ThOD	mg/L	ThOD
Test item	1	100	235	-	NA
Test item	2	100	234	-	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	100	235	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 7.4 and 7.5 in all test flasks. At the end of incubation, the pH was measured again in each test flask.

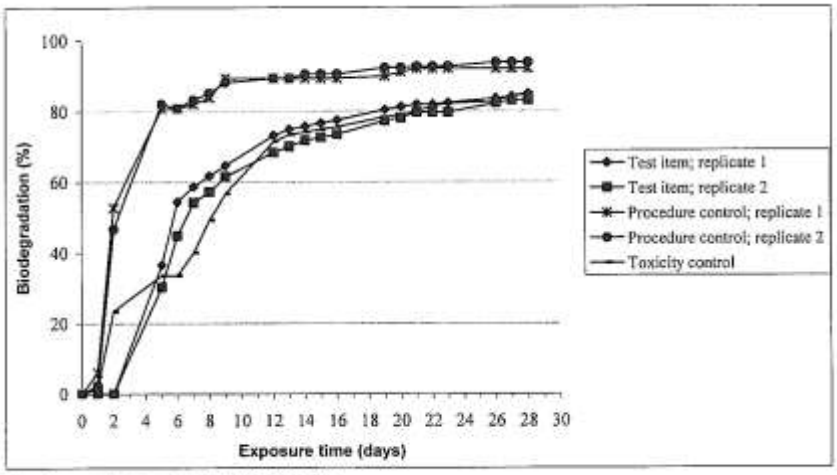
II. RESULTS

The percent biodegradation of the test item, eugenol, was calculated based on the theoretical oxygen demand of 2.34 mg O_2/mg test item.

The biochemical oxygen demand of eugenol 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 eugenol was 84%. Consequently, eugenol 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 90% by exposure day 14, thus confirming suitability of the activated sludge.

The toxicity control, contained both eugenol and the reference item sodium benzoate, showed that the test item had no inhibitory effect on activated sludge microorganisms at the tested concentration of 100 mg/L, because biodegradation in the toxicity control was >25% within 14 days.



(based on values presented in Table 2)

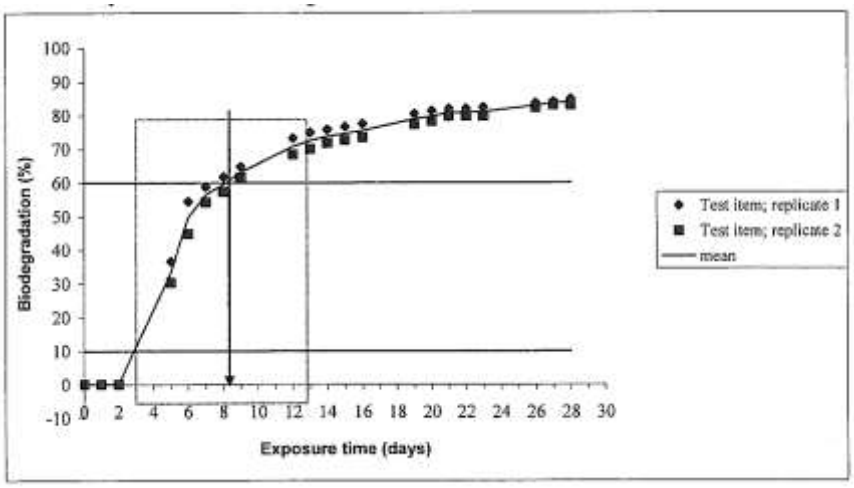


Figure 8.2.2.1 1: Biodegradation in the test flasks during the incubation period. Overview over the whole test series (up). 10-day window for the biodegradation of the test item (down).

III. CONCLUSION

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

3. Assessment and conclusions

Assessment and conclusion by applicant:
This study followed the OECD guidelines and is acceptable. Results show that eugenol is readily biodegradable under the test conditions.

Assessment and conclusion by RMS:
This study was previously evaluated and accepted in DAR 2011.
The ready biodegradability of eugenol was determined in a manometric respirometry test over 28 days in accordance with OECD test guideline 301 F and to GLP. Eugenol was readily biodegradable under the conditions of the test, the mean degradation was 84% in 28 days.
Eugenol is volatile and is readily biodegradable, one route of dissipation being microbial. It is unlikely that it will reach or impact on sewage treatment processes.
Outcome and conclusion of the study:
The RMS considers the study to be acceptable.

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	[^{14}C]-Eugenol: Aerobic Mineralisation in Surface Water
Report No.	Smither ERS Ltd No. 32025787
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 mineralisation and degradation rate of eugenol in a surface water system (Fountains Abbey) was studied at 20 ± 2°C in the dark. The test was performed using two concentration levels (95 and 10 µg/L). Sterilised samples were also tested at the higher concentration.
After test substance application, the air drawn over the surface of the vessels was passed through a polyurethane foam bung trap and two sodium hydroxide traps to collect evolved radiolabelled volatile organic compounds and carbon dioxide, respectively. Non-sterile samples were taken for analysis at 0, 0.25, 0.75, 1, 5, 14, 30 and 62 days after treatment (DAT) at the low application rate and at 0, 1, 1.25, 2, 7, 14, 30 and 62 DAT. The sterile vessels were sampled at 62 DAT.
The total recovery (mean values) at each sampling interval ranged from 85.7 % to 96.0 % and 87.7 % to 98.8 % at the non-sterile low and high application rates, respectively. The overall total radioactive recovery (mean value) was > 90 % at both application rates (non-sterile and sterile vessels).
Volatile radioactivity in alkaline hydroxide traps was confirmed to be carbon dioxide by precipitation with barium chloride.
The degradation of eugenol occurred quickly (DT_{50} values of 1.8 and 2.0 days) in surface water at both application rates under non-sterile conditions, with no detectable of levels observed after 14 DAT. Mineralisation to carbon dioxide is the major degradation product, with maximum levels exceeding 59% at the end of the 62 day incubation period.
The rate of mineralisation was significantly slower under sterile conditions, with detectable levels of carbon dioxide (1.6 %) present at 62 DAT, demonstrating that extent of mineralisation of [^{14}C]-eugenol in surface water is largely attributable to biotic activity.

