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

GIBBERELLINS (GA4/7)

Volume 3 – B.8 (AS)

Rapporteur Member State: Slovenia
Co-Rapporteur Member State: Slovakia

Version History

When	What
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B.8. ENVIRONMENTAL FATE AND BEHAVIOUR

Introduction

This document has been prepared to evaluate the European Gibberellins Task Force (Valent Biosciences Corporation (Sumitomo Chemical Agro Europe), Fine Agrochemicals Ltd, Globachem NV) application for EU renewal of the Annex I inclusion of active substance gibberellins (GA4, GA7). The document supplements and updates the corresponding Annex B section of the Draft Assessment Report produced during the first review of gibberellins (2005 - 2011).
Gibberelin has been identified as a presumed low-risk active substance in the Commission working document on the AIR-IV renewal programme (SANTE-2016-10616-rev 8). The EU Gibberellin Task Force (EGTF) proposes that Gibberelin is a low risk active substance according to Regulation (EC) 1107/2009 as amended by Commission Regulation 2017/1432.

In this report studies submitted for the first inclusion of gibberellins in Annex I to Directive 91/414/EEC and for the renewal of the approval of gibberellins have been evaluated.

Previous EU assessment

The dossier to support the first inclusion of gibberellins in Annex I to Directive 91/414/EEC was submitted to Hungary as the Rapporteur Member State in June 2005. The Draft Assessment Report is dated July 2006. Final Addendum to Draft Assessment Report, containing all individually submitted addenda on gibberellins, was compiled by EFSA in October 2011.

Structure of this document

Summaries of available data and overall assessments of each sub-section, as well as the exposure assessments, generally are not included in this document. Instead these parts of the assessment are included in Vol. 1, Level 2. The reason behind this structure is to avoid repetition and facilitate revisions of the assessment. As a result, this Annex B only contains the presentation and evaluation of individual study reports on the active substance.

In each section of this document, the following headings (a)-b)) occur:

a) Previous evaluation (2005-2011)
Under this heading study reports submitted for the first inclusion of gibberellins in Annex I to Directive 91/414/EEC are summarised. These studies have been re-evaluated for the purpose of the renewal in the light of current scientific and technical knowledge. The endpoints from the studies were also re-assessed and if considered relevant, re-calculated. However, full details from each study have not been repeated in this DRAR - therefore this DRAR is not a "stand-alone document" and for full reference sometimes the reader needs to consult the DAR (2005-2011).

b) Evaluation of additional data for the purpose of renewal of Annex I inclusion
Under this heading studies submitted prior to Annex I inclusion, but no evaluation of such material was presented in the form of Addenda to the DAR and studies that were submitted to support the application for renewal of Annex I inclusion are evaluated, i.e. new studies.

B.8.1. FATE AND BEHAVIOUR IN SOIL

The proposed use of the active substance gibberellins GA4/7 can potentially lead to amounts reaching soil, therefore the fate and behaviour of the active substance in soil under various conditions has been investigated in laboratory studies according to the data requirements laid down in EC Regulation 283/2013.

In accordance with the data requirements defined by EC Regulation 283/2013, for soil degradation studies included under Point CA 7.1.1, metabolites are considered major if they exceed 10% AR at a single time-point or exceed 5% on two consecutive sampling intervals or exceed 5% and are rising at the end of the study, otherwise metabolites are considered minor.

B.8.1.1. Route and rate of degradation in soil

Studies investigating the route of degradation of the active substance in soil sometimes also include the determination of the rate of degradation of the active substance and potential metabolites. For simplicity for each study investigating both the route and rate of degradation in soil, a single summary is presented and summarised under this Annex point (i.e. Points CA 7.1.2.1.1, CA 7.1.2.1.2, CA 7.1.2.1.3 and CA 7.1.2.1.4 will all refer back to the corresponding location under CA 7.1.1) rather than splitting the summary over two locations.

B.8.1.1.1. Route of degradation in soil

a) Previous evaluation (2005-2011)

Before, no data were available on the aerobic route of degradation of GA4/7 in soil. However since gibberellins in higher plants may be degraded in soil as the plants decay, it is highly likely that any metabolites would already be present in soil from natural sources.

RMS comments and conclusion:

Degradation studies were not conducted by either notifier. Gibberellins GA_4/GA_7 occurs naturally in plant seeds and shoots and as a consequence a continuous but variable background level of gibberellins GA_4/GA_7 will exist in the environment. Known information shows that gibberellins GA_4/GA_7 is readily degraded to carbon dioxide by microbial processes under the conditions of a ready bio-degradation test. In the soil environment, gibberellins GA_4/GA_7 is expected to behave in an identical manner with degradation resulting in rapid mineralisation. Extrapolation of the results from ready biodegradation tests is possible according to the EU Technical Guidance Document on Risk Assessment. This document states that a soil half-life of 30 days is appropriate for readily bio-degradable compounds having a $\log P_{ow}$ of ≤ 4.4 (GA_4 $\log P_{ow} = 2.34$; GA_7 $\log P_{ow} = 2.25$). A figure of 30 days may be considered a conservative estimate.
On the basis that gibberellins GA_4/GA_7 occurs naturally in the environment and will degrade readily to carbon dioxide, it was NOT considered necessary to conduct laboratory or field studies to determine the route and rate of degradation in soil for gibberellins GA_4/GA_7. Furthermore, metabolites formed in soil on the pathway to carbon dioxide may be regarded as transient and will also occur naturally and therefore laboratory or field studies to determine the rate of degradation of these metabolites in soil are not considered necessary.

b) Evaluation of additional data for the purpose of renewal of Annex I inclusion

The route and rate of degradation in soil of the individual components gibberellins GA4 and gibberellins GA7 in the active substance GA4/7 have been investigated in one “new” study (CA 7.1.1.1/01) which has been conducted since the original Annex I inclusion to fully address the requirements of Regulation EC 283/2013. The study is summarised below:

Data point addressed:	CA 7.1.1.1/01
Author(s) (year):	Hurst, L. (2014)
Title:	**[^{14}C]-GA4 and [^{14}C]-GA7: Aerobic Soil Metabolism and Transformation**
Document No:	Study No. 3200135
Testing facility:	Smithers Viscient (ESG) Ltd
Published:	Unpublished
Test guidelines used:	OECD Guideline 307 (Apr 2002)
Deviations:	No relevant deviations.
GLP:	Yes
Test material:	see below
Batch #/purity:	see below
Status:	**New submission**

EXECUTIVE SUMMARY

The route and rate of aerobic degradation of the individual components gibberellins GA4 and gibberellins GA7 of the active substance gibberellins GA4/7 was investigated in four soil types (ranging from loamy sand to clay loam) of varying origin in the dark under laboratory conditions at a temperature of 20°C and moisture content of 100% pF 2. The individual components gibberellins GA4 and gibberellins GA7 degrade extensively in soil. Numerous degradation products were observed but not fully identified. Ultimate degradation led to the formation of un-extracted soil residues and mineralisation to carbon dioxide. For both gibberellins GA4 and gibberellins GA7 the best-fit persistence half-lives were <0.5 days in all soil types.

I. MATERIALS AND METHODS

A. TEST AND REFERENCE MATERIALS

1 Test material 1	Radiolabelled gibberellins GA4 (structure listed in Appendix 1)
Radiolabel position	17-^{14}C-gibberellins GA4 (see Appendix 1)
Radiochemical purity	Details from Certificate of Analysis: >99% by HPLC RCP details determined analytically at the time of treatment: ≥ 95% (as quoted)
Lot/batch no.	TH-090413-^{14}C-GA4, specific activity 6.122 MBq/mg
Stability of test compound	Not stated
Test material 2	Radiolabelled gibberellins GA7 (structure listed in Appendix 1)
Radiolabel position	17-^{14}C-gibberellins GA7 (see Appendix 1)
Radiochemical purity	Details from Certificate of Analysis: >99% by HPLC RCP details determined analytically at the time of treatment: ≥ 95% (as quoted)
Lot/batch no.	TH-090413-^{14}C-GA7, specific activity 6.159 MBq/mg
Stability of test compound	Not stated
2 Reference material 1	Non radiolabelled gibberellins GA4 (note: name used in report gibberellic acid 4)
Purity	100%
Lot/batch no.	91-932-BD, expiry date 11-Jul-2017 (certificate provided)
Reference material 2	Non radiolabelled gibberellins GA7 (note: name used in report gibberellic acid 7)
Purity	92.1%
Lot/batch no.	65753-145, expiry date 11-Jul-2017 (certificate provided)
2 Reference material	Non radiolabelled gibberellins GA4/7 (mix)

Purity	60.4% GA4 and 30.2% GA7
Lot/batch no.	21-973-CD

Test System, soil

The study was conducted using 4 soils (2 from Germany and 2 of UK origin). The soil sampling sites were known to be clear from use of pesticides for the previous five years. Prior to use at the laboratory, the soils were stored in an incubator (4 ± 2°C) in accordance with ISO 10381-6. The soil characterisation details are summarised in Table 8.1.1.1-1.

Table 8.1.1.1-1: Summary of soil characteristics

Characteristic	Speyer 5M	Speyer 2.2	Brierlow	South Witham
Particle size distribution, % w/w (USDA) Clay (<2 µm)	11	7.3	16	31
Silt (2-50 µm)	30	13.8	54	27
Sand (50-2000 µm)	59	78.9	30	42
Textural classification (USDA)	sandy loam	loamy sand	silt loam	clay loam
Particle size distribution, % w/w (UK) Clay (<2 µm)	10	7	16	31
Silt (2-63 µm)	36	15	57	29
Sand (63-2000 µm)	54	78	27	40
Textural classification (UK)	sandy loam	loamy sand	sandy silt loam	clay loam
pH (H2O)	8.3	6.5	6.4	7.9
pH (CaCl2)	7.3	5.5	5.6	7.4
Organic matter content (%)	1.7	3.1	6.2	7.1
Organic carbon content (%)	1.0	1.8	3.6	4.1
CEC (meq/100g)	16.6	10.1	23.9	39.4
Moisture, pF 0 (w/w %)	39.5	41.8	63.3	32.6
Moisture, pF 2.0, MWHC (w/w %)	24.2	19.1	40.4	28.9
Microbial biomass, µg C/g soil (% TOC)	pre 153 (1.5) appln 254 (2.5) post 117 (1.2)	pre 503 (2.8) appln 434 (2.4) post 197 (1.1)	pre 465 (1.3) appln 422 (1.2) post 238 (0.7)	pre 488 (1.2) appln 829 (2.0) post 361 (0.9)

B. STUDY DESIGN

1 Conditions	Individual soil samples with individual traps (flow through system) in the dark. Traps – ethandiol (for volatile organic volatiles) and 2x 2 M sodium hydroxide. Airflow (moistened air) by vacuum pump and inlet manifold.
No. of soils	4
Temperature	20 ± 2°C (some deviations were noted, 2 occasions at 17.7°C and 1 occasion at 17.8°C)
Moisture	100% pF 2
Pre-acclimatisation	2-4 days dispensed into sample flasks at study temperature and moisture level
2 Test material application	Separate treatments were made for the two forms of the test substance. Separate treatment solutions for ^{14}C-gibberellins GA4 and ^{14}C-gibberellins GA7 were prepared dissolved in water (not augmented with non-radiolabelled material).
Application rate	For soils treated with ^{14}C-gibberellins GA4 an application rate of 0.041 mg/kg soil (dw) was used (equivalent to 31.1 g a.s./ha assuming a mixing depth of 5 cm and soil bulk density of 1.5 g/cm^3. For soils treated with ^{14}C-gibberellins GA7 an application rate of 0.015 mg/kg soil (dw) was used (equivalent to 11.5 g a.s./ha).
3 Sampling	Duplicate 50 g (dry weight)
Sampling intervals, main samples	0, 0.25, 1, 2, 7, 14, 30 and 58 days 20 samples treated with ^{14}C-gibberellins GA4 and ^{14}C-gibberellins GA7 (sufficient for duplicate analysis at each sampling interval and 4 spare samples). 4 control samples for each soil type were used for determination of microbial biomass at the end of the study.
Moisture maintenance	Maintained up to every 8 days by addition of water (reverse osmosis).
Collection of volatile trapping solutions	Trapping solutions were collected and quantified by LSC on the days of soil sample collection.
Biomass determination	Biomass determinations were made on each soil type prior to use, at the time of application and at the end of the study.

Method of analysis

1 Method of analysis for soil samples	
Extraction	Duplicate 50 g soil, extracted (shake, 1x 30 mins) with 0.01M phosphate buffer (pH 7.4 or 7.5, 100 mL) followed by methanol (2x 100 mL) and acetone (1x 100 mL) for drying. Extracts were separated by centrifugation, combined and quantified by LSC.
Sample work-up/concentration	Soil extracts were concentrated (rotary evaporation) and reconstituted in milli-Q water.

Analytical method, primary HPLC	HPLC: Hichrom ACE 5 C18 column (25 cm L x 4.6 mm id), mobile phase – isocratic system a) 0.2% aqueous hydrochloric acid, b) acetonitrile:methanol (1:1 v/v), 0 mins 40% a), 25 mins 40% a). Flow rate 1 mL/min. ^{14}C detection: β-ram, (Lablogic), liquid cell (Flow logic or Ultima-Flo M scintillant, 2 mL/min) or fraction collection (20 s). UV detection (210 nm). Co-chromatography with non-radiolabelled reference standards. LOD – <1% AR Column recoveries ranged 91.3-105.9%
Analytical method, secondary/ confirmatory	TLC: Whatman KC2F TLC plates using solvent system methanol/water/acetic acid (8:1.5:0.5 v/v). ^{14}C detection Fuji BAS 1500 Bio-image analyser using Tina evaluation software (v 2.09g). Co-chromatography with non-radiolabelled reference standards.
2 Method of analysis for extracted soil	Air dried, ground, oxidised by combustion and quantified by LSC.
Secondary extraction method - Harsh extraction	On selected samples only (30 d). Soil samples were refluxed (4 hrs) with 0.1% aqueous TFA (100 mL) i.e. acid reflux and then further refluxed (4 hrs) with 5M sodium hydroxide (100 mL) i.e. base reflux. The extracts were separated by centrifugation and quantified by LSC prior to analysis by HPLC and TLC, if needed.
Organic matter fractionation	Performed on selected samples only (30 d). The post extracted soil residue was shaken with sodium hydroxide solution (0.5 M, 100 mL, 24 h) and the residue (humin fraction) and supernatant were separated by centrifugation. The humin fraction was washed with further sodium hydroxide solution (0.5 M, 50 mL) and the washings were added to the original supernatant. The supernatant was acidified to *ca* pH 1 with hydrochloric acid (5 M) and the precipitate formed was separated from the supernatant by centrifuging and washed with hydrochloric acid (0.1 M, 50 mL). The supernatant and wash were combined (fulvic acid fraction) and the washed precipitate (humic acid fraction) was reconstituted in sodium hydroxide (0.5 M, 100 mL). Radioactivity in the fulvic acid and reconstituted humic acid fractions was determined by LSC. Radioactivity in the humin fraction was determined by combustion analysis followed by quantification by LSC.
3 Volatile components	Trapping solutions were collected and quantified by LSC.
Confirmation of carbon dioxide	By barium carbonate precipitation for each incubation group.

Degradation kinetics

1 Procedure followed	DT_{50} and DT_{90} values for the degradation of the individual components gibberellins GA4 and gibberellins GA7 in soil were determined following the recommendations of the FOCUS work group on kinetics FOCUS 2006[1] and FOCUS 2014[2] (determination of persistence endpoints i.e. degradation endpoints for use as triggers for additional work).
Software used	CAKE v1.1

[1] FOCUS (2006) "Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration" Report of the FOCUS Work Group on Degradation Kinetics, EC Document Reference Sanco/10058/2005 version 2.0, 434 pp.

[2] FOCUS 2014. Generic guidance for Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration, version 1.1, 18 December 2014.

Input data used	Input data for the degradation of gibberellins GA4 and gibberellins GA7 were taken from the individual values behind the data in Table CA 7.1.1.1-5 to Table CA 7.1.1.1-6. No corrections or modifications to data, all data points equally weighted. The models SFO, FOMC, DFOP and HS, where necessary, were considered in order to determine the best-fit kinetic model. SFO was selected as the only suitable model. The dissipation times DT_{50} and DT_{90} (time until 50% or 90% of disappearance) were calculated by the software from the optimized kinetic parameters. Metabolites were not considered.

II. RESULTS AND DISCUSSION

A. DISTRIBUTION OF APPLIED RADIOACTIVITY

The recovery and distribution of applied radioactivity is summarised in Tables 8.1.1.1-2 and 8.1.1.1-3.

Table 8.1.1.1-2: Mean recovery of gibberellins GA4 from soil

Sampling interval (days)	Soil extract	Soil residue	Soil (sub-total)	Total volatiles	Mass Balance
soil Speyer 5M					
0	98.1	0.8	98.8	n/a	98.8
0.25	95.7	3.4	99.0	0.3	99.3
1	70.9	21.6	92.5	1.8	94.2
2	42.4	42.2	84.6	5.2	89.8
8	20.7	50.3	71.0	10.7	81.7
14	10.3	48.8	59.1	14.2	73.3
30	6.3	42.3	48.6	48.8	97.4
58	4.7	38.5	43.1	55.4	98.7 (overall 91.7)
soil Speyer 2.2					
0	95.4	2.2	97.6	n/a	97.6
0.25	92.8	4.9	97.7	0.1	97.7
1	89.3	7.2	96.5	0.5	96.9
2	83.9	11.8	95.7	1.2	96.9
7	47.1	35.0	82.1	10.8	92.9
15	25.1	42.9	67.9	12.3	80.2
30	15.0	45.3	60.3	29.0	89.2
58	12.2	39.4	51.6	42.3	93.9 (overall 93.2)

Sampling interval (days)	Soil extract	Soil residue	Soil (sub-total)	Total volatiles	Mass Balance
soil Brierlow					
0	93.5	3.5	97.0	n/a	97.0
0.25	92.2	6.0	98.2	0.1	98.3
1	84.7	13.1	97.8	0.9	98.7
2	63.0	28.2	91.2	1.5	92.7
8	23.3	53.7	77.0	16.8	93.8
14	15.2	55.6	70.7	20.0	90.7
30	9.2	53.1	62.2	31.8	94.0
58	6.7	48.9	55.6	39.2	94.8 (overall 95.0)
soil South Witham					
0	96.3	3.1	99.4	n/a	99.4
0.25	91.4	6.6	98.0	0.1	98.0
1	80.5	14.6	95.1	1.4	96.4
2	54.3	32.8	87.0	6.9	93.9
8	12.6	42.8	55.4	36.8	92.2
14	8.4	40.8	49.2	46.3	95.4
30	4.4	39.3	43.7	51.2	94.9
58	3.4	37.0	40.4	55.2	95.6 (overall 95.7)

n/a = not applicable

Results are the mean of duplicate samples

Table 8.1.1.1-3: Mean recovery of gibberellins GA7 from soil

Sampling interval (days)	Soil extract	Soil residue	Soil (sub-total)	Total volatiles	Mass Balance
soil Speyer 5M					
0	101.1	1.7	102.8	n/a	102.8
0.25	90.0	9.7	99.7	0.3	100.0
1	66.3	27.9	94.2	3.9	98.1
2	29.3	52.2	58.5	11.7	93.2
8	13.9	55.3	69.2	26.4	95.6
14	12.5	53.2	65.7	18.7	84.3
30	9.4	48.5	57.9	42.9	100.7
58	7.1	42.0	49.1	48.8	97.8 (overall 96.6)
soil Speyer 2.2					
0	97.0	3.8	100.8	n/a	100.8
0.25	79.9	19.5	99.4	nd	99.4
1	73.0	24.3	97.3	0.7	98.0
2	64.4	30.1	94.5	0.8	95.3
7	36.6	50.0	86.5	9.0	95.5
15	23.8	53.8	77.6	15.3	92.9
30	15.6	52.2	67.8	19.8	87.5
58	11.3	49.6	60.8	27.8	88.6 (overall 94.8)

Sampling interval (days)	Soil extract	Soil residue	Soil (sub-total)	Total volatiles	Mass Balance
soil Brierlow					
0	93.5	7.3	100.8	n/a	100.8
0.25	85.3	14.6	99.9	nd	99.9
1	74.6	23.2	97.8	0.4	98.1
2	59.7	35.2	94.9	2.0	96.9
8	31.6	52.0	83.5	12.2	95.7
14	16.7	54.7	71.4	18.0	89.3
30	10.5	56.7	67.2	28.3	95.4
58	8.5	53.9	62.4	35.1	97.5 (overall 96.7)
soil South Witham					
0	91.5	10.5	102.0	n/a	102.0
0.25	80.1	20.0	100.1	0.1	100.2
1	67.3	28.3	95.6	1.7	97.3
2	43.8	45.7	89.5	2.2	91.6
8	14.4	50.2	64.6	28.9	93.4
14	8.8	43.3	52.1	15.3	67.3
30	6.2	47.3	53.5	41.6	95.0
58	3.4	42.3	45.6	50.4	96.0 (overall 92.9)

n/a = not applicable

Results are the mean of duplicate samples

Levels of non-extracted residue reached maximum levels of between 42.8-55.6 and 50.2-56.7% AR for gibberellins GA4 and gibberellins GA7, respectively. Levels of non-extracted residue declined in all soils by the end of the study.