I. MATERIALS AND METHODS

A MATERIALS

Test material 1

Name:	[ring-^{14}C(U)]-Eugenol
Description:	Clear solution in ethanol
Lot/Batch no.:	200925-RP1 and CFQ44613
Radiochemical Purity:	Radiochemical purity 96.3% and 96.7%
Specific activity:	27.99 and 9.27 MBq/mg.
Storage:	Frozen (nominally -20°C)

Non-labelled material (used in all tests)

Name:	Eugenol
Description:	Colourless to pale yellow
Lot/Batch no.:	ESTS 167/20 40002011619
Purity:	99.7%
CAS #:	97-53-0
Storage/stability:	Room temperature

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 a lake on the grounds of Fountains Abbey, Ripon, England, UK. The test system was named 'Fountains Abbey'.
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 eugenol

Soil parameter	Value
Source	Fountains Abbey
Test System code	CS02/21
pH	7.60
Sampling water O2 content mg/L	12.25
Total Organic carbon (TOC) (mg/L)	10.5
Total Phosphorus (mg/L)	0.069
Ammonia (mg/L)	0.055
Total Nitrogen (mg/L)	2.86
Suspended solids (mg/L)	9
Alkalinity (mg/L)	98
Hardness (mg/L $CaCO_3$)	71
Nitrite (mg/L)	<0.015
Nitrate (mg/L)	2.04

B STUDY DESIGN

Experimental conditions

Water samples (100 mL, non-sterilised) were dispensed into individual incubation vessels containing PTFE magnetic stirrer bars. Water samples (100 mL, sterilised) were dispensed under sterile conditions into individual sterilised

incubation vessels containing PTFE magnetic stirrer bars. Moistened air was drawn through the vessels and through a series of traps before and after test substance application. The first trap contained a polyurethane foam bung (foam bung) to collect volatile organic compounds. The second trap was an empty vessel, which was used as a safety trap. The third and fourth traps contained 2M sodium hydroxide solution (alkaline traps) to collect liberated carbon dioxide.

Sterilisation of an appropriate volume of water and number of vessels was performed by autoclaving two times, (autoclave set to reach 121°C for 15 minutes), with each occasion separated by *ca* 24 hours, and allowing the water and vessels to reach room temperature in between the procedures.
Control water vessels were prepared in the same manner as water vessels intended for test substance analysis.
Water samples were maintained under experimental conditions for eight days (sterile vessels) or nine days (non-sterile vessels) at 20 ± 2°C in the dark prior to test substance application.
Preliminary tests were performed to test the feasibility of the study prior to commencement of the definitive tests as eugenol was known to be volatile.
Test substance was applied dropwise onto the surface of the water to achieve concentrations of 10.2 µg/L in the low dose and 96.4 µg/L in the high dose.
Duplicate test vessels were removed for analysis from group D at 0, 0.25, 0.75, 1, 5, 14, 30 and 62 days after treatment (DAT), and from Group E at 0, 1, 1.25, 2, 7, 14, 30 and 62 DAT. Duplicate vessels from group F (sterile) were removed at 62 DAT. All these samples were analysed for radioactive content by LSC and were analysed for test substance and metabolites by HPLC.

Sample analysis

Method 1: Each incubation vessel was capped, shaken, and sonicated for *ca* 1 minute. The contents of the incubation vessel were transferred to a pre-weighed vessel (Extract 1). The glass incubation vessel and associated magnetic stirrer were washed (shaken) with RO water (10 mL) and the contents of the vessel (excluding the associated magnetic stirrer) were combined with 'Extract 1'.

Method 2: A subsample (Extract 1 - SS1) of water (*ca* 20 mL) was removed from the incubation vessel and the radioactivity in the subsample and associated 2M hydroxide traps quantified by LSC. Two new 2M sodium hydroxide traps were attached to the incubation vessel. The remaining water in the incubation vessel was acidified with HCl to *ca* pH 2 and the incubation continued for at least two hours. The incubation vessels were then processed following Method 1, to generate 'Extract 1 – Acidified'.

Method 3: The 2M sodium hydroxide traps associated with incubation vessel were removed for quantification by LSC. Two new 2M sodium hydroxide traps were attached to the incubation vessel. The water sample in the incubation vessel was acidified with HCl to *ca* pH 2 and the incubation continued for at least two hours. The incubation vessels were then processed following Method 1, to generate 'Extract 1 – Acidified'.
Aliquots of water samples (Extract 1, Extract 1 – SS1 and Extract 1 – Acidified), were quantified by LSC. Samples with high levels of radioactivity were analysed directly by HPLC. Low level samples were analysed by fraction collection as detailed in the Analytical Methods, HPLC section.

Volatile Traps
Alkaline traps attached to the vessels treated with [^{14}C]-eugenol were collected for analysis at the time the associated test vessel was removed from the incubation system. For group D and E vessels incubated for the duration of the test and group G vessels (reference / solvent controls)), the alkaline traps were also collected for analysis and replenished at 30 DAT. Group G and F (sterile) alkaline traps were collected for analysis at 62 DAT only.
After removal from the incubation system, the foam bung solid trap was transferred to into a vial and the foam bung extracted (10 minute shake) with acetonitrile (*ca* 8 mL).
The radioactivity in the alkaline traps and foam bung extract was quantified by LSC.
To representative alkaline traps (D9, D11, E9 and E11), barium chloride was added to the 2M sodium hydroxide samples to confirm the presence of carbon dioxide by precipitating barium carbonate.

<u>High performance Liquid chromatography (HPLC)</u>
The following HPLC method was used for the determination of the radiochemical purity of the application solutions and for sample analysis.

Table 8.2.2.2-2: HPLC method for primary sample analysis

HPLC System	Agilent 1260		
Column	YMC Triat C18, 5µm (250 mm x 4.6 mm)		
Column temperature	25°C		
Mobile phases	Eluent A: water Eluent B: methanol		
	Time (min)	% Eluent A	% Eluent B
	0	70	30
	30	50	50
	35	0	100
	40	0	100
	41	70	30
	45	70	30
Flow rate (mL/min)	1		

Table 8.2.2.2-3: HPLC method for LC-MS analysis

HPLC System	Agilent 1260		
Column	YMC Triat C18, 5µm (250 mm x 4.6 mm)		
Column temperature	25°C		
Mobile phases	Eluent A: 0.1% formic acid in water Eluent B: 0.1% formic acid in acetonitrile		
	Time (min)	% Eluent A	% Eluent B
	0	95	5
	30	50	50
	35	0	100
	40	0	100
	41	70	30
	45	70	30
Flow rate (mL/min)	1		

Samples were co-chromatographed with non-radiolabelled eugenol reference standard and chromatograms were evaluated using Laura (version 6.0.4.92 and 6.1.5.69) software (Lablogic).

II. RESULTS

A Radioactivity Recovery

The radioactivity associated with the surface water under non-sterile conditions decreased from 96.0 to 24.3 % at the low application rate and 98.8 % to 38.1 % at the high application rate, from 0 DAT to 62 DAT, respectively.
In the foam bung extracts, (for the collection of organic volatiles), < 1 % was detected at both application rates under non-sterile conditions by the end of the incubation period.
The total recovery (mean values) at each sampling interval ranged from 85.7 % to 96.0 % and 87.7 % to 98.8 % at the low and high application rates (non-sterile), respectively. The overall total radioactive recovery (mean value) was > 90 % at both application rates (non-sterile and sterile vessels).
The majority of radioactivity was detected in the surface water, (81.6 % at 62 DAT) in the sterile vessels. 9.1 % and < 2 % were detected in the foam bung extracts and alkaline traps, respectively.

Table 8.2.2.2-4: Mean Distribution and recovery of radioactivity from surface water treated with [^{14}C]-Eugenol at the low application rate

Sampling Interval	% Applied Radioactivity							
	0	0.25	0.75	1	5	14	30	62
Surface Water (Ext 1 or Ext 1 SS1)	96.0	93.2	84.3	81.2	41.7	3.7	NA	24.3
Surface Water (Ext 1 – acidified)	NA	NA	NA	NA	NA	26.4	24.3	NA
Surface water Total	96.0	93.6	84.3	81.2	41.7	30.1	24.3	24.3
Foam Bung	NA	ND	0.2	0.1	0.2	0.2	0.1	ND
Sodium hydroxide Traps (evolved $^{14}CO_2$	NA	0.7	4.9	4.5	47.0	56.3	62.8	69.1

Mass Balance	96.0	94.3	89.3	85.7	88.9	86.6	87.1	93.4
Overall Mean ±SD	90.1 ±4.1							

NA Not applicable; SS1 Sub-sample 1; ND Not detected (< 0.1% AR); SD Standard deviation

Table 8.2.2.2-5: Mean Distribution and recovery of radioactivity from surface water treated with [^{14}C]-Eugenol at the high application rate

Sampling Interval	% Applied Radioactivity							
	0	1	1.25	2	7	14	30	62
Surface Water (Ext 1 or Ext 1 SS1)	98.8	92.7	90.2	80.4	7.1	5.2	NA	38.1
Surface Water (Ext 1 – acidified)	NA	NA	NA	NA	39.8	32.9	31.4	NA
Surface water Total	98.8	92.7	90.2	80.4	46.8	38.1	31.4	38.1
Foam Bung	NA	0.4	0.4	0.4	0.3	0.5	0.1	0.1
Sodium hydroxide Traps (evolved $^{14}CO_2$)	-	2.2	3.3	9.8	46.1	52.3	56.2	59.4
Mass Balance	98.8	95.3	93.9	90.6	93.2	90.8	87.7	97.6
Overall Mean ±SD	93.5 ±4.0							

NA Not applicable; SS1 Sub-sample 1; ND Not detected (< 0.1% AR); SD Standard deviation

Table 8.2.2.2-6: Mean Distribution and recovery of radioactivity from sterilised surface water treated with [^{14}C]-Eugenol at the high application rate

Sampling Interval	% Applied Radioactivity
	62
Surface water Total	81.6
Foam Bung	9.1
Sodium hydroxide Traps (evolved $^{14}CO_2$)	1.6
Mass Balance	92.3

Table 8.2.2.2-7: Mean Distribution and recovery of radioactivity from reference / solvent control vessels treated with [^{14}C]-benzoate

Sampling Interval	% Applied Radioactivity			
	7	14	30	62
Surface water Total	-	-	-	13.8
Sodium hydroxide Traps (evolved $^{14}CO_2$)	76.4	6.9	4.7	3.7
Mineralisation[1] (Alkaline traps)	76.4	83.3	88.0	91.7
Mass Balance[2]	-	-	-	105.4

- Not applicable
1. Cumulative total of radioactivity recovered in alkaline traps over time
2. Summation of Extract 1 and Mineralisation at 62 DAT

B Mineralisation

The radioactivity recovered in the alkaline traps was confirmed to be evolved $^{14}CO_2$ as < 0.2% was present in the supernatant following precipitation of selected 2M sodium hydroxide samples with $BaCl_2$.

At 62 DAT, levels of evolved $^{14}CO_2$ recovered in the alkaline traps reached maximum mean values of 69.1% and 59.4% (non-sterile) at the low and high application rates. As a result, mineralisation to carbon dioxide is considered a major degradation pathway of [^{14}C]-eugenol in viable surface water, where the rate of mineralisation was slower at the high application rate by the end of the incubation period.

Conversely, the rate of mineralisation was significantly slower in the sterile vessels. 1.6 % was detected in the alkaline traps at 62 DAT, showing that the mineralisation of [^{14}C]-eugenol in surface water is largely attributable to biotic activity. Selected samples were acidified to check for the presence of dissolved carbon dioxide in the water. After acidification there was ≤ 0.5% AR present in the sodium hydroxide traps, therefore, the amount of dissolved carbon dioxide in the water was no significant.

C Characterisation

Under non-sterile conditions, levels of eugenol in the surface water decreased rapidly and eugenol was not detected after 14 DAT for the remainder of the incubation period. Eugenol decreased from 92.8% at 0 DAT to 6.4% at 5 DAT at the low application rate and from 95.7% at 0 DAT to < 1% by 7 DAT at the high application rate.
cis-Isoeugenol was only present in the water samples where eugenol was detected and reached maximum levels of 3.3% at 5 DAT at the high application rate. *cis*-Isoeugenol was not detected at 14 DAT and for the remainder of the incubation period at both application rates.
Impurity 1 was only present in the water samples where eugenol was detected and reached maximum levels of 6.9% at 5 DAT at the low application rate. Impurity 1 was not detected at 14 DAT and for the remainder of the 62 day incubation period at both application rates.
Decreases of eugenol in the water were accompanied by increasing levels of major degradation products. At the high application rate, Unknown 1 reached 9.0% by 62 DAT. At the low application rate, Unknown 2 increased to 23.4% at 14 DAT, decreasing to 9.4% by 62 DAT. At the high application rate, Unknown 2 reached 23.6% by 62 DAT. Transient degradations products, Unknown 3 and Unknown 4 were only detected at the high application rate, where the maximum level of Unknown 3 (10.7%) was reached at 2 DAT, decreasing to 2.5% by 14 DAT and Unknown 4 was present at 5.6% at 7 DAT declining to < 1 % for the remainder of the incubation period.

Table 8.2.2.2-8: Mean [^{14}C]-Residues present in surface water treated with [^{14}C]-Eugenol at the low application rate

	% Applied Radioactivity							
	0	0.25	0.75	1	5	14	30	62
Eugenol	92.8	88.5	77.7	73.3	6.4	ND	ND	ND
Cis-Isoeugenol	1.2	0.9	1.3	2.2	2.9	ND	ND	ND
Impurity 1	1.0	0.5	0.8	0.3	6.9	ND	ND	ND
Unk 1	ND	ND	ND	ND	2.1	1.0	ND	ND
Unk 2	ND	0.7	1.2	1.3	14.0	23.4	8.9	9.4
Other unknowns	0.6	2.5	2.3	3.8	8.6	5.4	15.1	14.6
Unresolved background	0.4	0.5	1.2	0.4	0.6	0.3	0.3	0.3
Total extractables	96.0	93.5	84.5	81.2	41.7	30.1	24.3	24.2

ND Not detectable (<0.1%AR)

Table 8.2.2.2-9: Mean [^{14}C]-Residues present in surface water treated with [^{14}C]-Eugenol at the high application rate