Levels of evolved carbon dioxide were significant in all soils reaching 39.2-55.4 and 27.8-50.4% AR for gibberellins GA4 and gibberellins GA7, respectively.

Table 8.1.1.1-4: Extraction of soil residue using secondary extraction methods (30 day samples only)

Soil	Soil residue (following primary extraction)	Secondary extraction (% AR)			Remaining soil residue after secondary extraction
		Acid reflux	Base reflux	sub-total	
gibberellins GA4					
Speyer 5M	42.3	3.7	22.5	26.2	16.1
Speyer 2.2	45.3	10.8	26.6	37.4	7.9
Brierlow	53.1	6.6	32.3	38.9	14.2
South Witham	39.3	6.4	18.7	25.1	14.2
gibberellins GA7					
Speyer 5M	48.5	5.1	22.8	27.9	20.6
Speyer 2.2	52.2	10.9	36.2	47.1	5.2
Brierlow	56.7	7.9	35.6	43.5	13.2
South Witham	47.3	7.0	23.8	30.8	16.5

For gibberellins GA4, harsh extraction under acidic and basic conditions released 3.7-10.8 and 18.7-32.3% AR, respectively.

For gibberellins GA7, harsh extraction under acidic and basic conditions released 5.1-10.9 and 22.8-36.2% AR, respectively.

D. DEGRADATION PROFILE

The profile of components extracted from the soil samples in the primary extracts is summarised in Tables 8.1.1.1-5 and 8.1.1.1-6.

The levels of gibberellins GA4 and gibberellins GA7 observed in soil declined rapidly leading to the formation of numerous degradation components.

For gibberellins GA4, up to eight degradation components accounting for >10% AR were observed. Maximum levels were in the range 11.6 to 45.7% AR after 0.25 to 2 days following treatment. By 30 days, maximum levels had declined to ≤5.2% AR.

For gibberellins GA7, up to nine degradation components accounting for >10% AR were observed. Maximum levels were in the range 10.2 to 54.3% AR after 0.25 to 2 days following treatment. By 30 days, maximum levels had declined to ≤6.2% AR.

Attempts were made to characterise the degradation components but due to the transient nature of the metabolites and fluctuating levels this was proven to be difficult and inconclusive.

The profile of components extracted from the soil samples in the secondary extracts is summarised in Tables 8.1.1.1-7 and 8.1.1.1-8. The levels of the individual components gibberellins GA4 and gibberellins GA7 found in the secondary extracts were ≤ 0.8% AR.

Table 8.1.1.1-5: Profile of extracted radioactivity of gibberellins GA4 (primary extracts)

Interval (days)	Degradation components (% AR)												Total
	GA4	UK4-1	UK4-2	UK4-3	UK4-4	UK4-5	UK4-6	UK4-7	UK4-8	Others	Largest other	Unres	
soil Speyer 5M													
0	93.7	nd	nd	nd	nd	nd	nd	nd	3.5	0.6	1.1	0.3	98.0
0.25	33.3	nd	nd	1.1	0.7	28.8	0.7	23.8	nd	6.9	3.5	1.3	96.5
1	3.5	7.4	nd	39.4	nd	3.4	0.2	2.2	14.2	0.1	0.2	0.6	70.9
2	nd	40.2	nd	nd	1.1	0.1	nd	nd	nd	0.2	0.5	0.7	42.4
8	0.1	6.3	nd	10.2	0.7	0.6	nd	nd	nd	2.6	4.6	0.1	20.7
14	0.3	1.0	4.6	nd	nd	0.1	0.1	nd	nd	4.1	4.1	nd	10.3
30	nd	nd	nd	nd	0.1	nd	nd	nd	nd	6.1	5.8	nd	6.3
soil Speyer 2.2													
0	85.2	nd	nd	nd	nd	nd	nd	nd	6.8	2.1	4.2	1.3	95.4
0.25	46.5	1.0	0.9	1.7	3.2	3.4	nd	0.8	29.0	5.2	2.5	1.1	92.8
1	11.6	3.0	1.7	32.7	nd	nd	11.6	2.1	24.5	1.8	1.2	0.2	89.3
2	16.5	nd	12.8	5.0	15.9	1.0	nd	28.7	nd	3.2	5.8	0.7	83.9
7	4.7	0.7	4.3	1.6	10.7	1.3	6.6	0.5	13.7	2.5	3.6	0.5	47.1
15	1.6	5.9	9.5	nd	0.3	0.9	nd	0.6	1.5	4.3	3.9	0.5	25.1
30	0.6	2.6	5.1	nd	0.6	nd	0.1	nd	1.3	4.6	4.6	0.2	14.9
soil Brierlow													
0	79.8	nd	nd	nd	nd	nd	nd	4.5	5.2	3.1	6.3	0.8	93.4
0.25	39.8	1.0	1.3	0.6	2.5	3.4	0.6	0.8	32.0	8.8	1.8	1.4	92.2
1	19.0	3.8	1.0	0.9	4.9	20.8	nd	nd	33.5	0.7	0.8	0.1	84.7
2	8.4	22.4	nd	11.1	nd	20.3	nd	nd	nd	nd	nd	0.8	63.0
8	2.6	nd	4.1	6.6	nd	1.8	nd	1.1	1.9	4.9	3.0	0.2	23.3
14	0.5	2.1	1.9	2.6	0.1	1.8	0.5	0.1	0.9	4.4	3.6	0.2	15.2
30	0.2	5.2	0.8	1.1	0.4	0.3	nd	nd	0.6	0.6	0.3	0.1	9.2
soil South Witham													
0	74.2	nd	nd	nd	9.0	1.2	nd	nd	11.5	nd	nd	0.4	96.3
0.25	13.6	40.1	3.2	1.2	nd	7.7	nd	4.6	nd	19.5	4.7	1.6	91.4
1	15.4	45.7	8.2	7.0	nd	nd	2.9	nd	nd	0.7	1.3	0.6	80.5
2	nd	15.2	27.7	3.8	2.2	nd	1.1	nd	0.2	3.6	7.2	0.5	54.3
8	0.1	nd	2.4	1.8	0.4	0.1	0.1	0.1	0.1	7.5	4.5	0.1	12.7
14	0.1	2.4	2.1	0.3	nd	0.1	nd	0.1	nd	3.2	6.5	0.1	8.4

nd = not detected

Table 8.1.1.1-6: Profile of extracted radioactivity of gibberellins GA7 (primary extracts)

Interval (days)	Degradation components (% AR)													Total
	GA7	UK7-1	UK7-2	UK7-3	UK7-4	UK7-5	UK7-6	UK7-7	UK7-8	UK7-9	Others	Largest other	Unres	
soil Speyer 5M														
0	91.1	nd	nd	nd	nd	nd	3.3	nd	nd	5.7	nd	nd	0.9	101.0
0.25	5.1	37.4	nd	0.5	1.8	10.9	7.5	0.7	6.5	nd	18.5	9.3	1.0	90.0
1	6.6	42.3	nd	12.2	nd	nd	nd	1.5	nd	1.0	1.9	2.5	0.8	66.3
2	nd	28.8	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.6	29.3
8	0.3	2.5	2.8	nd	2.0	0.7	nd	nd	0.2	nd	5.4	6.7	0.2	13.9
14	nd	2.3	7.7	nd	nd	0.1	nd	nd	nd	nd	2.4	4.2	nd	12.5
30	nd	3.5	nd	nd	0.1	0.1	nd	nd	nd	nd	5.2	4.4	nd	9.0
soil Speyer 2.2														
0	80.7	nd	nd	nd	nd	nd	nd	nd	nd	nd	13.5	6.0	0.3	94.4
0.25	5.3	15.4	nd	1.5	2.1	10.9	12.0	5.6	9.4	0.2	16.7	11.5	0.8	79.9
1	4.9	9.2	5.3	5.3	2.7	6.9	6.6	9.3	0.4	10.2	12.0	12.9	0.3	73.0
2	1.2	nd	28.1	3.4	2.2	17.0	10.6	0.5	nd	0.4	0.5	1.0	0.6	64.4
7	3.2	13.2	nd	2.8	4.7	2.7	0.5	nd	1.0	0.7	7.4	5.4	0.4	36.5
15	0.2	nd	10.2	nd	11.0	nd	0.8	0.4	nd	0.4	0.8	0.5	0.1	23.8
30	0.2	6.2	1.4	2.2	1.1	0.4	0.3	nd	0.1	0.2	3.4	2.1	0.1	15.6
soil Brierlow														
0	70.1	1.3	0.6	0.5	3.3	0.3	4.2	nd	0.2	2.9	9.1	3.6	1.0	93.5
0.25	18.3	11.4	4.3	0.9	0.8	23.2	4.2	1.6	0.9	nd	18.6	5.7	1.1	85.3
1	8.0	19.5	nd	nd	14.4	1.5	nd	15.1	14.6	nd	1.1	1.0	0.4	74.6
2	0.4	7.0	nd	41.0	2.6	4.9	1.1	0.2	0.7	0.4	1.2	0.6	0.2	59.7
8	0.3	3.8	nd	2.2	4.7	5.8	0.5	0.3	2.0	0.8	11.0	6.7	0.2	31.6
14	0.2	nd	nd	8.8	2.3	1.3	0.4	nd	0.2	0.2	2.9	3.5	0.2	16.6
30	0.1	1.5	1.0	1.2	1.5	nd	0.4	0.3	0.2	0.1	4.1	3.7	0.2	10.5
soil South Witham														
0	76.9	nd	nd	nd	nd	2.3	nd	nd	nd	nd	11.7	10.2	0.5	91.5
0.25	nd	54.3	nd	8.6	0.9	2.0	0.6	0.6	nd	nd	12.3	9.2	0.9	80.1
1	0.1	47.4	0.7	1.4	0.3	0.3	4.1	0.3	0.2	0.4	11.2	8.4	0.8	67.3
2	nd	31.0	6.0	1.5	0.8	0.3	1.5	0.6	0.2	nd	1.7	1.2	0.2	43.8
8	0.1	0.5	3.1	3.6	2.4	nd	0.1	nd	0.2	0.1	4.3	4.4	0.1	14.3
14	nd	2.6	1.4	0.4	nd	0.3	nd	0.1	0.1	nd	3.7	3.8	0.1	8.8
30	nd	0.6	0.6	0.9	0.2	0.1	0.1	nd	nd	0.1	3.6	3.1	0.1	6.2

nd = not detected

Table 8.1.1.1-7: Profile of extracted radioactivity of gibberellins GA4 (secondary extracts), 30 days samples only

Sampling interval (days)	Degradation components (% AR)												Total
	GA4	UK4-1	UK4-2	UK4-3	UK4-4	UK4-5	UK4-6	UK4-7	UK4-8	Others	Largest other	Unres	
Acidic reflux													
Speyer 5M	-	-	-	-	-	-	-	-	-	-	-	-	-
Speyer 2.2	0.2	0.3	5.9	0.4	0.5	0.4	0.1	nd	0.1	2.8	na	0.1	10.8
Brierlow	nd	0.2	4.3	0.2	0.7	0.3	nd	nd	0.1	0.8	na	nd	6.6
South Witham	0.1	nd	4.5	0.2	nd	0.3	nd	nd	nd	1.3	na	0.1	6.4
Basic reflux													
Speyer 5M	0.2	3.8	9.2	1.7	0.1	0.3	0.4	0.1	0.2	6.4	na	0.3	22.5
Speyer 2.2	0.1	1.7	18.7	0.4	0.3	0.2	0.2	0.1	nd	4.8	na	0.2	26.6
Brierlow	0.2	10.9	11.5	0.2	0.5	0.3	0.3	0.9	0.1	6.9	na	0.5	32.3
South Witham	0.4	nd	5.3	5.2	nd	0.3	0.2	0.1	nd	7.1	na	0.2	18.7

na = not applicable, nd = not detected

Table 8.1.1.1-8: Profile of extracted radioactivity of gibberellins GA7 (secondary extracts), 30 days samples only

Sampling interval (days)	Degradation components (% AR)													Total
	GA7	UK7-1	UK7-2	UK7-3	UK7-4	UK7-5	UK7-6	UK7-7	UK7-8	UK7-9	Others	Largest other	Unres	
Acidic reflux														
Speyer 5M	nd	nd	2.8	nd	0.3	nd	0.5	nd	nd	nd	1.4	na	nd	5.1
Speyer 2.2	0.7	nd	nd	6.0	0.8	0.2	1.4	nd	nd	nd	1.7	na	0.1	10.9
Brierlow	nd	nd	nd	5.6	0.7	nd	1.0	nd	nd	nd	0.6	na	nd	7.9
South Witham	nd	nd	nd	5.9	nd	1.0	nd	nd	nd	nd	nd	na	0.1	7.0
Basic reflux														
Speyer 5M	0.4	7.3	2.3	nd	nd	1.6	nd	nd	0.1	nd	10.8	na	0.3	22.8
Speyer 2.2	0.4	16.8	11.7	nd	2.3	nd	0.7	nd	nd	nd	4.1	na	0.2	36.1
Brierlow	0.8	3.1	18.9	5.4	2.2	0.6	0.3	nd	0.2	0.5	3.1	na	0.3	35.6
South Witham	0.5	nd	nd	10.4	6.1	0.4	0.6	nd	0.2	0.2	5.3	na	nd	23.8

nd = not detected

D. DEGRADATION RATE

Degradation rates for gibberellins GA4 and gibberellins GA7 in soil were calculated based on the concentrations observed in the primary extracts. The best fit i.e. persistence degradation rates are summarised in Table 8.1.1.1-9. Both gibberellins GA4 and gibberellins GA7 degraded rapidly in soil, with best-fit persistence half-lives of <0.5 days in all soil types.

Table 8.1.1.1-9: Degradation rate of gibberellins GA4 and gibberellins GA7 in soil

Soil	DT_{50}	DT_{90}	Chi2err (%)	Correlation coefficient, r^2
GA4				
Speyer 5M	0.171	0.568	3.188	0.9996
Speyer 2.2	0.347	1.153	20.75	0.9476
Brierlow	0.392	1.303	18.61	0.9492
South Witham	0.104	0.346	28.78	0.9504
GA7				
Speyer 5M	0.060	0.200	13.57	0.9927
Speyer 2.2	0.064	0.212	13.20	0.9830
Brierlow	0.132	0.440	16.67	0.9541
South Witham	0.011	0.036	1.005	0.9976

CAKE (version 1.1) software was used to determine the degradation rates and kinetic parameters. Graphical summaries of the data for each soil type showing the visual fits and plots of residuals are shown in Figures 8.1.1.1-1 to 8.1.1.1-4 for gibberellins GA4 and in Figures 8.1.1.1-5 to 8.1.1.1-8 for gibberellins GA7. Kinetics determined by SFO were not meaningfully improved by other models (FOMC, DFOP and HS). Full details of the kinetic evaluation are provided in Figures 28 and 29 of the study report (pages 94-117) and in Appendix 2 of this document.

Degradation rates for metabolites could not be reliably calculated as their observed levels fluctuated greatly throughout the study period, and in the majority of cases, because their transient nature resulted in the formation and degradation profile not being well described.

Figure 8.1.1.1-1: Kinetic assessment for gibberellins GA4 in the Speyer 5M soil (SFO)

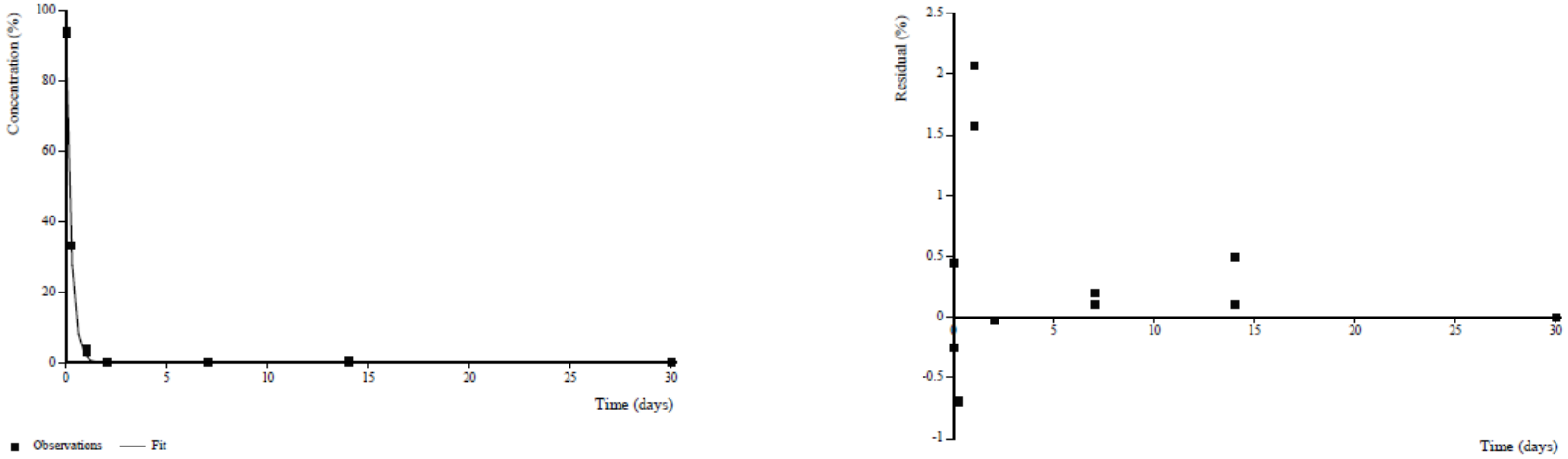


Figure 8.1.1.1-2: Kinetic assessment for gibberellins GA4 in the Speyer 2.2 soil (SFO)

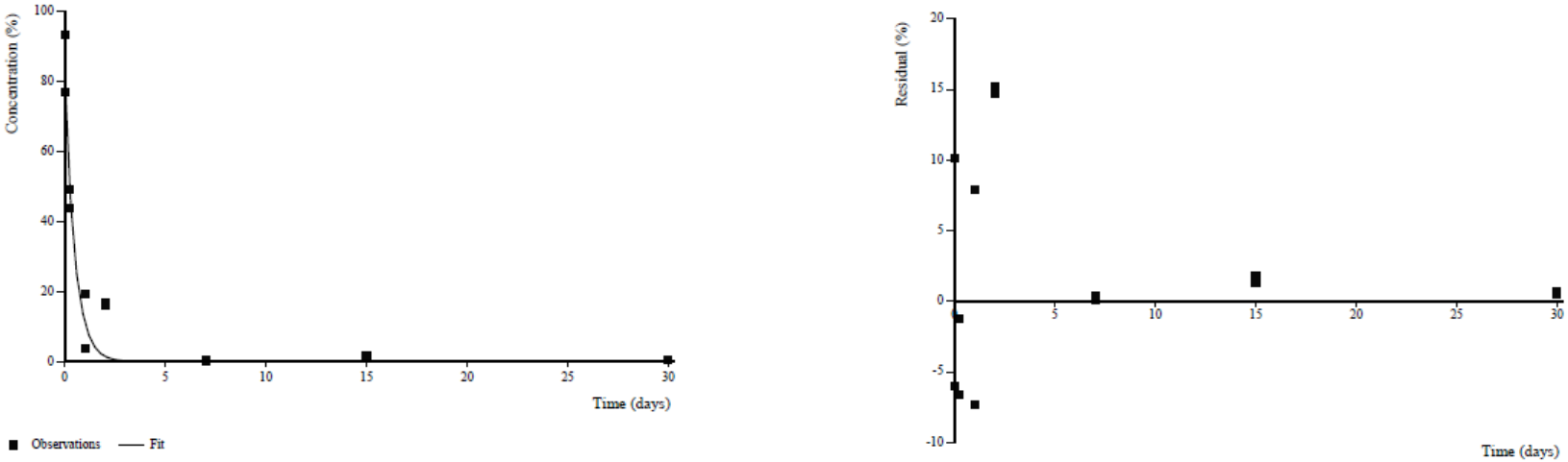


Figure 8.1.1.1-3: Kinetic assessment for gibberellins GA4 in the Brierlow soil (SFO)