	% Applied Radioactivity							
	0	1.0	1.25	2	7	14	30	62
Eugenol	95.7	83.8	73.9	46.0	0.8	ND	ND	ND
Cis-Isoeugenol	2.1	1.2	1.8	1.6	3.3	ND	ND	ND
Impurity 1	0.7	0.3	0.6	0.9	0.5	ND	ND	ND
Unk 1	ND	3.2	4.2	8.3	2.3	3.0	3.7	9.0
Unk 2	ND	1.9	2.5	7.8	18.3	23.1	21.1	23.6
Unk 3	ND	1.5	2.6	10.7	2.5	2.5	ND	2.2
Unk 4	ND	ND	ND	ND	5.6	0.6	ND	ND
Other unknowns	ND	0.4	3.2	4.8	13.1	8.5	6.3	3.0
Unresolved background	0.1	0.3	1.3	0.3	0.4	0.3	0.3	0.3
Total extractables	98.8	92.7	90.2	80.4	46.8	38.1	31.4	38.1

ND Not detectable
1. (<0.1%AR) Surface water (Extract 1 or Extract 1 SS1) retention time
2. Acidified surface water (Extract 1 acidified) retention time

Table 8.2.2.2-10: Mean [^{14}C]-Residues present in sterilised surface water treated with [^{14}C]-Eugenol at the high application rate

Sampling Interval	% Applied Radioactivity
	62
Eugenol	9.6

Cis-Isoeugenol	0.4
Impurity 1	6.1
Unk 1	1.1
Unk 2	32.8
Unk 3	14.9
Other unknowns	15.7
Unresolved background	1.0
Total extractables	81.6

D Kinetics

Concentrations of eugenol between 0 and 62 DAT were fitted to SFO kinetics. The degradation of eugenol occurred quickly (DT_{50} values of 1.8 and 2.0 days) at low and high application rates under non-sterile conditions.

Incubation Group		Degradation Rate (days)		χ^2	r^2
ID	Conc.	DT_{50}	DT_{90}		
D	Low	1.82	6.03	6.94	0.9807
E	High	2.04	6.79	11.5	0.9497

III. CONCLUSION

Eugenol degraded quickly in natural surface water under non-sterile conditions, with DT_{50} values of 1.8 and 2.0 days at the low and high application rate, respectively. No detectable levels of eugenol were observed by 14 DAT at both application rates. Degradation under sterile conditions was slower, with eugenol present at 9.6% at the end of the 62 day incubation period.

Degradation resulted in the formation of two major degradation products (> 10% AR). Unknown 2, (low and high application rate) and Unknown 3 (high application rate). Although attempts were made, it was not possible to characterise and identify the unknown degradation products by LC-MS/MS due to the nature of the metabolites, the matrix and compound ionisability.

Mineralisation was observed as the major route of degradation under non-sterile conditions, reaching maximum levels of ≥ 59.4% AR at 62 DAT. Under sterile conditions, mineralisation was a minor route of degradation (1.6 % AR at 62 DAT).

3. Assessment and conclusion

Assessment and conclusion by applicant:

This study was performed well and shows that eugenol mineralises to carbon dioxide in surface water with a DT50 of 1.8-2.0 days with carbon dioxide reaching a maximum of 59.4% at 62 days. This shows that eugenol is not persistent in water systems.

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 mineralisation and degradation rate of eugenol in a surface water test system (Fountains Abbey) 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 (95 µg/L).

The overall total radioactive recovery was ≥ 90% AR at both application rates for the non-sterile and sterile incubation groups. In surface water treated with [14C]-Eugenol at the low application rate, the individual recoveries for several sampling points fell below 90%AR. In surface water treated with [14C]-Eugenol at the high application rate, only one individual recovery fell below the 90% AR% (87.7% AR, 30 DAT). However, according to the OCD 309, the range of recovery between 90-110 % AR, should be interpreted as targets and should not be used as criteria for acceptance of the test.

Regarding to the other quality criteria, the sensitivity of the analytical method was considered acceptable (the limit of detection was *ca* 0.3% AR). Radiochemical purity chromatograms were run in duplicate to confirm the repeatability of the HPLC method, it is noted that OECD 309 recommends to use up to five replicates for this. Two replicates per sampling interval were also used to conduct the experiment.

According to the results, mineralisation to carbon dioxide was the major degradation route, with maximum levels reaching ≥ 59.4% AR in the sodium hydroxide traps at 62 DAT. Mineralisation was not a significant degradation product under sterile conditions, where 1.6% AR was detected in the sodium hydroxide traps at 62 DAT.

Eugenol decreased from 92.8% at 0 DAT to 6.4% at 5 DAT at the low application rate and from 95.7% at 0 DAT to < 1% by 7 DAT at the high application rate.

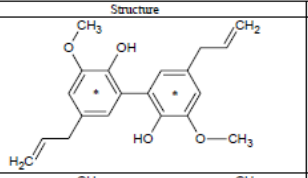
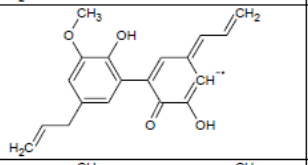
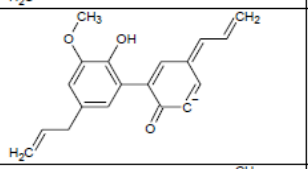
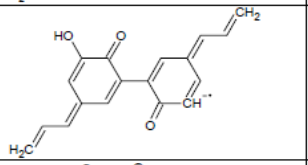
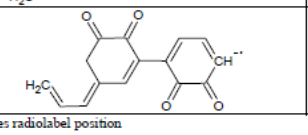
Major degradation products which accounted for more than 10% AR were found: unknown 2 and unknown 3, this last one presents only at the high application rate. The unknown 1 reached a maximum value of 9 %AR at high application rate (62 DAT). Since the plateau was not reached at the end of the experiment, it is considered that this metabolite should be included as relevant metabolite. Unknown 4, only present at the high application rate did not exceed 5.6% AR. Other unknown degradation products comprised of several compounds, with no single component representing more than 6.4% AR.

These ‘unknowns’ could not be characterized by LC-MS/MS due to the nature of the metabolites, the matrix and compound ionisability. However, applicant should provide an extension on the argumentation to not determine the degradation pathway.

cis-Isoeugenol and Impurity 1 were present in the application solution at the time of treatment and their identities confirmed by HPLC and LC-MS/MS, respectively. *cis*-Isoeugenol and Impurity 1 were not considered significant degradation products of eugenol (maximum levels < 7%) in surface water.

Impurity 1 was tentatively identified as dieugenol in the representative application solution. Impurity 1 was observed at a retention time of *ca* 38.3 minutes in the radiochromatogram. The full scan spectrum for the peak showed the molecular ions ([M-H]- and [14C3-M-H]-) measured as *m/z* 325.1445 and 331.1546 respectively with the isotope cluster which is consistent with application solution. The intensity of the 14C labelled peaks approximately doubled relative to the non-labelled de-protonated molecular ion (from *ca* 10% intensity to *ca* 20% intensity); this change in relative abundance suggests dimerization of the radiolabelled eugenol species.