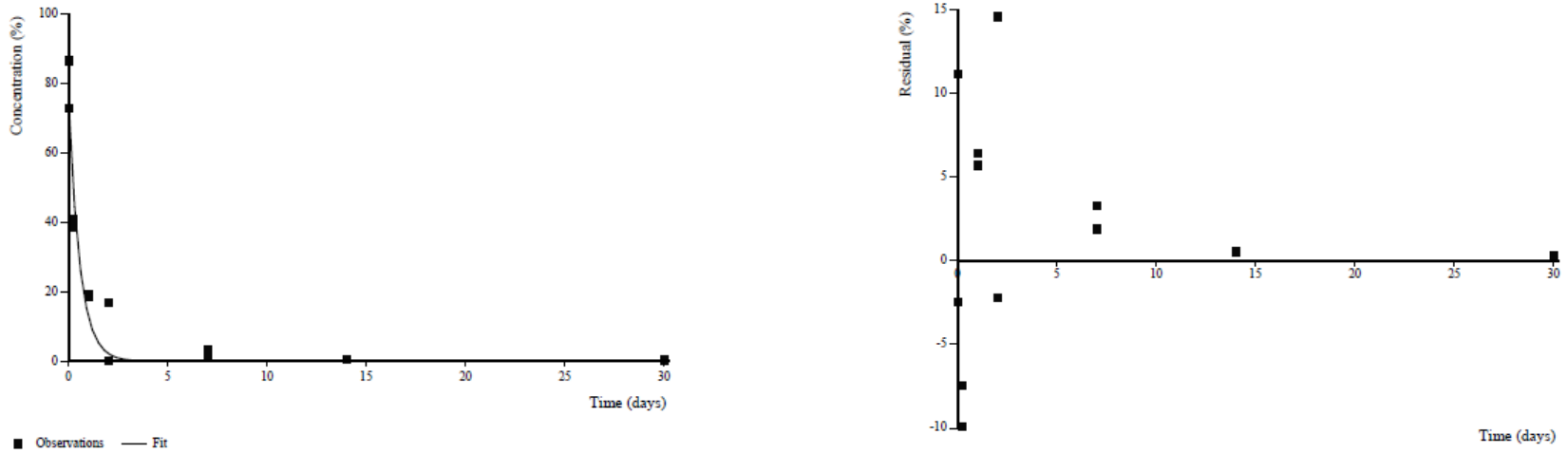


Figure 8.1.1.1-4: Kinetic assessment for gibberellins GA4 in the South Witham soil (SFO)

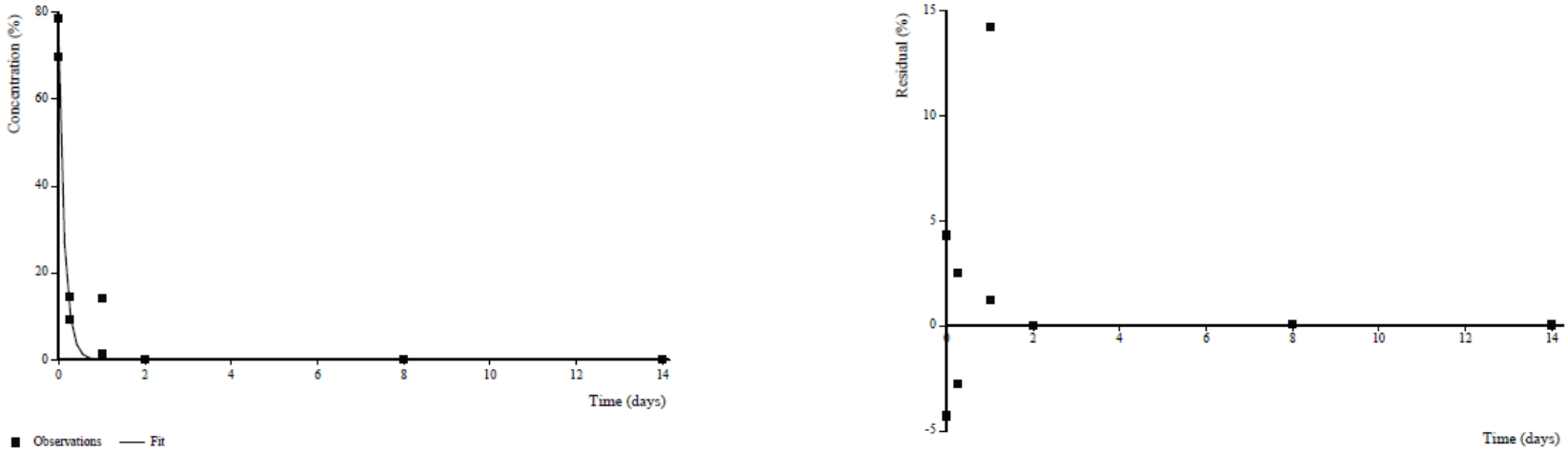


Figure 8.1.1.1-5: Kinetic assessment for gibberellins GA7 in the Speyer 5M soil (SFO)

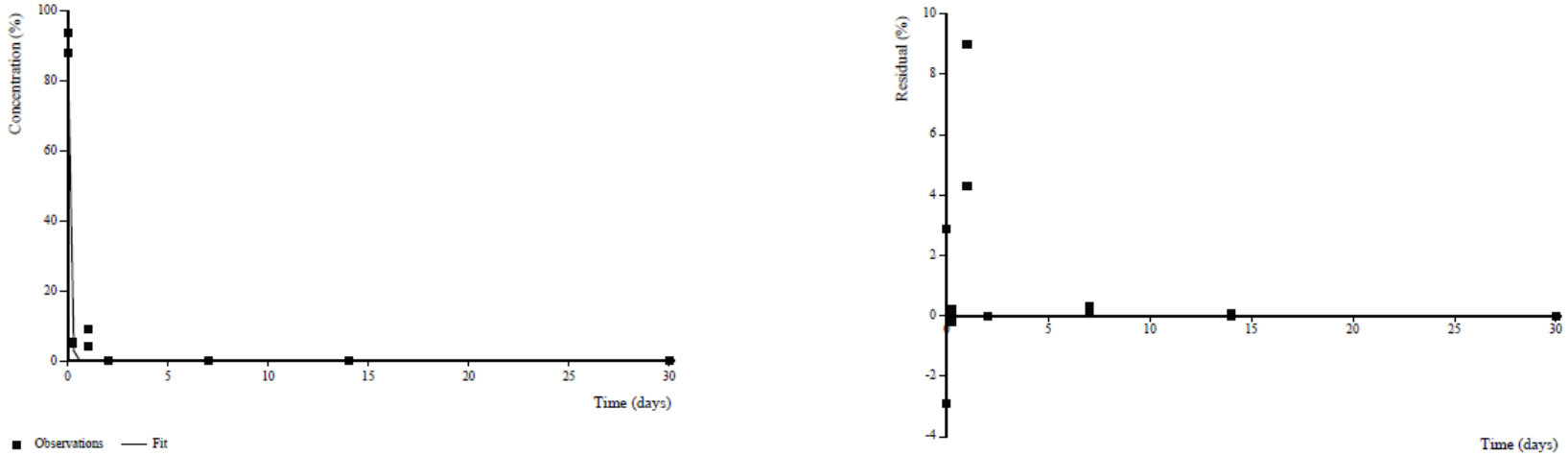


Figure 8.1.1.1-6: Kinetic assessment for gibberellins GA7 in the Speyer 2.2 soil (SFO)

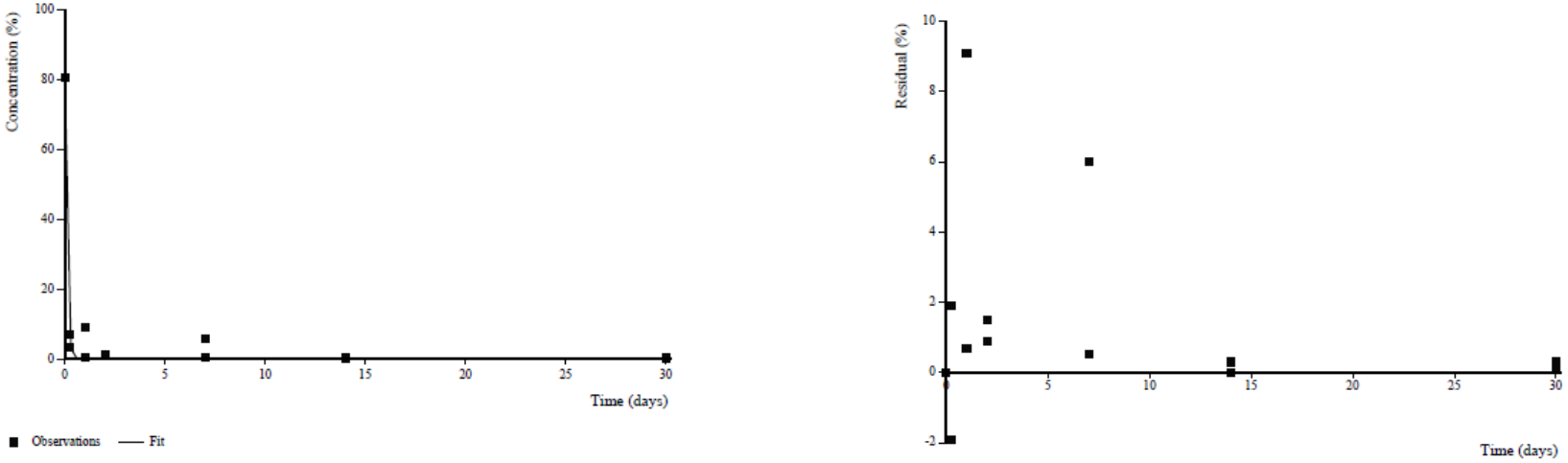


Figure 8.1.1.1-7: Kinetic assessment for gibberellins GA7 in the Brierlow soil (SFO)

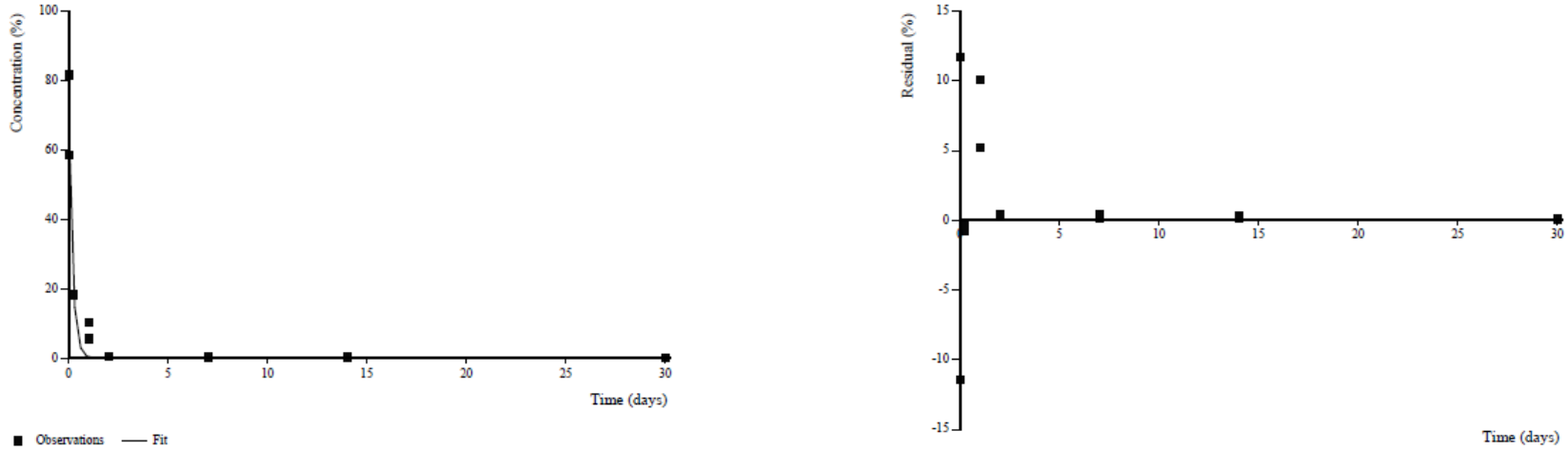
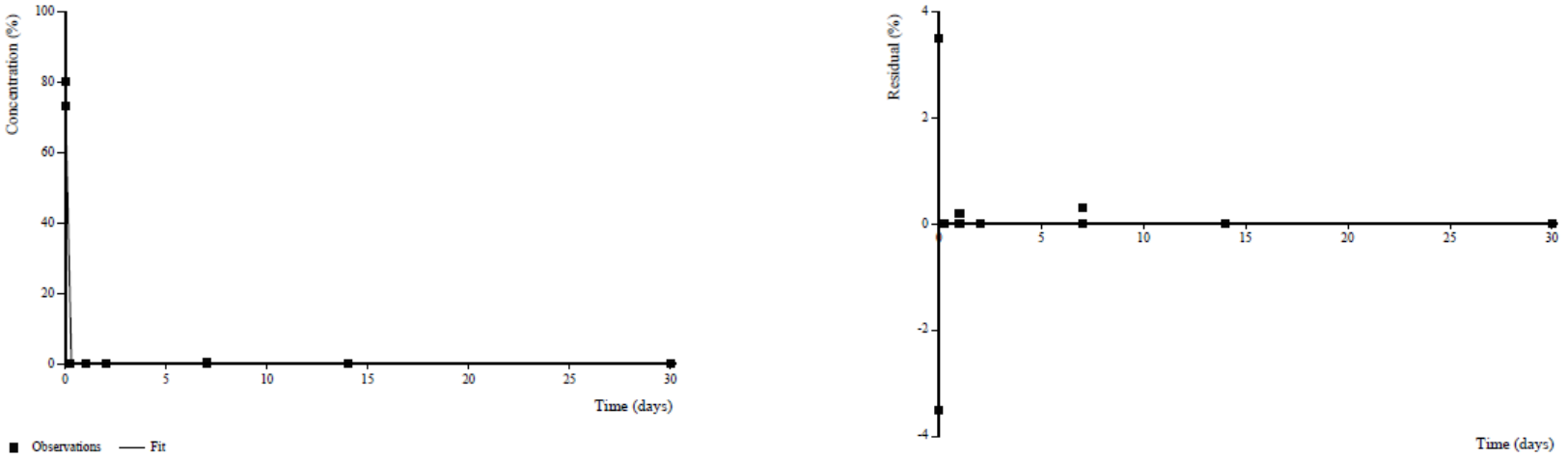


Figure 8.1.1.1-8: Kinetic assessment for gibberellins GA7 in the South Witham soil (SFO)



E. ORGANIC MATTER FRACTIONATION

The non-extractable radioactivity present in the residue was further characterised by organic matter fractionation.

Results showed that in soils treated with [^{14}C]-gibberellins GA4, the largest fraction was fulvic acid (mean of 60.1% of non-extractable radioactivity across all four soils), with humin being the next largest fraction (mean of 31.2%) and humic acid accounting for the remainder (mean of 2.0%).

Soils treated with [^{14}C]-gibberellins GA7 showed similar results with the largest fraction being fulvic acid (mean of 65.1% of non-extractable radioactivity across three soils), followed by humin (mean of 30.3%) and humic acid accounting for the remainder (mean of 4.1%).

III. CONCLUSIONS

The individual components of the active substance gibberellins GA4/7, gibberellins GA4 and gibberellins GA7 degrade extensively in soil. Numerous degradation products were observed but not fully identified, however, as these were formed and had declined during the short study period, they are considered to be extremely transient. Ultimate degradation led to the formation of un-extracted soil residues and mineralisation to carbon dioxide. For both gibberellins GA4 and gibberellins GA7 the best-fit persistence half-lives were <0.5 days in all soil types.

RMS comments and conclusion:

GA4 and GA7 are naturally occurring plant growth regulators. The route and rate of GA4 and GA7 in aerobic soil was studied at 20 ± 2°C using the radiolabelled test substances 17-14C-Gibberellin A4 and 17-14C-Gibberellin A7. The soils showed a range of different characteristics (i.e. organic carbon content, pH and clay content).
The nominal application rate was 0.041 mg/kg for soils treated with [14C]-GA4 and 0.015 mg/kg for soils treated with [14C]-GA7, both based on dry weight equivalent of soil. These concentrations correspond to an application rate of 31.1 and 11.5 g ai/ha respectively, assuming a bulk density of 1.5 g/cm3. Soil moisture was adjusted to the water holding capacity value at pF 2, up to every 8 days. Microbial biomass was determined in each soil prior to dispensing, in samples removed from incubation at application and in samples removed at the end of the study period. Although at the end of the study the microbial biomass in Brierlow and South Witham soils expressed as a percent of organic carbon was < 1, the actual biomass results were both >100 µg C/g soil and therefore the soils were still viable.
A representative sampling interval (30 DAT) was selected for harsher extraction methods. Samples were refluxed with 0.1% aqueous trifluroacetic acid (TFA), followed by centrifugation to separate the solid and liquid phases.
Degradation rates were determined by single first-order (SFO) kinetics according to FOCUS recommendations using CAKE software.).

Deviations from Protocol
No major deviations from the protocol were observed.

GA4:
Degradation rates could not be accurately calculated for the metabolites as their observed levels fluctuated greatly throughout the study period, and in the majority of cases, because their transient nature resulted in the formation and degradation profile not being well described. Following the primary extraction, maximal levels of all metabolites were observed between 0 and 2 DAT and levels had declined by a considerable margin by the end of the 30 DAT sampling interval such that levels were ≤ 5.2% AR. Identification of unknown major metabolites was not possible, however as they had formed and declined during the short study period, they can be considered as transient metabolites.
GA7:
Degradation rates could not be accurately calculated for the metabolites as their observed levels fluctuated greatly throughout the study period, and in the majority of cases, because their transient nature resulted in the formation and degradation profile not being well described. Following the primary extractions, maximal levels of the major metabolites were observed between 0 and 2 DAT (with the exception of UK7-4 in Speyer 2.2 soil at

14 DAT) and levels had declined by a considerable margin by the end of the 30 DAT sampling interval such that levels were ≤ 6.2% AR. Identification of unknown major metabolites was not possible, however as they had formed and declined during the short study period, they can be considered as transient metabolites.
Based on chemistry of the Gibberellin naturally occurring plant growth regulators and retention times of the metabolites found in this study, it was assumed that UK4-1 and UK7-1 are structurally similar.

General conclusions for both plant hormones:
By the end of the 58 day study period, GA4 and GA7 were rapidly and extensively metabolised mainly to carbon dioxide and bound residues. Bound residues accounted for maximums of 56% AR for soils treated with [14C]-GA4 and 57% for soils treated with [14C]-GA7). Harsh acid and base refluxes released up to 90.1% of this radioactivity.
Non-extractable radioactivity was mainly in the fulvic acid fraction of the soil. Using SFO kinetics, DT-50 values for the degradation of both GA4 and GA7 were in the range of 0.10 to 0.39 days (GA4) and 0.01 to 0.13 days (GA7). Up to eight degradates were formed in soils treated with GA4. The DT50 can be seen to be less than the study duration (although calculation via computer programming was not possible due to their transient nature and/ or their fluctuating concentrations). Up to nine degradates were formed in soils treated with GA7. The DT-50 can be seen to be less than the study duration (although calculation via computer programming was not possible due to their transient nature and/ or their fluctuating concentrations). The variation between replicates and sampling intervals time points was quite large and therefore the data was not amenable for more complex kinetic evaluations than SFO.
Characterisation of the unknown metabolites and unextractable radioactivity was extensive although identification could not be confirmed. Characterisation has shown that the metabolites are: generally more polar than parent GA4; transient (DT-50 reached during the study period) and unlikely to persist in the environment as they have an ultimate fate of CO2.

The study is considered acceptable and even results of estimated DT50/90 can be used in further assessment of the rate of degradation of GA4/7 in soil.
Kinetic output is shown in the Appendix 2 of this document.

Anaerobic degradation

Use of the active substance gibberellins GA4/7 in the representative formulation ‘Novagib’ involves applications to pomefruit. Consequently, anaerobic conditions are not relevant and a study has not been performed.

Soil photolysis

The molar absorbance coefficient of the individual components gibberellins GA4 and gibberellins GA7 in the active substance GA4/7 are <10 L/mol/cm at or above wavelength 298 nm, ref CA 2.4 and therefore the trigger value of 10 L/mol/cm is not exceeded i.e. photolysis is not expected to contribute significantly to the environmental degradation of the active substance due to low light absorbance. Consequently, a soil photolysis study has not been performed.

B.8.1.1.2. Rate of degradation in soil

a) Previous evaluation (2005-2011)

Degradation route and rate studies of GA_4/GA_7 were not performed. The DT_{50} was extrapolated from the result of the ready biodegradation test according to EU Technical Guidance Document on Risk Assessment and it was 30 days.

Further information is presented under Point B.8.1.1.1.

RMS comments and conclusion:

See RMS comments under Point B.8.1.1.1.

b) Evaluation of additional data for the purpose of renewal of Annex I inclusion

The same new laboratory study were provided as the previously submitted in the Route part of this document. The study was considered sufficient to meet current data requirements. A kinetic analysis of the experimental data in the laboratory aerobic soil degradation studies was conducted following the current EC guidance document to derive the rate of degradation of GA3 and its metabolites in aerobic soil.
The rate of degradation in soil of the individual components gibberellins GA4 and gibberellins GA7 in the active substance GA4/7 have been investigated in one "new" study (CA 7.1.2.1.1/01) which has been conducted since the original Annex I inclusion to fully address the requirements of Regulation EC 283/2013.

Data point addressed:	CA 7.1.2.1.1/01
Author(s) (year):	Traub, M. (2014)
Title:	**Gibberellins GA4, GA7: Aerobic degradation in four European soils**
Document No:	S14-01454
Testing facility:	Eurofins Agroscience Services, Germany
Published:	Unpublished
Test guidelines used:	OECD Guideline 307 (Apr 2002)
Deviations:	No deviations.
GLP:	Yes
Test material:	see below
Batch #/purity:	see below
Status:	**New submission**

Executive summary

The rate of aerobic degradation of the individual components gibberellins GA4 and gibberellins GA7 in the active substance gibberellins GA4/7 was investigated in four soil types (ranging from loamy sand to clay loam) of German origin in the dark under laboratory conditions at a temperature of 20°C and moisture content of 45% MWHC.

Gibberellins GA4 and gibberellins GA7 were shown to degrade rapidly in 4 soil types under aerobic conditions (20°C, 45% MWHC). As the study was conducted with non-radiolabelled materials any metabolites were not observed. The degradation rate was faster for gibberellins GA7 compared to gibberellins GA4. The degradation rate was dependent on soil type with the DT_{50} values for gibberellins GA4 and gibberellins GA7 ranging from 24-37 hrs and 4-14 hrs, respectively.

I. MATERIALS AND METHODS

A. TEST AND REFERENCE MATERIALS

1 Test material no. 1	Gibberellins GA4 (structures listed in Appendix 1)
Radiolabel position	Non radiolabelled
Radiochemical purity	Not applicable (chemical purity 99%)
Lot/batch no.	18603
Stability of test compound	Not stated
Test material no. 2	Gibberellins GA7
Radiolabel position	Non radiolabelled
Radiochemical purity	Not applicable (chemical purity 100%)
Lot/batch no.	18664
Stability of test compound	Not stated
2 Reference material	The test materials above were also used for the reference standards

Test System, soil

The study was conducted using 4 soils of German origin. The sites were known to be clear from use of pesticides for the previous five years. The soils were supplied already sieved to 2 mm. The soil characterisation details are summarised in Table 8.1.1.2-1.

Table 8.1.1.2-1: Summary of soil characteristics

Characteristic	LUFA 2.1	LUFA 2.2	LUFA 2.3	LUFA 6S
Particle size distribution, % w/w (USDA)				
Clay (<2 µm)	1.9	6.9	6.6	39.9
Silt (2-50 µm)	13.9	15.3	36.0	34.6
Sand (50-2000 µm)	84.2	77.8	57.4	25.5
Textural classification (USDA)	loamy sand	loamy sand	sandy loam	clay loam
pH (0.01M CaCl2)	5.12	5.85	6.22	7.14
Organic matter content (%)	1.09	2.45	1.24	3.22
Organic carbon content (%)	0.63	1.42	0.72	1.87
CEC (meq/100g)	4.5	9.3	7.8	20.0
Moisture, MWHC (w/w %)	30.5	42.6	36.8	41.7
Microbial biomass, µg C/g soil (% TOC)	-49 d - 49.0 0 d - 137.8 14 d - 144.8	123.2 144.6 151.3	91.3 127.9 124.9	162.1 293.7 287.4

% organic matter = % organic carbon x 1.724

B. STUDY DESIGN

1 Conditions	
No. of soils	4
Temperature	20 ± 2°C
Moisture	45% MWHC
Pre-acclimatisation	7 days
2 Test material application	Each soil sample was treated with both gibberellins GA4 and gibberellins GA7. The test materials gibberellins GA4 and gibberellins GA7 were added to the soil samples (3.56 µg and 1.78 µg, respectively) dropwise dissolved in acetonitrile (89 µL). Following application the soil samples were mixed by shaking the flasks.
Application rate	The treatment rate of the soil samples was equivalent to a field application rate of 26.70 g gibberellins GA4/ha and 13.35 g gibberellins GA7/ha (assuming no crop interception, a mixing depth of 5 cm and a soil bulk density of 1.5 g/cm^3). The application rate is equivalent to a field application rate of 40.04 g gibberellins GA4/7 per hectare (ratio of GA4:GA7 of 2:1).
3 Sampling	Duplicate 100 g (dry weight) in glass flasks (300 mL)
Sampling intervals, main samples	0, 6, 12, 24, 48, 72, 120, 168 and 336 hours (14 d) - 20 samples (for each soil type) treated with both gibberellins GA4 and gibberellins GA7 (sufficient for duplicate analysis at each sampling interval and 2 spare samples) - 10 additional untreated samples (for each soil type) used to determine microbial biomass content at the start, middle and end of the study - 10 additional treated samples (for each soil type) used to determine microbial biomass content at the start, middle and end of the study. - 30 untreated samples (for each soil type) used for the concurrent recoveries and blank
Collection of volatile trapping solutions	Not applicable (volatiles not collected/non-radiolabelled study)
Biomass determination	Conducted according to Anderson & Domsch (1978)

Method of analysis

1 Method of analysis for soil samples	
Sample extraction	Soil samples taken for analysis were worked up and analysed immediately. Soil samples were extracted (3x shake, at least 30 mins) with acetonitrile/water (100 mL, 80/20 v/v). Extracts were separated by centrifugation and combined. A further extraction was performed with acetonitrile/water (100 mL, 80/20 v/v) incubated at 55°C (20 mins) using a microwave. Afterwards the hot suspension was shaken (at least 30 mins) and the extract separated by centrifugation. All extracts were combined.
Sample work-up/concentration	A sub-sample of the combined extracts (1-2 mL) was diluted with HPLC water prior to chromatographic analysis by HPLC-MS/MS.
Analytical method, primary	HPLC: Thermo Betasil no. 35005-03 column (100 mm L x 2.1 mm id), injection volume 40 µL, column temperature 40°C, mobile phase – gradient system a) water + 0.1% acetic acid, b) methanol + 0.1% acetic acid, 0 mins 80% a), 1.00 mins 80% a), 2.5 mins 2% a), 6.5 mins 2% a), 6.51 mins 80% a), 9.50 mins 80% a). Flow rate 0.3 mL/min. Detection – MS: ionisation mode ESI –ve mode, fragment ions 257.2 and 243.1 m/z for GA4 and 223.0 and 211.1 m/z for GA7. Calibration 0.01 to 20 ng/mL. Controls and recovery samples included. LOD defined as 1% of applied dose.

4 Degradation kinetics

1 Procedure followed	Carried out according to FOCUS 2014 (determination of persistence endpoints) using KinGUI 2. The input data used were the individual values behind the data presented in Table CA 7.1.2.1.1-2. No data modifications were made, all data were equally weighted.

II. RESULTS AND DISCUSSION

A. VALIDATION

Method validation was conducted for both gibberellins GA4 and gibberellins GA7 at two levels in each soil (5 replicates each). For gibberellins GA4, the overall mean recoveries for the four soils (LUFA 2,1, LUFA 2.2, LUFA 2.3 and LUFA 6S) were 98.2 ± 7.4, 100.3 ± 3.3, 96.6 ± 5.1 and 91.3 ± 7.4%, respectively. For gibberellins GA7, the overall mean recoveries for the four soils were 87.3 ± 6.2, 88.3 ± 4.2, 88.3 ± 4.1 and 86.9 ± 6.5%, respectively.

Residues in the untreated soils were <30% of the assigned LOD.

B. DEGRADATION

The measured levels of gibberellins GA4 and gibberellins GA7 in the soil samples are presented in Table 8.1.1.2-2.

Table 8.1.1.2-2: Degradation of gibberellins GA4, gibberellins GA7 and overall gibberellins GA4/7 combined in soil

Sampling interval (hrs)	**Soil LUFA 2.1**			**Soil LUFA 2.2**		
	GA4	**GA7**	**GA4/7**	**GA4**	**GA7**	**GA4/7**
0	108.7 ± 3.8	102.8 ± 17.4	106.6 ± 8.2	96.6 ± 2.3	97.8 ± 0.3	97.0 ± 1.7
6	96.9 ± 7.4	69.1 ± 16.3	87.6 ± 9.7	81.2 ± 5.2	59.6 ± 14.2	74.0 ± 7.7
12	83.1 ± 1.6	61.2 ± 19.2	75.8 ± 6.3	69.7 ± 0.1	27.5 ± 15.6	55.8 ± 2.5
24	62.1 ± 1.8	18.5 ± 11.3	47.6 ± 0.1	44.7 ± 4.4	10.7 ± 16.6	33.3 ± 5.7
48	39 ± 13.4	3.4 ± 70.7	27.2 ± 10.2	44.7 ± 19.7	5.6 ± 5.2	31.5 ± 18.9
72	37.9 ± 9.5	3.54 ± 82.1	26.2 ± 12.4	29.5 ± 4.5	5.1 ± 5.8	21.3 ± 4.6
120	9 ± 1.6	<LOD	6 ± 1.2	28.9 ± 3.7	4.5 ± 10.9	20.8 ± 2.4
168	4.5 ± 18.9	<LOD	3 ± 18.9	20.5 ± 9.3	3.9 ± 5.8	15.0 ± 8.5
336 (14 d)	<LOD	<LOD	<LOD	5.1 ± 8.7	<LOD	3.4 ± 8.6
Recovery			n/a			n/a
LOQ	92.5-106.8	74.3-108.4		77.3-100.2	72.7-118.1	
22x LOQ	91.7-110.7	79.5-113.1		71.9-108.8	73.4-110.8	
Sampling interval (hrs)	**Soil LUFA 2.3**			**Soil LUFA 6S**		
	GA4	**GA7**	**GA4/7**	**GA4**	**GA7**	**GA4/7**
0	105.6 ± 1.2	106.7 ± 0.2	106.2 ± 0.9	94.1 ± 10.8	100.0 ± 5.9	96.1 ± 9.1
6	63.5 ± 4.3	39.3 ± 25.6	55.4 ± 2.7	81.7 ± 8.0	84.3 ± 8.6	82.6 ± 8.2
12	67.1 ± 10.4	14.0 ± 27.7	49.4 ± 6.9	62.6 ± 21.2	50.0 ± 15.5	58.4 ± 19.6
24	54.8 ± 4.9	5.1 ± 12.4	38.2 ± 4.1	50.0 ± 21.4	27.0 ± 5.8	42.1 ± 18.1
48	34.3 ± 1.9	1.7 ± 4.6	23.4 ± 2.1	29.5 ± 18.5	15.7 ± 16.5	24.9 ± 17.9
72	20.5 ± 15.3	<LOD	13.7 ± 15.0	10.7 ± 6.6	4.5 ± 25.7	8.6 ± 0.8
120	8.4 ± 0	<LOD	5.6 ± 1.3	5.3 ± 15.7	1.7 ± 53.6	4.1 ± 19.2
168	2.2 ± 22.0	<LOD	1.5 ± 24.4	3.7 ± 52.3	1.1 ± 0	2.8 ± 45.5
336 (14 d)	<LOD	<LOD	<LOD	<LOD	0 ± 0	<LOD
Recovery			n/a			n/a
LOQ	91.4-106.6	79.6-95.6		95.4-109.2	97.6-110.3	
22x LOQ	102.1-111.9	102.5-113.9		69.9-102.2	73.2-108.1	

n/a = not applicable

Concurrent recoveries were in the range of acceptability i.e. 70-110% for non-radiolabelled substances (except on 1 occasion where the high level recovery was 69.9%).

C. DEGRADATION RATE

A summary of the kinetic evaluation of the degradation of gibberellins GA4 and gibberellins GA7 in soil is provided in Table 8.1.1.2-3.

Table 8.1.1.2-3: DT_{50} and DT_{90} of gibberellins GA4, gibberellins GA7 and gibberellins GA4/7 combined in soil

Soil	Component	Model	DT_{50} (hrs)	DT_{90} (hrs)	Chi^2 error	Visual fit[1]
Soil LUFA 2.1	GA4	**SFO**	**37**	**122**	**7.2**	**o**
		DFOP	33	137	7.6	o
		FOMC	33	117	7.8	o
	GA7	**SFO**	**12**	**39**	**12.2**	**o**
		DFOP	10	42	16.5	o
		FOMC	1	622	57.2	o
	GA4/7	SFO	26	88	8.7	o
		DFOP	22	106	7.1	o
		FOMC	23	99	7.1	o
Soil LUFA 2.2	GA4	SFO	55	182	15.4	o
		DFOP	**27**	**302**	**7.2**	o
		FOMC	30	469	8.6	o
	GA7	**SFO**	**7**	**22**	**12.3**	o
		DFOP	7	26	12.8	o
		FOMC	1	216	44.9	o
	GA4/7	SFO	30	99	20.7	o
		DFOP	16	200	7.9	o
		FOMC	16	258	8.4	o
Soil LUFA 2.3	GA4	SFO	31	104	15.1	o
		DFOP	15	143	16.3	o
		FOMC	**24**	**128**	**14.7**	o
	GA7	**SFO**	**4**	**14**	**5.2**	o
		DFOP	4	14	5.7	o
		FOMC	0.3	27	38.5	o
	GA4/7	SFO	18	59	22.4	o
		DFOP	8	102	10.2	o
		FOMC	9	108	12.5	o
Soil LUFA 6S	GA4	**SFO**	**26**	**87**	**5.6**	**o**
		DFOP	26	88	6.2	o
		FOMC	22	88	7.0	o
	GA7	**SFO**	**14**	**46**	**11.3**	**o**
		DFOP	12	57	13.5	o
		FOMC	2	1440	53.0	o
	GA4/7	SFO	21	71	7.2	o
		DFOP	20	79	7.0	o
		FOMC	20	69	7.4	o

(1) Visual assessment: + good, o moderate, - poor

An overall summary of the degradation rate of gibberellins GA4 and gibberellins GA7 in soil is provided in Table 8.1.1.2-4.

Table 8.1.1.2-4: Overview of DT_{50} and DT_{90} of gibberellins GA4 and gibberellins GA7 in soil

Component	Soil	Model	DT_{50} (hrs)	DT_{90} (hrs)	Chi^2 error
GA4	Soil LUFA 2.1	SFO	37	122	7.2
	Soil LUFA 2.2	DFOP	27	302	7.2

Component	Soil	Model	DT_{50} (hrs)	DT_{90} (hrs)	Chi^2 error
	Soil LUFA 2.3	FOMC	24	128	14.7
	Soil LUFA 6S	SFO	26 (24-37, avg 28.5 hrs)	87	5.6
GA7	Soil LUFA 2.1	SFO	12	39	12.2
	Soil LUFA 2.2	SFO	7	22	12.3
	Soil LUFA 2.3	SFO	4	14	5.2
	Soil LUFA 6S	SFO	14 (4-14, avg 9.3 hrs)	46	11.3

The overall DT_{50} values (FOCUS persistence endpoint) of gibberellins GA4 and gibberellins GA7 in soil ranged from 24-37 and 4-14 hrs, respectively.

KinGUI (version 2) software was used to determine degradation rates and kinetic parameters. Graphical summaries showing the visual fits and plots of residuals for the best-fit kinetic model for each test soil are shown in Figures 8.1.1.2-1 to 8.1.1.2-4 for gibberellins GA4 and in Figures 8.1.1.2-5 to 8.1.1.2-8 for gibberellins GA7. Full details of the kinetic evaluation are provided in Appendices 4 to 7 of the study report (pages 78-151) and in Appendix 3 of this document.

Figure 8.1.1.2-1: Kinetic assessment for gibberellins GA4 in the LUFA 2.1 soil (SFO)

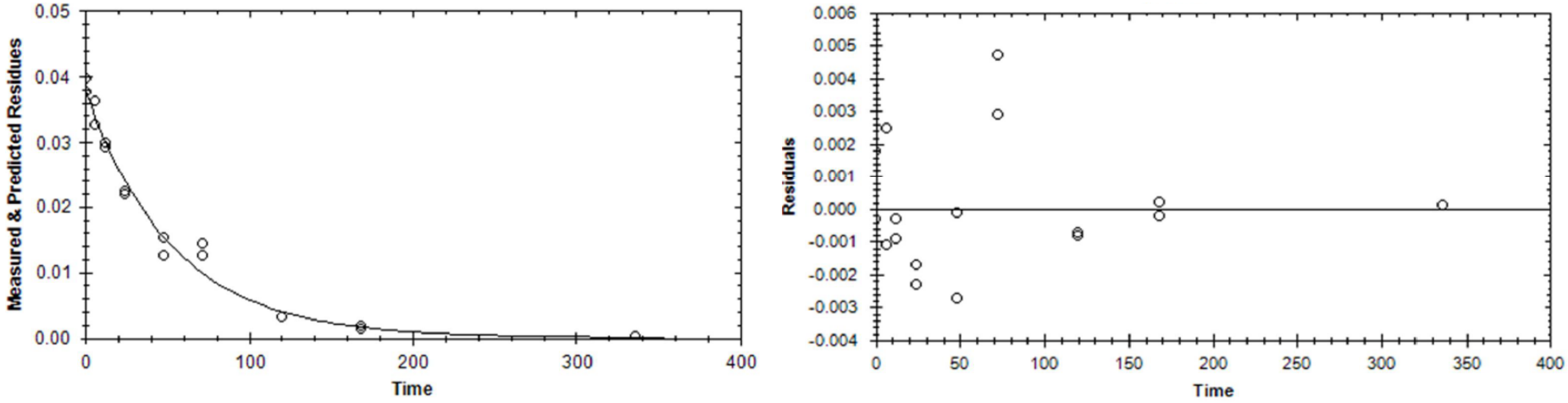


Figure 8.1.1.2-2: Kinetic assessment for gibberellins GA4 in the LUFA 2.2 soil (DFOP)

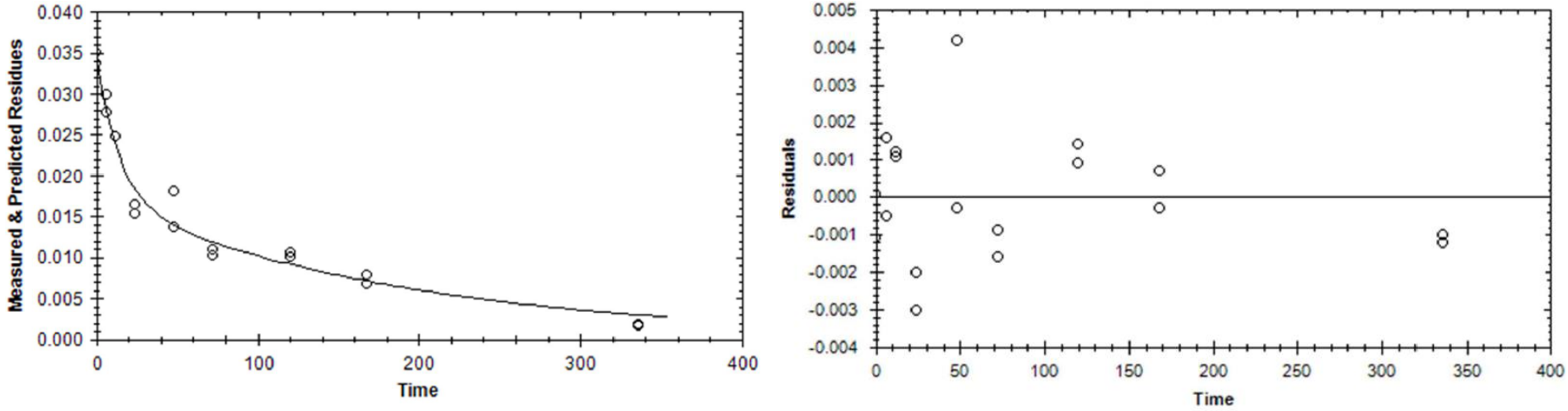


Figure 8.1.1.2.-3: Kinetic assessment for gibberellins GA4 in the LUFA 2.3 soil (FOMC)

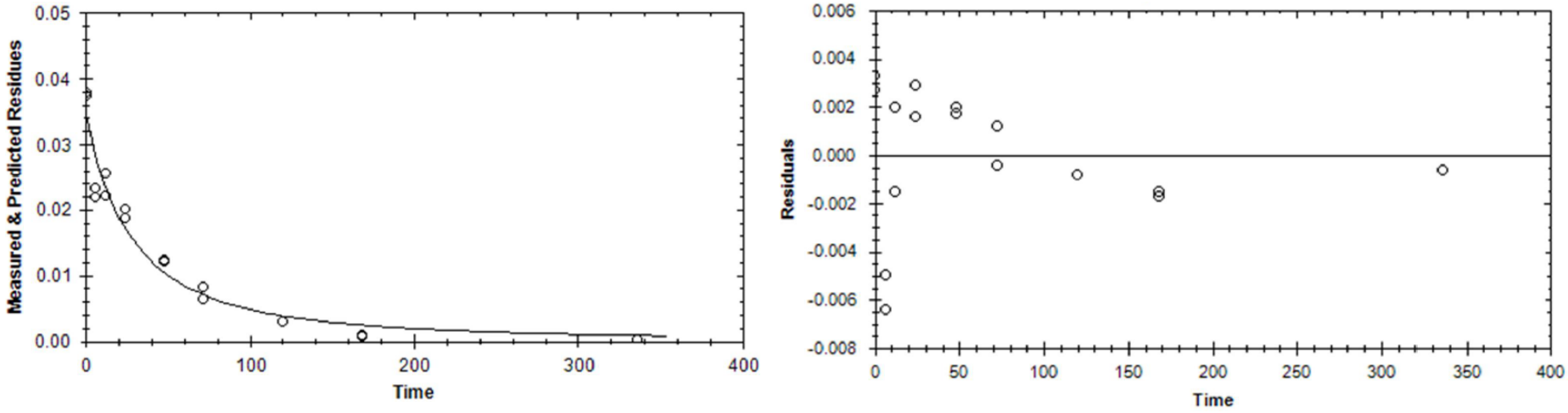


Figure 8.1.1.2-4: Kinetic assessment for gibberellins GA4 in the LUFA 6S soil (SFO)