Postulated structure of Impurity 1 and Fragments

Structure	Observed Ion (*m/z*)
	325.1445 [M-H]⁻ 331.1546 ([^{14}C-M-H])
	310.1215
	293.1184
	278.0950
	254.0588 (260.0687)

*- Denotes radiolabel position

The degradation rates of eugenol, the parameters used to calculate them and parameter statistics were determined using CAKE version 2.0 software. The provided kinetic analysis was considered acceptable by RMS. Eugenol degraded quickly in natural surface water under non-sterile conditions, with DT50 values of 1.8 and 2.0 days at the low and high application rate, respectively.

Outcome and conclusion of the study:
The study is considered acceptable. However, more information regarding to the inability to determine the identity of the reaction products (unknown 1, 2 and 3) is needed.
Mineralisation was observed as the major route of degradation under non-sterile conditions.
The following comment was added by applicant during the co-RMS-applicant´s initial revision: *The CRO will be contacted for more information on the determination of the identity of the reaction products.*

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.**
Applicant to address the route and rate of degradation of eugenol in natural sediment water systems. Any information provided would need to demonstrate methyl-eugenol is not formed.

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

For information only, the following paper has been summarised on the environmental fate of methyleugenol in water

1. **Information on the study**

Data point	CA 7.2.2.3/01
Report author	T.N.Shaver and D.L.Bull
Report year	1980

Report title	Environmental Fate of Methyl Eugenol
Report No.	NA
Document No.	Environ. Contam. Toxicol. 24,619-626 (1980)
Guidelines followed in study	None
Deviations from current test guideline	No guideline followed. No mass balance data reported.
Previous evaluation	No
GLP/Officially recognised testing facilities	No
Acceptability/Reliability	No, supplementary data only

2. Full summary

The environmental fate of methyleugenol was examined in water. 100 mL water was treated with 10 mg of radiolabelled methyleugenol and the samples were stored in an environmental chamber at 32°C or 22°C. Three samples were taken at 0, 24, 48, 72 and 96 hours.
Water was extracted 3 times with ca 50 ml portions of dichloromethane. Combined extracts were concentrated and analysed by GC. Analyses was performed with gas chromatography (GC). 2m x 2 mm ID glass column packed with 1.5% SP2250 + 1.95% SP2401 on 10-Supelcoport at a column temperature of 140°C.
Aqueous layer was analysed for residual methyleugenol by high performance liquid chromatography with a 24 cm x 0.5 mm, µ-Bondapak/C-18 column and methanol-water (70:30) as solvent
Methyleugenol dissipated rapidly from water with 98% of the material being lost within 96 hours at 32°C, and 77% being lost after 96 hours at 22°C. TLC analyses revealed methyleugenol as the only radiolabelled compound.
The half-life of methyleugenol was calculated to be 6 and 34 hours at 32 and 22°C respectively.

3. Assessment and conclusions

Assessment and conclusion by applicant:
This data is shown for information only. Methyleugenol was not seen in the aqueous studies previously submitted and this data is shown for information only and indicates that any methyleugenol that may be formed in water will dissipate rapidly with a half-life of <2 day.

Assessment and conclusion by RMS:
This study has been included for completeness.

Outcome and conclusion of the study:
Supplementary information.

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**

No data submitted.

B.8.3. FATE AND BEHAVIOUR IN AIR

Adequate data to assess the route and rate of degradation of eugenol in air were evaluated during the first EU review and no further data are considered necessary.
The vapour pressure of eugenol is 2.7 Pa at 20°C and the water solubility at 20°C is 1.85 mg/l. A Henry's Law constant of 0.24 $Pa.mol^{-1}.m^3$ was derived. A DT_{50} of 1.975 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 eugenol degradation in air

Process	Rate constant (s^{-1})	DT_{50} in the troposphere (12 hr sunlight, days)
Direct photodegradation	-	-
Reaction with hydroxy radicals	64.9894×10^{-12}	1.975
Reaction with $O(^3P)$ species	1.2×10^{-17}	22.92
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	Eugenol: Estimated of Atmospheric Oxidation Rate
Report No.	EDR/02/01c
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 eugenol 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 eugenol due to gas phase hydroxyl radical reaction was 0.165 days (1.975 hours) under environmental conditions with a diurnal cycle of 12 hours. The estimated half-life of eugenol due to reaction with ozone was 0.955 days (22.920 hours). On this basis, it can be concluded that eugenol 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 eugenol 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: O(c1c(O)ccc(c1)CC=C)C

Molecular formula: $C_{10}H_{12}O_2$

II. RESULTS

AOPWIN estimated the following rate constants for the various processes.

Hydrogen abstraction	1.7636 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	26.3 x 10^{-12} cm^3 $molecule^{-1}$ sec^{-1}
Addition to aromatic rings	36.7858 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 1.975 hours, equivalent to 0.165 days under environmental conditions with a diurnal cycle of 12 hours.
The half-life due to reaction with ozone is therefore calculated to be 22.92 hours, equivalent to 0.955 days under environmental conditions.

III. CONCLUSIONS

On this basis, it is concluded that eugenol 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 eugenol degrades quickly in the atmosphere with DT50 of 0.165 days with hydroxyl radicals and 0.955 days with reaction to ozone.

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 0.165 days.
Outcome and conclusion of the study:
Accepted.

1. Information on the study

Data point	CA 7.3.1/02
Report author	Y. Sun, X. Chen, L. Liu, F. Xu, X. Zhang
Report year	2021
Report title	Mechanisms and kinetics studies of the atmospheric oxidation of eugenol by hydroxyl radicals and ozone molecules
Report No.	-
Document No.	Science of the Total Environment 770 (2021) 145203
Guidelines followed in study	NA
Deviations from current test guideline	
Previous evaluation	No
GLP/Officially recognised testing facilities	No
Acceptability/Reliability	Supplementary information only

ABSTRACT (copied from original literature)
Eugenol is a representative methoxyphenol derived from the pyrolysis of lignin containing a branched alkene group. Its concentration in the atmosphere is equivalent to guaiacol and syringol. In this present paper, the gas phase reaction mechanisms and kinetic parameters of eugenol with hydroxyl radicals (OH) and ozone molecules (O_3) were calculated at the M06-2×/6-311+G(3df,2p)//M06-2×/6-311+G(d,p) level. There are two distinct reaction types between eugenol and OH. In particular, Path2 is most favorable in the OH additions, whereas IM16 is most advantageous in H atom abstraction pathways. OH additions have more advantages than H abstraction reactions. Thus, the comprehensive and detailed

reaction schemes for the further reactions of IM2 were presented. The main products generated by IM2 are methyl (Z)-3-(2-formylpenta-1,4-dien-1-yl)-2-hydroxyoxirane-2-carboxylate (P2B-4), 2-methoxy-2-oxoacetic acid (P2B-10), 2-allylmalealdehyde (P2B-11) and other carbonyl or carboxyl compounds. As for the reaction of eugenol with O_3, the cycloaddition reactions and subsequent oxidative degradation processes were also explored, which yielded the most dominant product 2-(4-hydroxy-3- methoxyphenyl) acetaldehyde (P8-1). The reaction constants of the primary reactions for eugenol with OH and O_3 under the temperature range of 225– 375 K were successively calculated by POLYRATE and MESMER program. At 298 K and 1 atm, the respective rate coefficients are 5.91×10^{-11} and 5.48×10^{-16} cm^3 $molecule^{-1}$ s^{-1} and the corresponding atmospheric lifetimes are 4.70 h and 0.72 h. The short lifetimes suggest that once eugenol enters the atmosphere, it is likely to be rapidly degraded. This work aims to provide theoretical guidance for the photochemical reaction mechanisms of eugenol with OH and O_3, and present a reference for more experimental researches.

CONCLUSION

In this paper, the gas phase reaction mechanisms of eugenol with OH and O_3 were investigated at the M06-2×/6-311+G(3df,2p)//M06-2×/ 6-311+G(d,p) level. Based on the thermodynamic data, kinetic calculations were performed with POLYRATE and MESMER program. Some valuable conclusions are summarized as follows:

(1) For the primary reactions of eugenol with OH, IM2 is most favorable among all the OH-adducts and IM16 presents most advantageous during all the H atom abstraction reactions. Most importantly, OH radical additions are easier to occur than H abstraction pathways. Thus, intermediate IM2 was selected as the representative to discuss the subsequent degradation processes in the atmosphere. The main products generated by IM2 are methyl (Z)-3-(2-formylpenta-1,4-dien-1-yl)-2-hydroxyoxirane -2-carboxylate (P2B-4), 2-methoxy-2-oxoacetic acid (P2B-10), 2-allylmalealdehyde (P2B-11) and other carbonyl or carboxyl compounds.

(2) Seven elementary addition pathways for ozonolysis of eugenol were considered. IM1 is the most predominant POZ. Particularly, IM8 is more favorable than other Criegee intermediates, which could proceed isomerization or experience bimolecular reactions with small molecules such as NOx and COx. It's remarkable that 2- (4-hydroxy-3-methoxyphenyl) acetaldehyde (P8-1) is the most main product in the further reactions of IM8.

(3) The total and individual rate coefficients were calculated in the range of 225– 375 K and 1 atm. In particular, the total reaction constant of eugenol with OH is 5.91×10^{-11} cm^3 $molecule^{-1}$ s^{-1} under 298 K, which is consistent with the experimental data. As for eugenol with O_3, the rate coefficient is 5.48×10^{-16} cm^3 $molecule^{-1}$ s^{-1} , which is supported by the investigated values of styrene with ozone. Additionally, the atmospheric lifetimes of eugenol with OH and O_3 are 4.70 h and 0.72 h, respectively. According to the above analysis, the reaction constant of eugenol with OH is faster than that of O_3. And once eugenol enters the atmosphere, it may be quickly oxidized and degraded by oxidants (OH and O_3) in the troposphere, which could indicate that OH radicals and O_3 molecules play a certain role in the atmospheric reactivity.

Assessment and conclusion by RMS:
This study from the open literature was identified as a relevant study included after detailed assessment of full-text documents for relevance and found to be not reliable
Outcome and conclusion of the study:
Not GLP study.
Supplementary information

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 0.165 hours. This indicates that any volatilised eugenol will be extremely short-lived in the atmosphere. Any eugenol 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 0.165 hours. This indicated that any volatilised eugenol will be extremely short-lived in the atmosphere, therefore there be no local or global effects. Any eugenol 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:	Eugenol
Groundwater:	Eugenol
Surface water:	Eugenol
Sediment:	Eugenol
Air:	Eugenol

B.8.4.2. **Definition of the residue for monitoring**

Soil:	Eugenol
Groundwater:	Eugenol
Surface water:	Eugenol
Sediment:	Eugenol
Air:	Eugenol

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 eugenol 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 eugenol, 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 eugenol 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 eugenol and between 1/1/1995 and 16/02/2021 for methyleugenol.

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 Appendix I, 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	**Eugenol**			
	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)	28 270			
Total number of documents retrieved (without duplicates) after focused search strategy (combining terms for active substance and specific data requirements)	1191	487	733	1865
Number of publications excluded after rapid assessment for relevance (non-relevant, excluded literature)	1166	473	730	1756
Number of publications for which detailed assessment of full-text required after rapid assessment for relevance	25	13	3	109
Number of publications excluded from further consideration after detailed assessment for relevance (non-relevant, excluded literature)	13	3	1	66
Number of publications considered relevant following the two-step process (i.e. after detailed assessment of full-text)	12	10	2	**43**

For environmental fate 2 publications, respectively, considered relevant following the two-step process are summarized in this document.

Study selection process	**Methyl-eugenol**			
	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)	4 133			

Total number of documents retrieved (without duplicates) after focused search strategy (combining terms for active substance and specific data requirements)	191	102	147	329
Number of publications excluded after rapid assessment for relevance (non-relevant, excluded literature)	176	98	147	275
Number of publications for which detailed assessment of full-text required after rapid assessment for relevance	15	4*	0	54
Number of publications excluded from further consideration after detailed assessment for relevance (non-relevant, excluded literature)	1	1*	0	50
Number of publications considered relevant following the two-step process (i.e. after detailed assessment of full-text)	14	3*	0	**4**

* The same papers were also identified in the eugenol literature search.

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 Eugenol dealing with toxicological and toxicokinetic studies, operator exposure, residues, fate and behavior 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 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.

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 below.

The literature search was conducted on 2nd July 2020 and 16 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 eugenol and in the last 25 years (from 1/1/1995 to 16/02/2021) for methyleugenol.