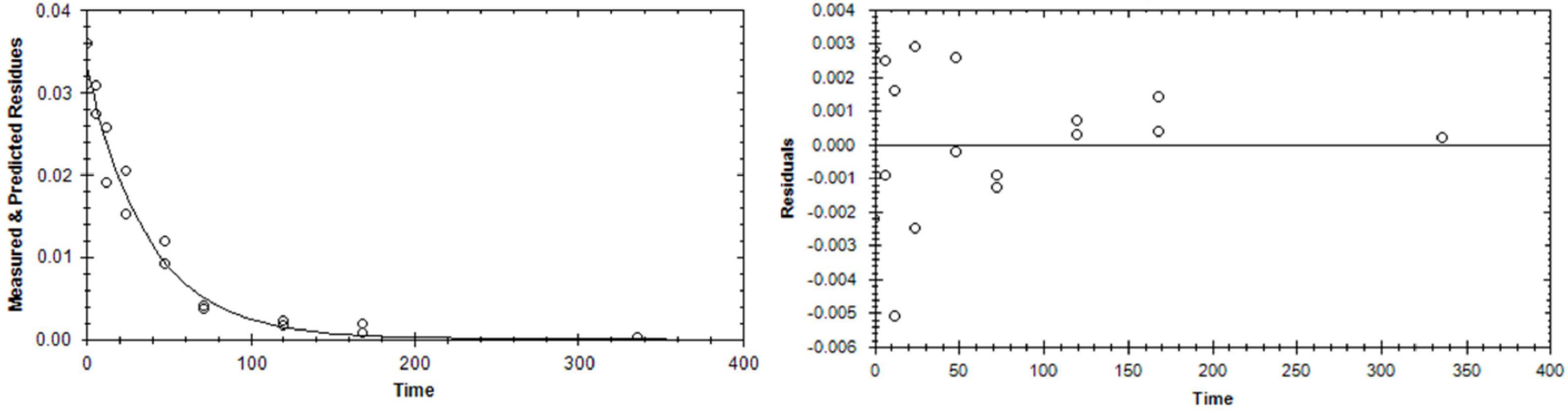


Figure 8.1.1.2-5: Kinetic assessment for gibberellins GA7 in the LUFA 2.1 soil (SFO)

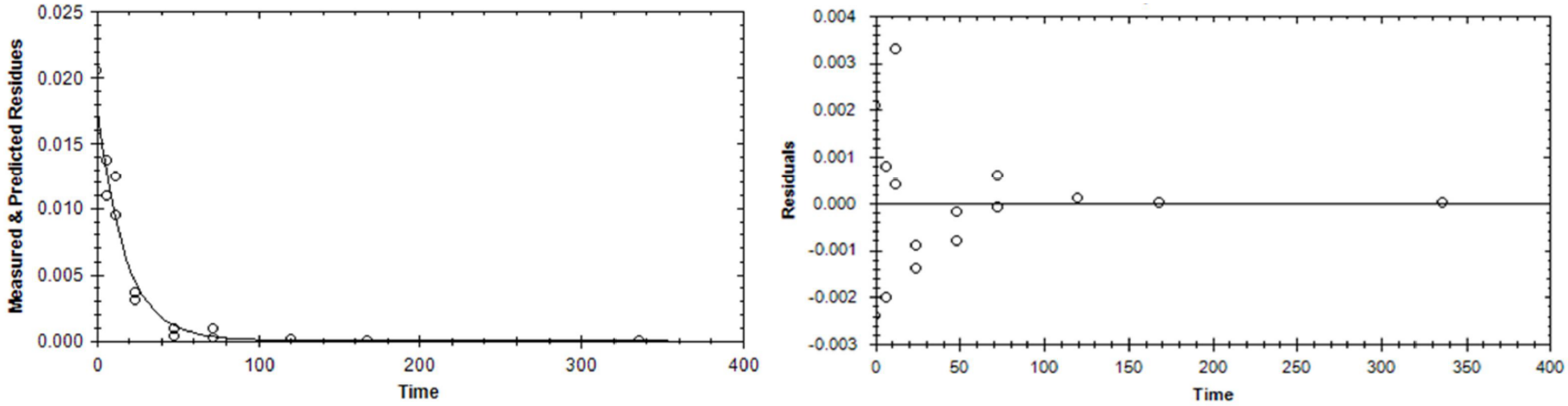


Figure 8.1.1.2-6: Kinetic assessment for gibberellins GA7 in the LUFA 2.2 soil (SFO)

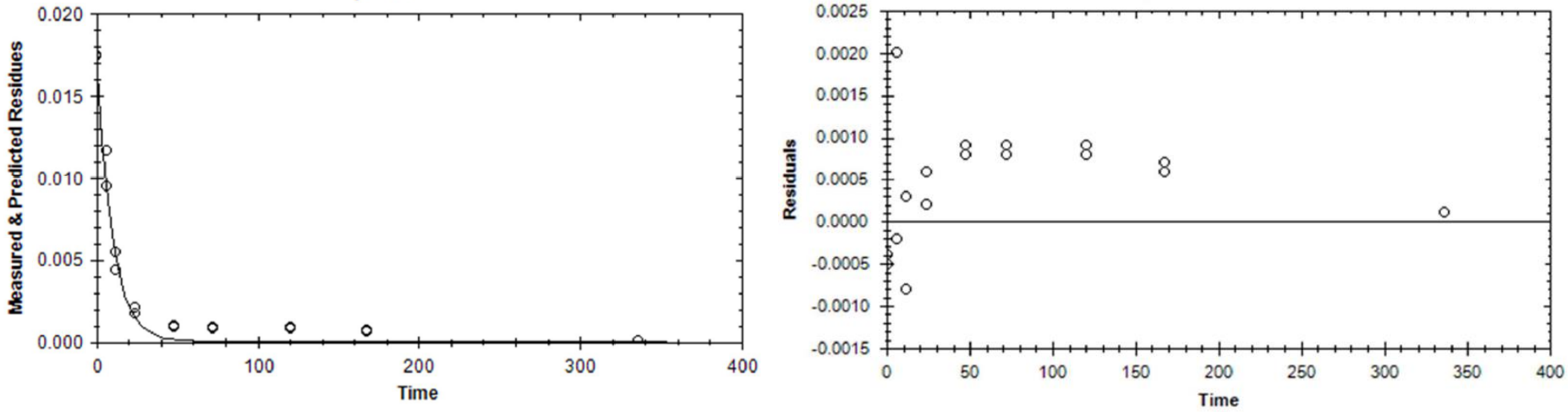


Figure 8.1.1.2-7: Kinetic assessment for gibberellins GA7 in the LUFA 2.3 soil (SFO)

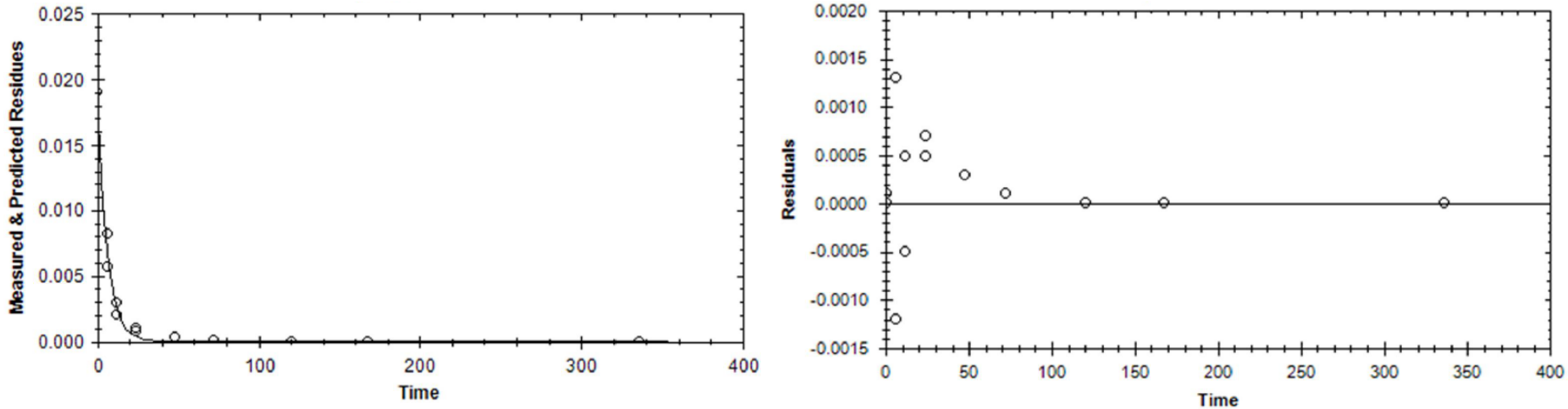
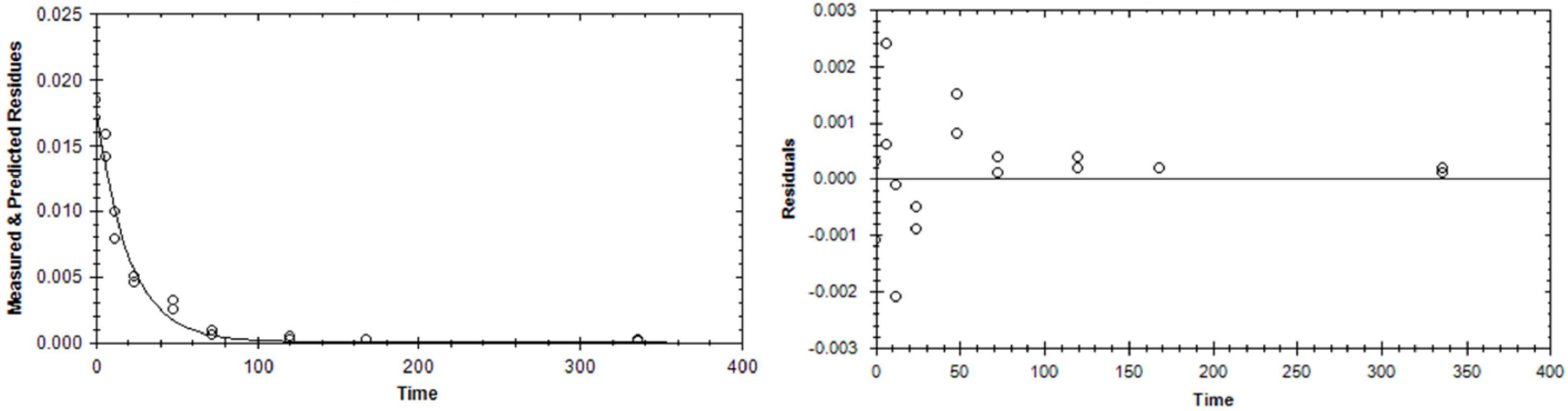


Figure 8.1.1.2-8: Kinetic assessment for gibberellins GA7 in the LUFA 6S soil (SFO)



III. CONCLUSIONS

Gibberellins GA4 and gibberellins GA7 were shown to degrade rapidly in 4 soil types under aerobic conditions (20°C, 45% MWHC). As the study was conducted with non-radiolabelled materials any metabolites were not observed. The degradation rate was faster for gibberellins GA7 compared to gibberellins GA4. The degradation rate was dependent on soil type with the DT_{50} values for gibberellins GA4 and gibberellins GA7 ranging from 24-37 hrs and 4-14 hrs, respectively.

RMS comments and conclusion:

The aim of this study was to determine the degradation rate of the test items in four different soils (German Standard Soil LUFA 2.1, 2.2, 2.3 and 6S) under aerobic conditions at 20°C in the dark. The study was performed with nonlabelled Gibberellins GA4 and GA7 over a period of 14 days. The average soil moisture content was 45% of the maximum water holding capacity (MWHC) over the entire period of the study. The Cation Exchange Capacity was quite low for 3 soils (Lufa 2.1, Lufa 2.2 and Lufa 2.3). The biological activity was checked 14 days after treatment. The application rate of Gibberellins GA4 and GA7 was 5.34 µg (ratio GA4:GA7; 2:1) per vessel containing 100 g (dried weight) soil, which was equivalent to 0.0534 mg Gibberellins/kg soil. Duplicate test systems were worked-up per sampling interval and soil type. The entire soil per flask was extracted three times at ambient temperature and one additional microwave treatment. The combined extracts were analysed for GA4 and GA7 residues by reversed phase high performance liquid chromatography/mass spectrometry (HPLC-MS/MS). The method validation was conducted according the guideline SANCO/3029/99, rev. 4. The limit of quantification (LOQ) of the method was 0.0018 mg/kg for GA4 and 0.0009 mg/kg for GA7. The limit of detection (LOD) was defined as 1% of applied dose and equals 0.356 µg/kg for GA4, respectively 0.178 µg/kg for GA7. The extraction efficiency during the study was demonstrated by concurrent recovery samples. Untreated samples of each soil (LUFA 2.1, 2.2, 2.3 and 6S) were applied with GA4 at 5% of the application amount (0.0018 mg/kg) and with 110% of the application amount (0.0416 mg/kg) and with GA7 at 5% of the application amount (0.0009 mg/kg) and with 110% of the application amount (0.0196 mg/kg). The values of all concurrent recoveries were between 69.9 – 118.1%. In soil LUFA 2.1 Gibberellins were degraded from 106.6% of applied amount to values below 1% of applied amount within 14 days. The degradation of GA7 was faster (values below 1% within 5 days incubation) than the degradation of GA4 (values below 1% within 14 days incubation). In soil LUFA 2.2 Gibberellins were degraded from 97.0% of applied amount to values at 3.7% of applied amount within 14 days. The degradation of GA7 was faster (values below 1% within 14 days incubation) than the degradation of GA4 (values at 5.6% within 14 days incubation). In soil LUFA 2.3 Gibberellins were degraded from 106.2% of applied amount to values below 1% of applied amount within 14 days. The degradation of GA7 was faster (values below 1% within 3 days incubation) than the degradation of GA4 (values below 1% within 14 days incubation). In soil LUFA 6S Gibberellins were degraded from 96.1% of applied amount to values below 1% of applied amount within 14 days. The degradation of GA7 was faster (values below 1% within 7 days incubation) than the degradation of GA4 (values below 1% within 14 days incubation). The analytical results were evaluated according to Focus Guidelines by applying three kinetic models (single first order (SFO), double first order in parallel (DFOP), first order multi compartment (FOMC)) using non-averaged values for each time point.
The DT50 and DT90 values were determined properly.
In the original study even DT50 values with Sum of GA4 and GA7, with ratio 2:1 were presented. RMS considered this results only as supplemental info.

	Gibberellins (Sum of GA4 and GA7; ratio 2:1)			
LUFA Soil	2.1	2.2	2.3	6S
DT_{50} [hours]	22	16	8	20
DT_{90} [hours]	106	200	102	79
Best Fit Kinetic Model	DFOP	DFOP	DFOP	DFOP
Chi^2 Error [%]	7.1	7.9	10.2	7.0

Deviations from Protocol
No major deviations from the protocol were observed.

The study is considered acceptable.
Kinetic output is shown in the Appendix 3 of this document.

Aerobic degradation of metabolites, breakdown and reaction products

Based on the information under Point 7.4.1, studies on the aerobic degradation of metabolites of the active substance gibberellins GA4/7 are not required as no major metabolites that are considered to be of environmental concern (due to the natural occurrence of the active substance), are formed in soil under aerobic conditions.

Anaerobic degradation of the active substance

The degradation of the active substance gibberellins GA4/7 in soil under anaerobic conditions has not been investigated as anaerobic conditions are not relevant for uses of the representative formulation.

Anaerobic degradation of metabolites, breakdown and reaction products

The rate of anaerobic degradation of any metabolites of the active substance gibberellins GA4/7 is addressed under previous points.

B.8.1.1.3. Photo degradation on soil

a) Previous evaluation (2005-2011)

No data were available for photodegradation on gibberellins GA4/7 acid with their metabolites.

b) Evaluation of additional data for the purpose of renewal of Annex I inclusion

No new data were presented by applicant for photodegradation on gibberellins GA4/7 with their metabolites. The molar absorbance coefficients of the individual components gibberellins GA4 and gibberellins GA7 are <10 L/mol/cm at or above wavelength 298 nm and therefore the trigger value of 10 L/mol/cm is not exceeded i.e. soil photolysis is not expected to contribute significantly to the environmental degradation of the active substance due to low light absorbance.

B.8.1.1.4. Field studies

a) Previous evaluation (2005-2011)

Based on the information presented under previous points DT_{50} and DT_{90} values presented, the DT_{50} values for the degradation of the separate components gibberellins GA4 and gibberellins GA7 in the active substance gibberellins GA4/7 in soil (20°C, pF 2) do not exceed 60 days and the DT_{90}s do not exceed 200 days.

The active substance gibberellins GA4/7 can potentially be used in colder climates and the DT_{50} value for the degradation of the separate components gibberellins GA4 and gibberellins GA7 in soil (10°C, pF 2) does not exceed 90 days. Correspondingly, a soil dissipation study for the active substance gibberellins GA4/7 are not triggered.

RMS comments and conclusion:

Agree. No further comment.

b) Evaluation of additional data for the purpose of renewal of Annex I inclusion

No new data were presented by applicant. RMS agree with applicant, that new field data are NOT necessary.

Soil accumulation studies

As described under previous points, soil dissipation studies for the active substance gibberellins GA4/7 are not triggered.

Correspondingly, soil accumulation studies for the active substance gibberellins GA4/7 are not triggered.

Summary of degradation in soil

The route and rate of aerobic degradation of the individual components gibberellins GA4 and gibberellins GA7 of the active substance gibberellins GA4/7 was investigated in four soil types (ranging from loamy sand to clay loam) of varying origin in the dark under laboratory conditions at a temperature of 20°C and moisture content of 100% pF 2. The individual components gibberellins GA4 and gibberellins GA7 were degraded extensively in soil. Numerous degradation products were observed but not fully identified. Ultimate degradation led to the formation of un-extracted soil residues and mineralisation to carbon dioxide.

For both gibberellins GA4 and gibberellins GA7 the best-fit persistence half-lives were <2 days in all soil types (and a worst-case value of 2 days is used for PEC generation). The DT_{50} and DT_{90} values are summarised in Table 8.1.1.4-1. The available data clearly indicate that the active substance gibberellins GA4/7 and its individual components gibberellins GA4 and gibberellins GA7 are not persistent in soil. Therefore, in accordance with Regulation (EC) No. 1272/2008, gibberellins GA4/7 fulfils the criteria for consideration as a low-risk active substance in this regard.

Table 8.1.1.4-1: DT_{50} of gibberellins GA4 and gibberellins GA7 (FOCUS persistence endpoints)

Soil	Kinetic Model	Degradation rate (days)		Chi2err (%)
		DT_{50}	DT_{90}	
gibberellins GA4				
CA 7.1.1.1/01 (conducted at 20°C and pF 2 moisture content)				
Speyer 5M	SFO	0.171	0.568	3.188
Speyer 2.2	SFO	0.347	1.153	20.75
Brierlow	SFO	0.392	1.303	18.61
South Witham	SFO	0.104	0.346	28.78
CA 7.1.2.1.1/01 (conducted at 20°C and 45% MWHC)				
Soil LUFA 2.1	SFO	1.542	5.083	7.2
Soil LUFA 2.2	DFOP	1.125	12.583	7.2
Soil LUFA 2.3	FOMC	1.000	5.333	14.7
Soil LUFA 6S	SFO	1.083	3.625	5.6
gibberellins GA7				
CA 7.1.1.1/01 (conducted at 20°C and pF 2 moisture content)				
Speyer 5M	SFO	0.060	0.200	13.57
Speyer 2.2	SFO	0.064	0.212	13.20
Brierlow	SFO	0.132	0.440	16.67
South Witham	SFO	0.011	0.036	1.005
CA 7.1.2.1.1/01 (conducted at 20°C and 45% MWHC)				
Soil LUFA 2.1	SFO	0.500	1.625	12.2
Soil LUFA 2.2	SFO	0.292	0.917	12.3
Soil LUFA 2.3	SFO	0.167	0.583	5.2
Soil LUFA 6S	SFO	0.583	1.917	11.3

The degradation of the active substance gibberellins GA4/7 in soil under anaerobic conditions has not been investigated as anaerobic conditions are not relevant for uses of the representative formulation.

The molar absorbance coefficients of the individual components gibberellins GA4 and gibberellins GA7 are <10 L/mol/cm at or above wavelength 298 nm and therefore the trigger value of 10 L/mol/cm is not exceeded i.e. soil photolysis is not expected to contribute significantly to the environmental degradation of the active substance due to low light absorbance.

B.8.1.2. Adsorption and desorption in soil

The proposed use of plant protection products containing the active substance gibberellins GA4/7 will result in contact with soil, therefore the soil sorption characteristics of the active substance and any major metabolites (as defined under Point CA 7.4.1) are investigated, respectively in laboratory studies according to the data requirements laid down in EC Regulation 283/2013.

a) Previous evaluation (2005-2011)

The Notifier Globachem NV has submitted only a Koc calculation using the model KOCWIN as part of EPISUITE software package (Syracuse Research Corporation, SRC). Calculations were performed from log Kow. The study is summarised below:

Data point addressed:	CA 7.1.3.1.1/01
Author(s) (year):	Ville C., Beltrán E. and Adrian P., (2005).
Title:	**QSAR (Quantitative Structure-Activity Relationship) – Gibberellins GA4/7.**
Document No:	**/**
Testing facility:	CEHTRA, France
Published:	Unpublished
Test guidelines used:	The group contribution method outperforms traditional estimation methods based on octanol/water partition coefficients and water solubility. (calculations)
Deviations:	/
GLP:	Not applicable (calculations)
Test material:	GA4/GA7
Batch #/purity:	/
Status:	**Previously submitted**

Conclusion of the previous review (2005-2011): The study was considered as acceptable.

The Koc value of Gibberellins was determined using the PCKOCWIN™ software which is part of the EPIWIN software, owned by US EPA. The Soil Adsorption Coefficient Program (PCKOCWIN) estimates the soil adsorption coeffiecient (Koc) of organic compounds. Koc can be defined as "the ratio of the amount of chemical adsorbed per unit weight of organic carbon (oc) in the soil or sediment to the concentration of the chemical in solution at equilibrium"; it is represented by the following equation (Lyman, 1990):
Koc = (µg adsorbed/g organic carbon) / (µg/mL solution)
Koc provides an indication of the extent to which a chemical partitions between solid and solution phases in soil, or between water and sediment in aquatic ecosystems. Estimated values of Koc are often used in environmental fate assessment because measurement of Koc is expensive. Traditional estimation methods rely upon the octanol/water partition coefficient or related parameters, but recently the first-order molecular connectivity index (1-MCI) has been used successfully to predict Koc values for hydrophobic organic compounds. PCKOCWIN uses 1-MCI and a series of group contribution factors to predict Koc. The group contribution method outperforms traditional estimation methods based on octanol/water partition coefficients and water solubility.
Later on The Notifier Globachem NV submitted a new Koc calculation using the model KOCWIN v2.00 as part of EPISUITE 4.0 software package (Syracuse Research Corporation, SRC). Calculations were performed from logKow. The endpoint concerned by the KOCWIN v2.00 prediction was the soil adsorption/desorption. The predicted variable is Koc, which is the soil/water partition coefficient related to soil organic carbon.
Koc calculated from logKow (from Doucette, 2000):

For non-polar compound (no correction factor):
log Koc = 0.8679 Log Kow - 0.0004
(n = 68, r2 = 0.877, std dev = 0.478, avg dev = 0.371)

With correction factors:
log Koc = 0.55313 Log Kow + 0.9251 + ΣPfN
where ΣPfN is the summation of the products of all applicable correction factor coefficients multiplied by the number of times (N) that factor is counted for the structure.

Results obtained with KOCWIN v2.00

Chemical name	CAS number	M (g/mol)	logKow Measured	KOCWIN v2.00 – logKow method
Gibberellins GA4	468-44-0	332.40	0.146	0.5747
Gibberellins GA7	510-75-8	330.38	0.146	0.5747

It is to note that the Koc of this structure may be sensitive to pH. The estimated Koc represents a best-fit to the majority of experimental values however; the Koc may vary significantly with pH.

RMS comments and conclusion:

For the Annex I inclusion this calculation was acceptable, but in now days more information is needed and new study is necessary.

b) Evaluation of additional data for the purpose of renewal of Annex I inclusion

The sorption properties of the individual components gibberellins GA4 and gibberellins GA7 in the active substance gibberellins GA4/7 have been investigated in one "new" study (CA 7.1.3.1.1/01) which has been conducted since the original Annex I inclusion to fully address the requirements of Regulation EC 283/2013. The study is summarised below:

Data point addressed:	CA 7.1.3.1.1/01
Author(s) (year):	Hurst, L (2013).
Title:	**[^{14}C]-GA4 and [^{14}C]-GA7: Adsorption/Desorption in soil**
Document No:	3200134
Testing facility:	Smithers Viscient (ESG) Ltd., UK
Published:	Unpublished
Test guidelines used:	OECD Guideline 106 (January 2000)
Deviations:	None
GLP:	Yes
Test material:	GA4/GA7
Batch #/purity:	see below
Status:	**New submission**

EXECUTIVE SUMMARY

The soil sorption properties of the individual components gibberellins GA4 and gibberellins GA7 of the active substance gibberellins GA4/7 were investigated in four soils (UK origin) at five concentrations (0.05 – 5 µg/mL) using the batch equilibrium technique with a soil to solution ratio of 1:1 w/v and a 3 hr equilibration time. The sorption parameters determined were Kf (0.19-1.32 mL/g), K_{FOC} 4-165 mL/g and 1/n (0.9611-0.9706) for

gibberellins GA4 and Kf (0.22-1.33 mL/g), K_{FOC} (4-166 mL/g) and 1/n (0.9802-1.0464) for gibberellins GA7.

I. MATERIALS AND METHODS

A. TEST AND REFERENCE MATERIALS

1 Test material 1	Radiolabelled gibberellins GA4 (structure listed in Appendix 1)
Radiolabel position	17-^{14}C-Gibberellins GA4 (see Appendix 1)
Radiochemical purity	Details from Certificate of Analysis: >99% by HPLC RCP details determined analytically at the time of treatment: 99.72% (based on chromatogram shown)
Lot/batch no.	TH-090413-^{14}C-GA4, specific activity 6.122 MBq/mg
Stability of test compound	Not stated
Test material 2	Radiolabelled gibberellins GA7 (structure listed in Appendix 1)
Radiolabel position	17-^{14}C-Gibberellins GA7 (see Appendix 1)
Radiochemical purity	Details from Certificate of Analysis: >99% by HPLC RCP details determined analytically at the time of treatment: 98.33% (based on chromatogram shown)
Lot/batch no.	TH-090413-^{14}C-GA7, specific activity 6.159 MBq/mg
Stability of test compound	Not stated
2 Reference material 1	Non-radiolabelled gibberellins GA4 (note: name used in report gibberellic acid 4)
Purity	100%
Lot/batch no.	91-932-BD, expiry date 11-Jul-2017 (certificate provided)
Reference material 2	Non-radiolabelled gibberellins GA7 (note: name used in report gibberellic acid 7)
Purity	92.1%
Lot/batch no.	65753-145, expiry date 11-Jul-2017 (certificate provided)

Test soils

The soils were selected to provide an appropriate range of organic carbon contents, soil pH values, clay contents and soil classifications. Soil characteristics are presented in Table 8.1.2-1. Prior to use soils were air-dried, sieved through a 2 mm sieve and stored in the dark at room temperature. Prior to application of the test item 10 g of soils were dispensed into individual Teflon test vessels, sterilised by autoclaving, and equilibrated with 9 mL of sterilised 0.01 M calcium chloride solution by shaking overnight. No pesticides had been applied to the test soils in the last 5 years.

Table 8.1.2-1: Summary of soil characteristics

Characteristic	Empingham	Kenslow	Brierlow	Worsop
Particle size distribution, % w/w (UK)[1]				
Clay (<2 µm)	26	16	15	4
Silt (2-63 µm)	39	45	61	7
Sand (63-2000 µm)	35	39	24	89
Textural classification (UK)	clay loam	sandy silt loam	sandy silt loam	sand
pH (H2O)	7.8	7.8	6.4	4.8
pH (CaCl2)	7.4	7.3	5.6	3.8

Characteristic	Empingham	Kenslow	Brierlow	Worsop
Organic matter content (%)	9.0	6.6	4.7	1.4
Organic carbon content (%)	5.2	3.8	2.7	0.8
CEC[2] (meq/100g)	46.1	19.5	22.3	7.4
Moisture, pF 0 (w/w %)	70.5	61.8	33.2	34.9
Moisture, pF 2.0 (w/w %)	36.0	32.3	27.2	7.0

(1) UK particle size distribution, (2) Cation exchange capacity

B. STUDY DESIGN

1 Preliminary investigations	Preliminary tests below were conducted to determine the experimental conditions to be used for the definitive test (i.e. the adsorption and desorption isotherms).
Solubility	Maximum concentration used in 0.01 M calcium chloride was 5 µg/mL (quoted pure water solubility at 20°C was 127 mg/L). Solubility of gibberellins GA4 and gibberellins GA7 was confirmed in 50 µg/mL 0.01 M calcium chloride (by centrifugation and quantification of the supernatant).
Adsorption to experimental containers	Using 0.05 µg/mL gibberellins GA4 and gibberellins GA7 in 0.01 M calcium chloride in plastic and Teflon tubes. Solutions were shaken for 24 hrs and then re-quantified by LSC.
Determination of soil to solution ratio	The soil:solution ratio of 1:1 (w/v) was pre-determined in the definitive protocol due to previously known properties of the test item.
Adsorption equilibrium time determination	Duplicate soil samples (*ca* 10 g dw) for each soil and each test substance were pre-equilibrated with 0.01 M calcium chloride (9 mL) overnight prior to treatment with the test substances. Following pre-equilibration, treatment solutions (1 mL) for each test substance were added to the soil slurries. Slurries were shaken for 3, 6, 24 and 48 hrs and taken for analysis. Slurries were centrifuged and the water layer quantified by LSC.
Stability over test duration	The soil samples (following extraction with 2x 20 mL methanol and 20 mL acetone) and supernatant solutions from the 48 hr samples recovered from the preliminary adsorption equilibration time determination above were analysed by HPLC. Due to stability issues, further soil samples and supernatant solutions were analysed as above (Kenslow soil, 3, 6 and 24 hrs). On the basis of these results, the adsorption equilibration time was chosen.
Desorption equilibrium time determination	Based on adsorption equilibration time i.e. not determined experimentally.

2 Definitive Investigations	
Soil to solution ratio	1:1 for all soils
Pre-equilibration	Duplicate soil samples (*ca* 10 g dw) were sterilised (autoclave) and pre-equilibrated with 0.01 M calcium chloride (9 mL, sterilised) overnight prior to treatment with the test substance.
Treatment solutions	Appropriate treatment solutions were prepared in 0.01 M calcium chloride for each treatment level.
Concentrations	0.05, 0.1, 0.5, 1.0 and 5 µg/mL
Equilibration time	Adsorption phase (3 hrs) and desorption phase (3 hrs)
Adsorption phase	Following pre-equilibration, treatment solution (1 mL) was added to the soil slurries. After the equilibration period (adsorption step) the layers were separated by centrifugation. The water layer was quantified directly by LSC, the soil layer was quantified indirectly (OECD 106).
Desorption phase	The water layer was replenished with fresh 0.01 M calcium chloride (sterilised), the soil shaken to break up the compacted layer and shaken further for the equilibration period (desorption step). The layers were separated by centrifugation. The water layer was quantified by LSC. The soil layer was quantified indirectly.
Mass balance	Mass balance determination was performed on the post-desorption samples for each soil type for the highest concentration only. Following desorption, soil samples were extracted with methanol (3 x 20 mL) and rinsed with acetone (assist drying) prior to being air-dried and ground. The methanol extracts were combined and quantified by LSC. The level of radioactivity in the soil was quantified by combustion and LSC.

3 Method of analysis

1 Method of analysis for buffer samples	
Sample extraction and/or work-up/concentration	No extraction or sample concentration was performed.
Analytical method, primary	HPLC: Hichrom ACE 5 C18 column (25 cm L x 4.6 mm id), mobile phase – isocratic system a) 0.2% aqueous hydrochloric acid, b) acetonitrile:methanol (1:1 v/v), 0 mins 40%A, 25 mins 40% A. Flow rate 1 mL/min. ^{14}C detection β-ram, (Lablogic), liquid cell (Flow logic or Ultima-Flo M scintillant, 2 mL/min). UV detection (210 nm). Co-chromatography with non-radiolabelled reference standards. LOD – <1% AR.
Analytical method, secondary/ confirmatory	TLC: Whatman KC2F TLC plates using solvent system methanol/water/acetic acid (8:1.5:0.5 v/v/v). ^{14}C detection Fuji BAS 1500 Bio-image analyser using Tina evaluation software (v 2.09g). Co-chromatography with non-radiolabelled reference standards.

II. RESULTS AND DISCUSSION

A. PRELIMINARY INVESTIGATIONS

Solubility test
Solubility of both gibberellins GA4 and gibberellins GA7 were confirmed in 0.01 M calcium chloride at a concentration of 50 µg/mL.

Adsorption to containers
No adsorption was noted to Teflon tubes when using a soil to solution ratio of 1:1 w/v and concentration of 0.05 µg/mL.

Soil to solution ratio
A soil to solution ratio of 1:1 w/v was used.

Stability and equilibration time determination
The analysis of soil extracts and supernatant solutions following 48 hrs is presented in Table 8.1.2-2.

Table 8.1.2-2: Preliminary experiment: Stability of test substances after 48 hrs equilibration

Test substance	Soil type	% of chromatogram attributed		
		Parent	Number of degradates	Largest degradate
gibberellins GA4	Empingham	95.8	4	1.1
	Kenslow	98.1	1	1.7
	Brierlow	93.7	5	2.6
	Warsop	86.2	3	8.4
gibberellins GA7	Empingham	86.3	6	5.1
	Kenslow	85.8	5	6.2
	Brierlow	87.9	4	4.8
	Warsop	84.1	4	8.5

Most degradation was observed in the Warsop soil for the gibberellins GA7 samples. As the level of test substance remaining after 48 hrs was *ca* 85% in the Warsop soil, shorter equilibration times were investigated, as summarised in Table 8.1.2-3.

Table 8.1.2-3: Preliminary experiment: Stability of gibberellins GA7 in Warsop soil after 3-24 hrs equilibration

Sampling interval	% of chromatogram attributed		
	Parent	Number of degradates	Largest degradate
24 hr	91.6	2	4.0
6 hr	96.1	2	2.6
3 hr	96.5	2	1.8

Based on these results, a 3 hr adsorption equilibration time was used for the definitive experiment.

B DEFINITIVE TEST

Isotherms
The concentration of gibberellins GA4 and gibberellins GA7 in the soil and water layers for the isotherms are provided in the study report. The proportions of gibberellins GA4 adsorbed to the soil layer were 13.8-20.6, 15.5-

18.9, 30.5-35.0 and 55.8-61.5% for the Empingham, Kenslow, Brierlow and Warsop soils, respectively. The proportions of gibberellins GA7 adsorbed to the soil layer were 14.4-22.5, 15.0-19.5, 31.8-35.7 and 54.5-59.8% respectively.

A summary of the partition and Freundlich coefficients for the adsorption step is provided in Table 8.1.2-4.

Table 8.1.2-4: Summary of partition and freundlich coefficients (adsorption step)

Soil	Concentration (µg/mL)	Partition coefficient		Freundlich coefficient			
		Kd	Koc	Kf	Kfoc	1/n	r2
Gibberellins GA4							
Empingham	5	0.17	3	0.19	4	0.9614	0.9960
	1	0.20	4				
	0.5	0.21	4				
	0.1	0.19	4				
	0.05	0.23	4				
Kenslow	5	0.19	5	0.19	5	0.9673	0.9985
	1	0.19	5				
	0.5	0.20	5				
	0.1	0.21	5				
	0.05	0.22	6				
Brierlow	5	0.45	17	0.46	17	0.9706	0.9990
	1	0.46	17				
	0.5	0.49	18				
	0.1	0.50	19				
	0.05	0.51	19				
Warsop	5	1.26	157	1.32	165	0.9611 (avg 0.97)	0.9997
	1	1.38	172				
	0.5	1.45	181				
	0.1	1.44	180				
	0.05	1.54	193				

Soil	Concentration (µg/mL)	Partition coefficient		Freundlich coefficient			
		Kd	Koc	Kf	Kfoc	1/n	r2
Gibberellins GA7							
Empingham	5	0.25	5	0.22	4	1.0464	0.9956
	1	0.17	3				
	0.5	0.21	4				
	0.1	0.19	4				
	0.05	0.21	4				
Kenslow	5	0.23	6	0.22	6	1.0296	0.9985
	1	0.20	5				
	0.5	0.21	6				
	0.1	0.21	5				
	0.05	0.19	5				
Brierlow	5	0.50	19	0.50	18	0.9822	0.9994
	1	0.49	18				
	0.5	0.49	18				
	0.1	0.52	19				
	0.05	0.54	20				
Warsop	5	1.27	159	1.33	166	0.9802 (avg 1.01)	0.9992
	1	1.42	177				
	0.5	1.36	170				
	0.1	1.46	183				
	0.05	1.37	171				

The Freundlich soil sorption coefficient, normalised for organic carbon content (K_{FOC}), ranged from 4 to 165 mL/g (n=4) for gibberellins GA4 and from 4 to 166 mL/g for gibberellins GA7.

The corresponding partition and Freundlich coefficients for the desorption step are also provided in the study report.

Mass Balance
The recovery of applied radioactivity from the isotherm (determined at the highest concentration only) ranged from 96.0 to 100.1% AR for gibberellins GA4 and from 95.3 to 98.2% AR for gibberellins GA7.

III CONCLUSIONS

The soil sorption properties of the individual components of the active substance gibberellins GA4/7, gibberellins GA4 and gibberellins GA7 were investigated in four soils (UK origin) at five concentrations (0.05 – 5 µg/mL) using the batch equilibrium technique with a soil to solution ratio of 1:1 w/v and a 3 hr equilibration time. The sorption parameters determined were Kf (0.19-1.32 mL/g), Koc 4-165 mL/g and 1/n (0.9611-0.9706) for gibberellins GA4 and Kf (0.22-1.33 mL/g), Koc (4-166 mL/g) and 1/n (0.9802-1.0464) for gibberellins GA7.

RMS comments and conclusion:

The adsorption/desorption characteristics of [14C]-GA4 and [14C]-GA7 were determined in four soils. All soils were UK; Empingham (clay loam); Kenslow (sandy silt loam); Brierlow (sandy silt loam); and Warsop (sand). The amount of organic matter in soil was in average in 3 soils quite high, with exception of Warsop soil. From our point of view such soils do not clearly represents typical agricultural soils in Europe, but the best soils in Europe. Following interpretation of the results obtained during the preliminary tests, the main isotherm test was performed using Teflon® tubes, a soil : solution ratio of 1:1 (w/v, 10 g dry weight equivalent of soil and 10 mL solution) and adsorption and desorption times of 3 hours each. Stability was verified by high performance liquid chromatography (HPLC) of

adsorption supernatants and soil extracts of samples from each soil that had been mixed for a 6 hour adsorption equilibrium period. Radioactivity could, therefore, be used to determine GA4 and GA7 concentrations.

The definitive adsorption assessment was conducted in the dark at 20 ± 2°C. Soil samples (10 g dry weight equivalent) were pre-equilibrated with 0.01 M calcium chloride solution (9 mL) overnight. The equilibrated soils were then treated with calcium chloride solutions of [14C]-GA4 or [14C]-GA7 (1 mL) to produce duplicate samples per soil. Concentrations of test item in the aqueous phase were initially 0.05, 0.1, 0.5, 1 and 5 µg/mL. The adsorption phase was followed by a single desorption phase.

Freundlich adsorption coefficients (KFOC) were in the range 4 to 166 L/kg for both GA4 and GA7. The range of 1/n values was 0.9611 to 1.0464 (for GA4 and GA7). Freundlich desorption coefficients KFOCdes were in the range 3 to 230 L/kg, with 1/n values in the range of was 0.9335 to 1.0245. All values quoted are for both GA4 and GA7.

Overall recoveries (mass balance) of applied radioactivity from the highest concentration treated soil samples following a 3 hour adsorption and 3 hour desorption phase were in the range 95 to 100%, with an average of 98%.

Using the McCall Classification scale to assess the potential mobility of a chemical in soil (based on KFOC), both GA4 and GA7 can be classified as having “very high potential mobility” in Empingham, Kenslow and Brierlow soils and as having “medium mobility” in Warsop soil.

The study is considered acceptable and can be used for further assessment.