B.8.6.1.2.1. Bibliographic database 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. "eugenol" in addition to common variants such as eugenic acid, and "clove oil". Where the number of records for, say, eugenol 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 8.6.1.2.2-1a: List search terms used to identify all literature related to the active substance (eugenol), its metabolites and plant protection products containing the active substance

Common name	Eugenol Eugenic acid Eugenol methyl ether Allylguaiacol Eugenic acid Caryophyllic acid p-Allylguaiacol p-Eugenol 1,3,4-Eugenol allylveratrol Caryophyllic acid Clove oil Nutmeg oil Cinnamon oil Basil oil Bay leaf Phenol, 2-methoxy-4-(2-propenyl)-
Chemical name (IUPAC and CA)	2-methoxy-4-prop-2-enylphenol 2-methoxy-4-(2-propen-1-yl)phenol 2-Methoxy-4-(2-propenyl)phenol 2-Methoxy-4-allylphenol 4-Allyl-2-methoxyphenol 4-Allylguaiacol 1-Hydroxy-2-methoxy-4-allylbenzene 1-Hydroxy-2-methoxy-4-prop-2-enylbenzene 1-Hydroxy-2-methoxy-4-propenylbenzene 2-Methoxy-1-hydroxy-4-allylbenzene 2-Methoxy-4-(2-propen-1-yl)phenol 2-Methoxy-4-allylphenol 2-methoxy-4-(2-propenyl)phenol 2-Methoxy-4-prop-2-enylphenol 2-Metoksy-4-allilofenol 4-Allyl-1-hydroxy-2-methoxybenzene 4-Allyl-2-methoxyphenol 4-Allylcatechol 2-methyl ether 4-Allylcatechol-2-methyl ether 4-Allylguaiacol 4-Hydroxy-3-methoxy-1-allylbenzene 4-Hydroxy-3-methoxyallylbenzene 5-Allylguaiacol 1-Allyl-3-methoxy-4-hydroxybenzene
Metabolite(s)	-
Product name	Mevalone, Andromeda, 3Logy, ES-00108, Trigemol, Cagenoleta, Eugeti
CAS No.	97-53-0
EC No.	202-589-1

Table 8.6.1.2.2-1b: List search terms used to identify all literature related to the compound methyleugenol

Common name	Methyl Eugenol Methyl eugenol Methyleugenol methyl-eugenol ME Benzene, 1,2-dimethoxy-4-(2-propenyl)- O-Methyl eugenol O-Methyleugenol
Chemical name (IUPAC and CA)	1,2-dimethoxy-4-prop-2-enylbenzene 1,2-Dimethoxy-4-(2-propen-1-yl)benzene 1,2-Dimethoxy-4-(2-propenyl)benzene 1,2-Dimethoxy-4-allylbenzene 1,3,4-Eugenol methyl ether 1-(3,4-Dimethoxyphenyl)-2-propene 1-Allyl-3,4-dimethoxybenzene 3,4-Dimethoxyallylbenzene 4-Allyl-1,2-dimethoxybenzene 4-Allylveratrole Benzene, 1,2-dimethoxy-4-(2-propenyl)- Benzene, 4-allyl-1,2-dimethoxy- 4-Allyl-1,2-dimethoxybenzene
Metabolite(s)	-
Product name	Mevalone
CAS No.	93-15-2
EC No.	202-223-0

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 8.6.1.2.2-2 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 for eugenol and KCA 9.3/02 for methyleugenol.
The peer-reviewed literature search strategy highlighted 28 270 documents of potential interest for eugenol and 4 133 documents of potential interest for methyleugenol 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)

Databases (through online STN)	Number of hits Eugenol
1. AGRICOLA	97
2. AGRIS (FAO)	423
3. PASCAL	508
4. PubMed MEDLINE (including TOXNET)	4 509
5. Science Direct	10 512
6. Springer	7 801
7. Wiley	4 420
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)	**28 270**

Table 9.11.1.3-2: Numbers of records (before removing duplicates) for the search (based on search terms according to Table 8.6.1.2.2-1)

Databases (through online STN)	Number of hits Methyleugenol
1. AGRICOLA	18
2. AGRIS (FAO)	128
3. PASCAL	-
4. PubMed MEDLINE (including TOXNET)	500
5. Science Direct	1 599
6. Springer	900
7. Wiley	988
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)	**4 133**

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 9.11.1.3-3: Number of documents identified as potentially relevant after text mining and removal of duplicates

All databases	Number of hits* Eugenol	Number of hits* Methyleugenol
Mammalian Toxicity	1 191	191
Residues	487	102
Environmental fate and behaviour	733	147
Ecotox	**1 865**	**329**

*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.

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.

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

Study selection process	Eugenol			
	Toxicology (including human exposure)	Residues	Environmental fate and behaviour	Ecotoxicology
Number of publications excluded after rapid assessment for relevance (non-relevant, excluded literature)	1166	474	730	1756
Number of publications for which detailed assessment of full-text required after rapid assessment for relevance	25	13	3	109

Table 8.6.1.4.1-2: Numbers of publications excluded/identified after rapid assessment for relevance for methyleugenol

Study selection process	Methyleugenol			
	Toxicology (including human exposure)	Residues	Environmental fate and behaviour	Ecotoxicology

Number of publications excluded after rapid assessment for relevance (non-relevant, excluded literature)	176	98	147	275
Number of publications for which detailed assessment of full-text required after rapid assessment for relevance	15	4*	0	54

* The same papers were also identified in the eugenol literature search.

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.

Table 8.6.1.4.2-1: Criteria for Relevance – Detailed Screening

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

Table 8.6.1.4.2-2: Numbers of publications excluded/identified after detailed assessment for relevance for eugenol

Study selection process	Eugenol			
	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)	13	3	1	66
Number of publications considered relevant following the two-step process (i.e. after detailed assessment of full-text)	12	10	2	**43**

Table 8.6.1.4.2-3: Numbers of publications excluded/identified after detailed assessment for relevance for methyleugenol

Study selection process	Methyleugenol

	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)	1	1*	NA**	50
Number of publications considered relevant following the two-step process (i.e. after detailed assessment of full-text)	**14**	**3***	**0**	**4**

* The same papers were also identified in the eugenol literature search.
** Not applicable as none of the publications were considered as relevant following rapid assessment of the title or abstract.

All publications excluded following the detailed assessment are presented in the tables below, with a reason for not including in the dossier.

Table 8.6.1.4.2-4: Publications excluded from the risk assessment after detailed assessment of full-text documents – results for eugenol

Author(s)	Year	Title	Source	Reason for not including in dossier (based on relevance criteria)	RMS conclusion
ENVIRONMENTAL FATE AND BEHAVIOUR					
Chaiane Regina Rech a; Kelly Cristina da Silva Brabes b; Bárbara Ellen Bagnara e Silva a; Paulo Rodrigo Stival Bittencourt c; Marivane Turim Koschevic a; Tayla Fernanda Serantoni da Silveira d; Marco Antonio Utrera Martines d; Thiago Caon e; Silvia Maria Martelli b (No 5b in CA 9.4/02a)	2020	Biodegradation of eugenol-loaded polyhydroxybutyrate films in different soil types.	Case Studies in Chemical and Environmental Engineering Volume 2 September 2020 100014	Not a PPP and no useable efate endpoints	Not relevant to fate endpoint

Table 8.6.1.4.2-4: Publications excluded from the risk assessment after detailed assessment of full-text documents – results for methyleugenol

Author(s)	Year	Title	Source	Reason for not including in dossier (based on relevance criteria)	RMS conclusion
ENVIRONMENTAL FATE AND BEHAVIOUR					
Not applicable as none of the publications were considered as relevant following rapid assessment of the title or abstract.					