Summary of K_d, K_{OC}, K_F, K_{FOC} and 1/n values from the adsorption step

GA4

Soil	Nominal Rate Applied Aqueous Phase (µg/mL)	Partition Coefficient		Freundlich Coefficient			
		K_d	K_{OC}	K_F	K_{FOC}	1/n	r^2
Empingham	5	0.17	3	0.19	4	0.9614	0.9960
	1	0.20	4				
	0.5	0.21	4				
	0.1	0.19	4				
	0.05	0.23	4				
Kenslow	5	0.19	5	0.19	5	0.9673	0.9985
	1	0.19	5				
	0.5	0.20	5				
	0.1	0.21	5				
	0.05	0.22	6				
Brierlow	5	0.45	17	0.46	17	0.9706	0.9990
	1	0.46	17				
	0.5	0.49	18				
	0.1	0.50	19				
	0.05	0.51	19				
Warsop	5	1.26	157	1.32	165	0.9611	0.9997
	1	1.38	172				
	0.5	1.45	181				
	0.1	1.44	180				
	0.05	1.54	193				

GA7

Soil	Nominal Rate Applied Aqueous Phase (µg/mL)	Partition Coefficient		Freundlich Coefficient			
		K_d	K_{OC}	K_F	K_{FOC}	1/n	r^2
Empingham	5	0.25	5	0.22	4	1.0464	0.9956
	1	0.17	3				
	0.5	0.21	4				
	0.1	0.19	4				
	0.05	0.21	4				
Kenslow	5	0.23	6	0.22	6	1.0296	0.9985
	1	0.20	5				
	0.5	0.21	6				
	0.1	0.21	5				
	0.05	0.19	5				
Brierlow	5	0.50	19	0.50	18	0.9822	0.9994
	1	0.49	18				
	0.5	0.49	18				
	0.1	0.52	19				
	0.05	0.54	20				
Warsop	5	1.27	159	1.33	166	0.9802	0.9992
	1	1.42	177				
	0.5	1.36	170				
	0.1	1.46	183				
	0.05	1.37	171				

All units (except for 1/n and r^2) are L/kg

Summary of K_d, K_{OC}, K_F, K_{FOC} and 1/n values from the desorption step

GA4

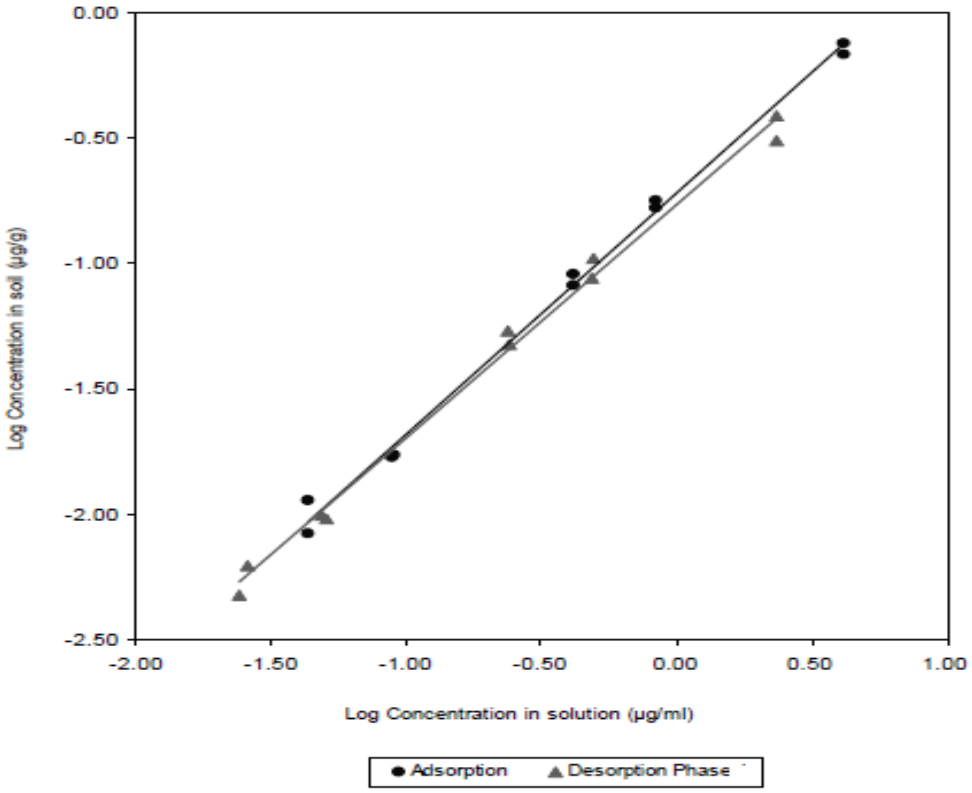
Soil	Nominal Rate Applied Aqueous Phase (µg/mL)	Partition Coefficient		Freundlich Coefficient			
		K_d	K_{OC}	K_F	K_{FOC}	1/n	r^2
Empingham	5	0.15	3	0.17	3	0.9335	0.9940
	1	0.20	4				
	0.5	0.21	4				
	0.1	0.20	4				
	0.05	0.22	4				
Kenslow	5	0.18	5	0.18	5	0.9407	0.9982
	1	0.20	5				
	0.5	0.20	5				
	0.1	0.21	6				
	0.05	0.24	6				
Brierlow	5	0.50	19	0.52	19	0.9625	0.9997
	1	0.54	20				
	0.5	0.56	21				
	0.1	0.58	21				
	0.05	0.60	22				
Warsop	5	1.69	211	1.75	218	0.9534	0.9996
	1	1.90	237				
	0.5	1.98	248				
	0.1	2.02	253				
	0.05	2.14	268				

GA7

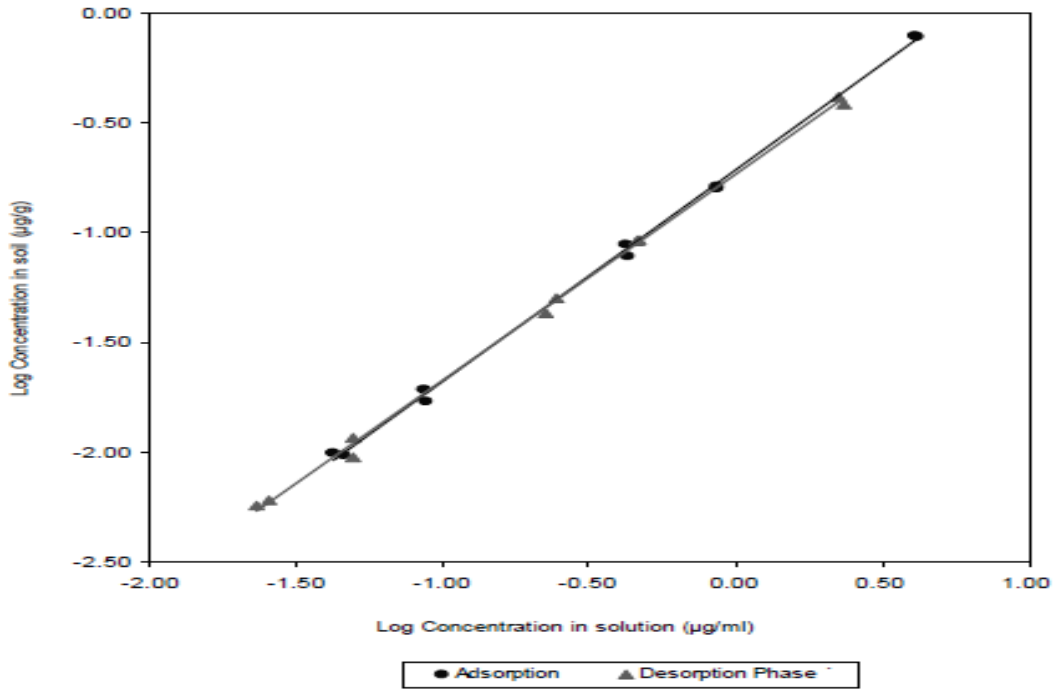
Soil	Nominal Rate Applied Aqueous Phase (µg/mL)	Partition Coefficient		Freundlich Coefficient			
		K_d	K_{OC}	K_F	K_{FOC}	1/n	r^2
Empingham	5	0.25	5	0.23	4	1.0245	0.9956
	1	0.18	3				
	0.5	0.22	4				
	0.1	0.20	4				
	0.05	0.23	4				
Kenslow	5	0.25	7	0.22	6	1.0130	0.9969
	1	0.20	5				
	0.5	0.21	5				
	0.1	0.22	6				
	0.05	0.23	6				
Brierlow	5	0.60	22	0.58	22	0.9724	0.9988
	1	0.56	21				
	0.5	0.59	22				
	0.1	0.64	24				
	0.05	0.67	25				
Warsop	5	1.83	229	1.84	230	0.9885	0.9992
	1	1.92	239				
	0.5	1.84	229				
	0.1	2.00	250				
	0.05	1.88	235				

All units (except for 1/n and r^2) are L/kg

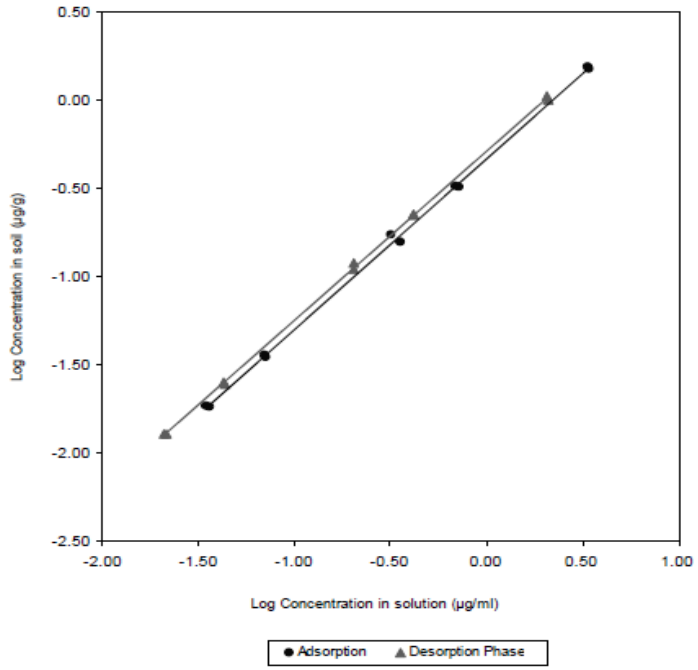
Adsorption and desorption isotherms for [^{14}C]-GA4 on Empingham soil



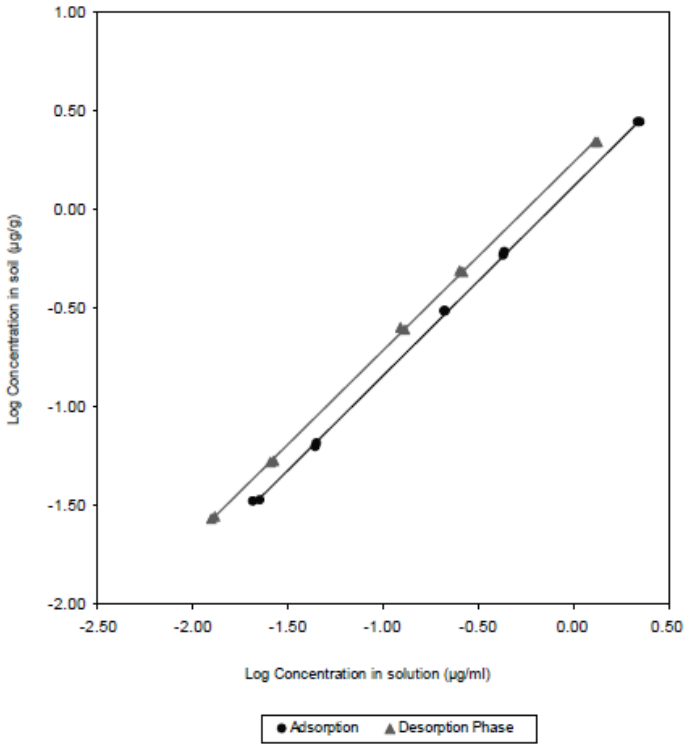
Adsorption and desorption isotherms for [^{14}C]-GA4 on Kenslow soil



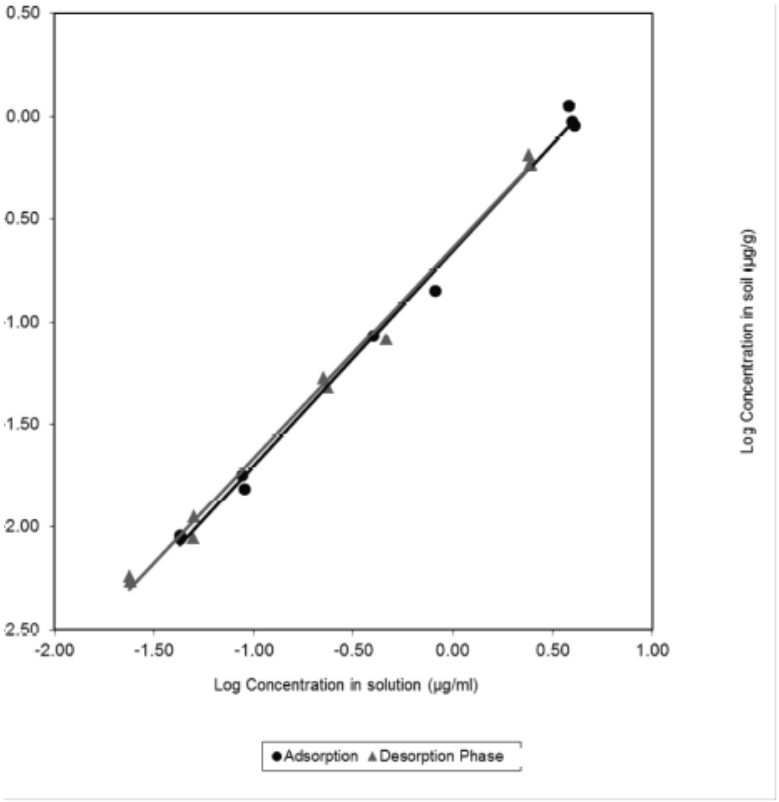
Adsorption and desorption isotherms for [^{14}C]-GA4 on Brierlow soil



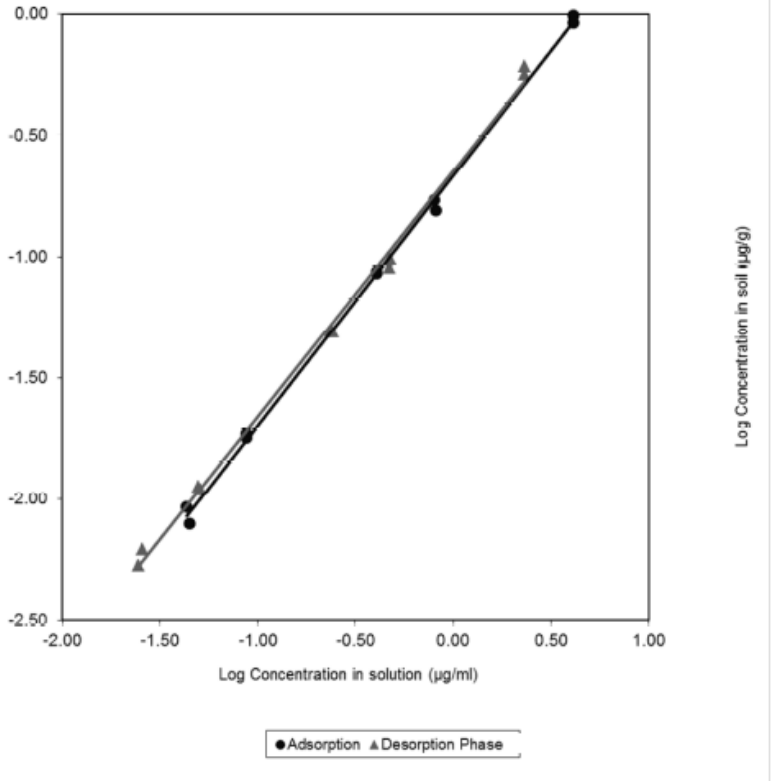
Adsorption and desorption isotherms for [^{14}C]-GA4 on Warsop soil



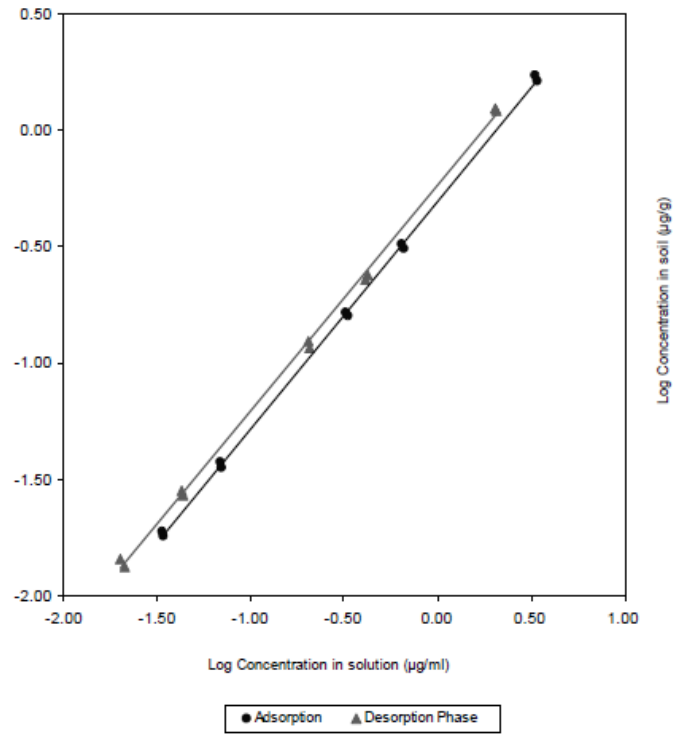
Adsorption and desorption isotherms for [^{14}C]-GA7 on Empingham soil

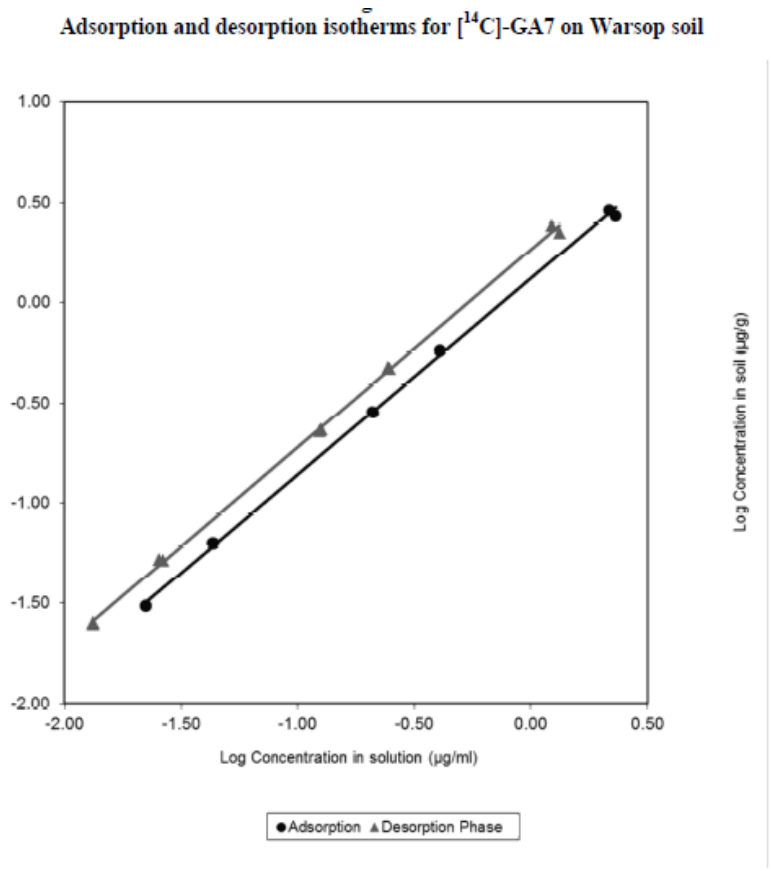


Adsorption and desorption isotherms for [^{14}C]-GA7 on Kenslow soil



Adsorption and desorption isotherms for [^{14}C]-GA7 on Brierlow soil





B.8.1.2.1 Adsorption and desorption of metabolites, breakdown and reaction products
Information on the aerobic soil metabolism of the active substance GA4/7 is provided under Point CA 7.1.1. Although several major metabolites of gibberellins GA4 and gibberellins GA7 were observed in soil, these are considered to be of no environmental concern due to the natural occurrence of the active substance and are therefore not considered further.

Aged sorption

Studies investigating the aged sorption of the active substance are a higher tier option and are not currently triggered or considered necessary.

B.8.1.3. Mobility in soil

Leaching studies
Lysimeter studies investigating the leaching potential of the active substance are a higher tier option and are not currently triggered or considered necessary.