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.

Eugenol
For environmental fate: 2 publications considered relevant following the two-step process are summarized in respective MCAs.

Methyleugenol
For environmental fate: no publication has been considered as relevant following the two-step process.

Table 8.6.1.4.2-5: Relevant studies included in the supplementary dossier after detailed assessment of full-text documents for relevance in the Fate Section for eugenol

Data requirement (indicated by the corresponding CA and CP data point)	Author(s)	Year	Title	Source
CA 7.2.1.2/02	Ye Z., Zhuang Y., Chen Y., Zhao Z., Ma S., Huang H., Chen Y., Ge X.	2020	Aqueous-phase oxidation of three phenolic compound by hydroxyl radical: Insight into secondary organic aerosol formation yields, mechanisms, products and optical properties	Atmospheric Environment 223 (2020) 117240
CA 7.3.1/02	Xiaochen Zhang a; Yanhui Sun a b; Xiaoxiao Chen a; Lin Liu a; Fei Xu c	2021	Mechanisms and kinetics studies of the atmospheric oxidation of eugenol by hydroxyl radicals and ozone molecules.	Science of The Total Environment Volume 770 20 May 2021 145203

Table 8.6.1.4.2-6: Relevant studies included in the supplementary dossier after detailed assessment of full-text documents for relevance: sorted by data requirement(s) for methyleugenol in the Fate Section

Data requirement (indicated by the corresponding CA and CP data point)	Author(s)	Year	Title	Source
Not applicable as none of the publications were considered as relevant following rapid assessment of the title or abstract.				

B.8.6.1.5. Conclusion

In this literature search for the active substance eugenol and methyleugenol, 28 270 references (for eugenol) and 4 133 references (for methyleugenol) 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 Eugenol	**Number of publications considered to be relevant Methyleugenol**
Toxicology, including human exposure	12	14
Residues	10	3*
Environmental fate	2	0
Ecotoxicology	**43**	**4**

* The 3 papers were also identified in the eugenol literature search

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.

Eugenol
For environmental fate: 2 publications considered relevant following the two-step process are summarized in respective MCAs.

Methyleugenol
For environmental fate: no publication has been considered as relevant following the two-step process.

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 literature search was done considering eugenol and methyleugenol.
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, 2 publications with eugenol were considered relevant and therefore summarized under their corresponding data requirement point.

-Aqueous-phase oxidation of three phenolic compound by hydroxyl radical: Insight into secondary organic aerosol formation yields, mechanisms, products and optical properties. Ye Z., et.al. 2020.

-Mechanisms and kinetics studies of the atmospheric oxidation of eugenol by hydroxyl radicals and ozone molecules. Xiaochen Zhang a., 2021.

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** **Report No.** **Document No.** **Source (where different from company)** **GLP/ Officially recognised testing facilities[2,3]** **Published or not**	**Vertebrate study Y/N**	**Data protection claimed Y/N**	**Justification if data protection is claimed**	**Owner**	**Previously used[1]** **Y/N** **If yes, for which data point?**
CA 7.1.1.1/01 CA 7.1.2.1.1/01	A, Jones	2015a	Eugenol: Aerobic soil metabolism Envigo Study No. PIF0002 GLP Unpublished	N	N	-	Eden Research plc	Y In EFSA Confirmatory Data 2016
CA 7.1.1.3/01	D. Kelly	2021a	Eugenol - Soil Photolysis of [^{14}C]-Eugenol Smithers ERS Ltd 3203050 GLP Unpublished	N	Y	Study not previously submitted	Eden Research plc	N
CA 7.1.2.1.2/01 CA 7.2.2.3/01	T.N.Shaver and D.L.Bull	1980	Environmental Fate of Methyl Eugenol Environ. Contam. Toxicol. 24,619-626 (1980) Not GLP Published Paper	N	N	-	Public	N
CA 7.1.3.1.1/01	A.Jones	2015b	Eugenol: Adsorption/desorption in Five soils Envigo Study No. PIF0003 GLP Unpublished	N	N	-	Eden Research plc	Y In EFSA Confirmatory Data 2016
CA 7.1.3.1.1/02	D. Kelly	2021b	[^{14}C]-Eugenol: Adsorption/Desorption in Soil Smithers ERS Ltd	N	Y	Study not previously submitted	Eden Research plc	N

			3202786 GLP Not published					
CA 7.2.1.1/01	D. Kelly	2021c	Eugenol – Hydrolysis of [^{14}C]-Eugenol in Sterile Buffer at pH 4,7 and 9 Smithers ERS Ltd 3202784 GLP Not published	N	Y	Study not previously submitted	Eden Research plc	N
CA 7.2.1.2/01	P. Yeomans	2021	Eugenol – Photolysis of [^{14}C]-Eugenol in Sterile Aqueous Solution Smithers ERS Ltd 3202785 GLP Not published	N	Y	Study not previously submitted	Eden Research plc	N
CA 7.2.1.2/02	Z. Ye, Y. Zhuang, Y. Chen, Z. Zhao, S. Ma, H. Huang, Y. Chen, X. Ge	2020	Aqueous-phase oxidation of three phenolic compound by hydroxyl radical: Insight into secondary organic aerosol formation yields, mechanisms, products and optical properties Atmospheric Environment 223 (2020) 117240 Non-GLP Published Paper	N	N	-	Public	N
CA 7.2.2.1/01	B. Seyfried	2008	Eugenol: Ready Biodegradability in a Manometric Respirometry Test RCC Study number B65160 GLP Not published	N	N	-	Eden Research plc	Y In DAR 2011
CA 7.2.2.2/01	L. Simmonds	2021	[^{14}C]-Eugenol: Aerobic Mineralisation in Surface Water Smithers ERS Ltd 3202787 GLP Not Published	N	Y	Study not previously submitted	Eden Research plc	N
CA 7.3.1/01	V. Thomas	2007	Eugenol: Estimated of Atmospheric Oxidation Rate EDR/02/01c No GLP required Not published	N	N	-	Eden Research plc	Y In DAR 2011
CA 7.2.1/02	Y. Sun, X. Chen, L. Liu,	2021	Mechanisms and kinetics studies of the atmospheric oxidation	N	N	-	Public	N

	F. Xu, X. Zhang		of eugenol by hydroxyl radicals and ozone molecules Science of the Total Environment 770 (2021) 145203 Non GLP Published					

[1] In order to facilitate the compilation of the final list of the tests and studies relied upon and the corresponding data protection, indicate whether the study was used in the previous DAR/RAR or, when the information is available, whether the study was already submitted in the framework of national authorisations.
[2] See Art.3 of Annex of Regulation No 283/2013 and 284/2013
[3] The RMS shall check that the GLP statement has been properly signed in the study report, that the study results are properly reported in accordance with GLP standards and following the relevant guidance by OECD on the review of the GLP status of non-clinical safety data (currently under development).