The outcome of the groundwater modelling conducted, where exposure to groundwater is negligible with a high margin of protection, is considered adequate to address the leaching behaviour of the active substance. Therefore a column leaching study is not considered necessary.

Column leaching of metabolites, breakdown and reaction products
Information on the aerobic soil metabolism of the active substance GA4/GA7 is provided under previous points.

Although several major metabolites were observed in soil, these are considered to be of no environmental concern due to the natural occurrence of the active substance and are therefore not considered further.

Lysimeter and field leaching studies
Field studies investigating the leaching potential of the active substance are a higher tier option and are not currently triggered or considered necessary.

B.8.1.4. Predicted environmental concentrations in soil (PEC_{SOIL})

See separate Annex B.8 for product related data.

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)

Use of the active substance can potentially lead to amounts reaching surface water during treatments or via soil run-off, therefore the fate and behaviour if the active substance in the aquatic environment has been investigated in laboratory studies according to the data requirements laid down in EC Regulation 283/2013.

B.8.2.1.1. Hydrolysis

a) Previous evaluation (2005-2011)

One existing study (CA 7.2.1.1/01) investigating the aqueous hydrolysis of the two components gibberellins GA4 and gibberellins GA7 in the active substance is available and was included in the original DAR for Annex I inclusion. The study summary provided below is summarised in more detail than provided in the original DAR. The study is considered still adequate to address the data point.

In accordance with the data requirements defined by EC Regulation 283/2013, for hydrolysis studies included under Point CA 7.2.1.1, metabolites are considered major if they exceed 10% AR, otherwise metabolites are considered minor.

Data point addressed:	CA 7.2.1.1/01
Author(s) (year):	Van der Kolk, J. (2000)
Title:	**Gibberellin A4/A7: Determination of the Hydrolysis as a Function of pH**
Document No:	1042.003.715
Testing facility:	Springborn Laboratories (Europe) AG, Switzerland
Published:	Unpublished
Test guidelines used:	OECD guideline 111 and EC L383A - Part C.7
Deviations:	None
GLP:	Yes
Test material:	Giberilin
Batch #/purity:	/
Status:	**Previously submitted**

Conclusion of the previous review (2005-2011): The was study considered as acceptable.

EU Agreed Endpoint:	pH 4: GA4: Stable to hydrolysis at 50ºC (extrapolated DT_{50} >1 yr at 20ºC) GA7: Declined to ca. 50% of initial value after 5 days at 50ºC pH 7: GA4: Stable to hydrolysis at 50ºC (extrapolated DT_{50} >1 yr at 20ºC) GA7: Stable to hydrolysis at 50ºC (extrapolated DT_{50} >1 yr at 20ºC) pH 9: GA4: Stable to hydrolysis at 50ºC (extrapolated DT_{50} >1 yr at 20ºC) GA7: Completely degraded after 5 days at 50ºC

Executive Summary
The hydrolysis of the two individual components gibberellins GA4 and gibberellins GA7 of the active substance gibberellins GA4/7 was investigated at a temperature of 50°C in sterile aqueous buffer solutions at pH values of 4, 7 and 9. The study was conducted by treating solutions with both ^{14}C-gibberellins GA4 and ^{14}C-gibberellins GA7 (augmented with non-radiolabelled gibberellins GA4/7). Gibberellins GA4 was stable to hydrolysis at pH 4, 7 and 9 (i.e. half-life values of > 1 year at 20°C). Gibberellins GA7 was stable to hydrolysis at pH 7 but was hydrolysed at pH 4 and pH 9 at 50°C. However, the rate of hydrolysis at 20°C is expected to be slow. Degradation products were not identified, but may include the diol derivative of gibberellins GA7 following hydroxylation at the double bond position in the gibberellins GA7 unsaturated ring.

I. MATERIALS AND METHODS

A. TEST AND REFERENCE MATERIALS

1 Test material	Gibberellins GA4 (structure listed in Appendix 1)
Radiolabel position	See Appendix 1
Radiochemical purity	Not stated. Specific activity 57.2 mCi/mmol
Lot/batch no.	^{14}C-GA4 - batch, 64794-GR-17-A
Stability of test compound	Not stated
Test material	Gibberellins GA7 (structure listed in Appendix 1)
Radiolabel position	See Appendix 1
Radiochemical purity	97%. Specific activity 57.2 mCi/mmol
Lot/batch no.	^{14}C-GA7 - batch, 64794-GR-18-A
Stability of test compound	Not stated
2 Reference material	Non radiolabelled gibberellins GA4/7 (mix)
Purity	90.8% (72.5% GA4 and 27.5% GA7)
Lot/batch no.	CSL-89-216-7-11

Test System, buffer solutions
Sterile aqueous buffer solutions were prepared at pH values 4, 7 and 9, as follows:

Preparation of test system, buffer solutions:	Acidic (pH 4, 0.1M tri sodium citrate), neutral (pH 7, 0.1M triethylamine hydrochloride) and alkaline (pH 9, 0.025M sodium tetraborate decahydrate) buffer solutions were prepared using deionised water. The buffer solutions were adjusted to the desired pH by addition of either 0.1M sodium hydroxide or 0.1M hydrochloric acid, as necessary.
Buffer sterilisation	Bulk buffers solutions were sterilised by filtration (0.2 µm pore size)

B. STUDY DESIGN

1 Conditions	Solutions of gibberellins GA4/7 (*ca* 25 µg/mL) were prepared in Erlenmeyer flasks by dissolving non-radiolabelled gibberellins GA4/7 (*ca* 2.5 mg) in each buffer (100 mL) and augmenting the resulting solutions with ^{14}C-gibberellins GA4 (39.3 µg) and ^{14}C-gibberellins GA7 (14.9 µg), dissolved in isopropanol (total volume, 16 µL). The final solvent content was <0.1% by volume. The test solutions were sterilised by filtration (0.2 µm), purged with nitrogen and covered with aluminium foil to exclude light. Traps containing ethylene glycol and 0.5M sodium hydroxide were attached to the sample flasks to collect volatile compounds and carbon dioxide, respectively. The samples were incubated at 50°C in a temperature controlled water bath.
pH of buffer solutions	pH values 4, 7 and 9
Temperature	50°C maintained by water bath
Application rate	Nominal 25 µg/L
2 Sampling	Samples were taken for analysis at 0 and 5 days. At each sampling interval, sub-samples of the buffer solution were analysed for radioactivity by LSC. The gibberellins GA4/7 content was determined by HPLC.
pH readings	The pH of the buffer solutions was checked at each sampling occasion.
Collection of volatile trapping solutions	The study did not collect evolved volatile components (not needed as minimal degradation observed).

Method of analysis

1 Method of analysis for buffer samples	
Sample extraction and/or work-up/concentration	No extraction or sample concentration was performed.
Analytical method, primary	HPLC was conducted using a reverse phase system, consisting of a C_{18} stationary phase and a potassium phosphate (pH 4.8)/acetonitrile (70/30 v/v) mobile phase. Non-radiolabelled gibberellins GA4/7 was visualised using UV light (210 nm). Radioactive regions were quantified using a flow through radio-detector. LOD – not assessed as part of report, but adequate for purposes of study
Analytical method, secondary/ confirmatory	Not applicable
2 Confirmation of incubation conditions	-
pH testing	pH was measured at each sampling interval: pH 4 – range 4.06 - 4.07 pH 7 – range 6.67 – 6.72 pH 9 – range 8.89 - 8.85
3 Volatile components	No trapping of any evolved volatile components

Degradation kinetics

1 Procedure followed	No DT_{50} values were determined.

II. RESULTS AND DISCUSSION

A. RECOVERY OF APPLIED RADIOACTIVITY

The recovery of applied radioactivity from the buffer solutions is summarised in Table 8.2.1.1-1.

Table 8.2.1.1-1: Recovery of applied radioactivity from the buffer solutions

Interval (days)	pH 4			pH 7			pH 9		
	GA4	GA7	Total	GA4	GA7	Total	GA4	GA7	Total
0	76.6	23.4	100.0	77.0	23.0	100.0	79.8	20.2	100.0
5	72.3	12.6	84.9	82.2	17.8	100.0	76.5	0.0	76.5
Difference	- 4.3	- 10.8	- 15.1	+ 5.2	- 5.2	-	- 3.3	- 20.2	- 23.5
Overall recovery at Day 5	106.0			104.0			109.0		

Distribution of GA4 and GA7 expressed as a percent of radioactivity analysed by HPLC
Overall recovery at Day 5 expressed as a percent of applied radioactivity

B FINDINGS

The distribution of radioactivity in the buffer solutions is summarised in Table 8.2.1.1-1.

In pH 7 buffer solution, no change in the overall amount of combined gibberellins GA4 and gibberellins GA7 was observed after incubation for 5 days at a temperature of 50°C. The level of gibberellins GA7 observed after 5 days was reduced slightly, but this was accounted for by an increase in the level of gibberellins GA4. In pH 4 and pH 9 buffer solutions, the overall amount of gibberellins GA4 and gibberellins GA7 was reduced by 15.1 and 23.5% AR, respectively after 5 days incubation at a temperature of 50°C. The reduction was due primarily to degradation of gibberellins GA7, which declined by 10.8 and 20.2% AR at pH 4 and 9, respectively. The degradation of gibberellins GA4 was slow by comparison, declining by only 4.3 and 3.3% AR at pH 4 and 9, respectively.

C HYDROLYSIS RATE

The combined gibberellins GA4 and gibberellins GA7 was stable to hydrolysis at pH7 and a temperature of 50°C, indicating that the half-life at environmentally relevant temperatures (20°C) will be > 1 year. Gibberellins GA4 was stable to hydrolysis at pH 4 and pH 9 at 50°C (degradation less than 10% AR), indicating that the half-life value at environmentally relevant temperatures (20°C) will be > 1 year. Gibberellins GA7 was degraded at pH 4 and pH 9, at 50°C. At pH 4 after 5 days, the level of GA7 present had declined to ca 50% of the initial value, whilst at pH 9; GA7 was completely degraded after 5 days.

D TRANSFORMATION OF PARENT COMPOUND

Hydrolysis of gibberellins GA7 led to the formation of one polar component at pH 4 and two polar components at pH 9. These compounds were not identified. However, considering the relative stability of gibberellins GA4 and the structural differences between the two gibberellins, hydrolysis of gibberellins GA7 must have occurred at the double bond position in the unsaturated ring, resulting in hydroxylated derivatives which may include the diol of gibberellins GA7.

III CONCLUSIONS

The hydrolysis of the two individual components gibberellins GA4 and gibberellins GA7 of the active substance gibberellins GA4/7 was investigated at a temperature of 50°C in sterile aqueous buffer solutions at pH values of 4, 7

and 9. The study was conducted by treating solutions with both ^{14}C-gibberellins GA4 and ^{14}C-gibberellins GA7 (augmented with non-radiolabelled gibberellins GA4/7). Gibberellins GA4 was stable to hydrolysis at pH 4, 7 and 9 (i.e. half-life values of > 1 year at 20°C). Gibberellins GA7 was stable to hydrolysis at pH 7 but was hydrolysed at pH 4 and pH 9 at 50°C. However, the rate of hydrolysis at 20°C is expected to be slow. Degradation products were not identified, but may include the diol derivative of gibberellins GA7 following hydroxylation at the double bond position in the gibberellins GA7 unsaturated ring.

RMS comments and conclusion:

No detailes on the recovery, data on transformation products, volatile components or carbon dioxide were reported, so it cannot be possible to check the recovery or mass balance. However the results indicate a complete recovery from the buffer solutions and therefore levels of evolved volatile components, including carbon dioxide, are not significant. One (at pH4) or two polar components (at pH9) were detected, but not identified, although the amount of them can exceed the limit of 10%. However, considering the relative stable of GA_4 and the structural differences between the two gibberellins, hydrolysis of GA_7 must have occurred at the double bond position in the unsaturated ring, resulting in hydroxylated derivatives which may include the diol of GA_7, moreover GA_7 is presented only 27.5% from the mixture of Gibberellins GA_4/GA_7. All in one the study was regarded as a pilot study and the results were accepted.

Gibberellins GA_4/GA_7 was stable to hydrolysis at pH7 and a temperature of 50°C, indicating that the half-life value at environmentally relevant temperatures (20°C) will be > 1 year. Gibberellin GA_4 was stable to hydrolysis at pH4 and pH9 at 50°C (degradation less than 10% AR), indicating that the half-life value at environmentally relevant temperatures (20°C) will be > 1 year. Gibberellin GA_7 was degraded at pH4 and pH9, at 50°C. At pH 4 after 5 days, the level of GA_7 present had declined to *ca* 50% of the initial value, whilst at pH9; GA_7 was completely degraded after 5 days. The study is considered acceptable.

b) Evaluation of additional data for the purpose of renewal of Annex I inclusion

No new data were presented by applicant. RMS agree with applicant, that new studies are NOT necessary.

B.8.2.1.2. Photo degradation in water

a) Previous evaluation (2005-2011)

Direct photochemical degradation

The molar absorbance coefficients of the individual components gibberellins GA4 and gibberellins GA7 of the active substance gibberellins GA4/7 are <10 L/mol/cm at or above wavelength 298 nm, ref CA 2.4 and therefore the trigger value of 10 L/mol/cm is not exceeded i.e. photolysis studies are not required.

However, one existing study (CA 7.2.1.2/01) investigating the aqueous photolysis of the active substance gibberellins GA4/7 is available and was included in the original DAR for Annex I inclusion. The study summary provided below is summarised in more detail than provided in the original DAR. Although the study is not required, it is included as useful supporting information.

In accordance with the data requirements defined by EC Regulation 283/2013, for photochemical degradation studies included under Point CA 7.2.1.2, metabolites are considered major if they exceed 10% AR, otherwise metabolites are considered minor.

Data point addressed:	CA 7.2.1.2/01
Author(s) (year):	McLaughlin, S.P. (2003)
Title:	**Gibberellins A4 and A7 Combined - Photodegradation in Water an Experimental Screening Test Based on the OECD Direct Photolysis Draft Guideline, Tier II**
Document No:	12709.6214
Testing facility:	Springborn Smithers Laboratories, USA
Published:	**Unp**ublished
Test guidelines used:	OECD Direct Photolysis Draft Guideline, Tier II
Deviations:	/
GLP:	YES
Test material:	Gibberellins
Batch #/purity:	/
Status:	**Previously submitted**

Conclusion of the previous review (2005-2011): The study was considered as acceptable.

Executive Summary

The molar absorption coefficient of the individual components of the active substance gibberellins GA4/7, gibberellins GA4 and gibberellins GA7 was less than 10 L/mol/cm and therefore a measurement of the photolytic half-life and quantum yield is not required.

Nevertheless, the rate of photolysis of non-radiolabelled gibberellins GA4/7 in aqueous buffer solutions at pH 5, 7 and 9 was investigated using artificial sunlight (Heraeus CPS+). The photochemical degradation of the active substance gibberellins GA4/7 was slow with a half-life in the range of 104 to 267 days. The photo-degradation of gibberellins GA4 and gibberellins GA7 measured separately was 101 to 163 days and 57 to 145 days, respectively. Photolysis of gibberellins GA4 and gibberellins GA7 can therefore be regarded as a slow process and not a significant route of degradation in the environment. Degradation products were not identified in the study; however information obtained from dark control samples showed that breakdown resulted primarily from hydrolysis reactions.

I. MATERIALS AND METHODS

A. TEST AND REFERENCE MATERIALS

1 Test material	Gibberellins GA4/7 (structures listed in Appendix 1)
Radiolabel position	Non-radiolabelled
Radiochemical purity	Not applicable (chemical purity 92.17%)
Lot/batch no.	81-097-CD
Stability of test compound	Not stated
2 Reference material	Non-radiolabelled gibberellins GA4/7 (mix)
Purity	60.4% GA4 and 30.2% GA7
Lot/batch no.	21-973-CD

Test System, buffer solutions

Sterile aqueous buffer solutions were prepared at pH values 5, 7 and 9, as follows:

Preparation of test system, buffer solutions:	Buffer solutions at pH 5, 7 and 9 were prepared as follows:
pH 5	Prepared by mixing 0.2M acetic acid (148 mL) with 0.2M sodium acetate (352 mL) and diluting to 1 L with pure water
pH 7	Prepared by mixing 0.1M potassium phosphate (1000 mL) with 0.1M sodium hydroxide (580 mL) and diluting to 2 L with pure water
pH 9	Prepared by mixing 0.1M hydrochloric acid (92 mL) with 0.025M sodium borate (1000 mL) and diluting to 2 L with pure water
Buffer sterilisation	All buffer solutions were sterilised by autoclave

B. STUDY DESIGN

1 Conditions	Buffer solutions containing gibberellins GA4/7 at a concentration of 5 µg/mL were irradiated with artificial sunlight in quartz glass vessels at a temperature of 25°C. Control samples were maintained in the dark at the same temperature. Irradiation was performed using a Heraeus CPS+ instrument equipped with xenon arc lamp (650 W/m^2) and a UV filter system with a cut-off at 290 nm. The spectral energy distribution of the xenon lamp was comparable to that of natural sunlight measured from 300 to 700 nm at the test facility (42°N, 70°W). The output of the light source was measured using a radiometer and the results showed that exposed samples received an intensity of 1.16 x 10^{-2} W/cm^2 compared to a natural sunlight intensity of 1.67 x 10^{-2} W/cm^2. The UV-visible absorbance of a solution (5 µg/mL) of gibberellins GA4/7 was also measured between 290 and 800 nm and the resulting molar absorption was calculated.
pH of buffer solutions	pH values 5, 7 and 9
Temperature	25 ± 1°C
2 Test material application	The test solutions were prepared by placing 1.25 mL of the 1.00 mg/mL gibberellins GA4/7 combined stock solution in 250 mL volumetric flasks and bringing to volume with the corresponding buffer solution.
Application rate	5 µg/mL
3 Sampling	At sampling intervals of 0, 1, 3, 4, 7, 14 and 30 days, triplicate irradiated vessels were taken for analysis. Sampling intervals for the dark controls were 7, 14 and 30 days. At each interval the level of the individual components gibberellins GA4 and gibberellins GA7 in solution was quantified directly by HPLC.
Collection of volatile trapping solutions	The study was conducted with non-radiolabelled test material. Therefore, evolved volatile components were not collected.

Method of analysis

1 Method of analysis for buffer samples	
Sample extraction and/or work-up/concentration	No extraction or sample concentration was performed. Buffer sub-samples were quantified by HPLC for content.
Analytical method, primary	Analysis was performed by reversed phase HPLC, using a methanol/0.2% potassium phosphate (43/57 v/v) mobile phase and a Waters micro-Bondapak C18 stationary phase. Gibberellins GA4 and gibberellins GA7 were detected by UV absorption at 204 nm.

Degradation kinetics

1	Procedure followed	First order plot and linear regression. Not carried out according to FOCUS 2014 (determination of persistence endpoints).

II. RESULTS AND DISCUSSION

A. FINDINGS

Little or no absorption of light was observed for combined gibberellins GA4/7 at wavelengths longer than 290 nm. Molar absorption ranged from 1.85 x 10^{-5} at 297.5 nm to 2.25 $mol^{-1}cm^{-1}$ at 800 nm at a test concentration of 5 µg/mL.

The mean recovery of gibberellins GA4/7 from the irradiated and dark control samples is shown in Table 8.2.1.2-18.2.1.2-1.

Table 8.2.1.2-1: Mean recovery of gibberellins GA4 and gibberellins GA7 from irradiated and dark control sterile aqueous buffer solutions

Interval (days)	pH 5			pH 7			pH 9		
	GA4	GA7	Com-bined	GA4	GA7	Com-bined	GA4	GA7	Com-bined
Irradiated									
0	120	80.5	104	120	82.3	105	120	81.7	104
1	118	72.8	99.6	120	73.6	99.6	118	65.1	96.8
3	130	58.2	101	120	56.5	95.1	120	45.9	90.8
7	118	45.2	88.4	120	40.7	87.9	108	28.3	75.4
14	112	20.2	74.9	116	18.1	76.5	102	n.a	64.1
30	98.6	n.a	59.1	103	n.a	61.6	108	n.a	64.6
Dark control									
7	114	47.9	86.6	119	47.9	89.8	116	34.5	82.6
14	117	34.7	83.7	124	34.8	88.3	123	12.9	78.6
30	119	12.6	76.1	123	11.6	77.0	123	n.a	73.3

n.a = not applicable
Results are the mean of triplicate samples, except 14 day dark which is a single sample
Values expressed as a percent of the theoretical day 0 value

Combined levels of gibberellins GA4/7 in the irradiated samples declined from between 104 and 105% of the theoretical initial concentration at Day 0 to between 59.1 and 64.6% at Day 30, at each pH value. A similar level of decline was observed in the corresponding dark controls, indicating that degradation in the irradiated samples was due to hydrolysis rather than photolysis processes.

Levels of gibberellins GA4 in the irradiated samples declined from 120% of the theoretical initial concentration at Day 0 to between 98.6 and 108% at Day 30 at each pH value, showing that photolysis was limited. Gibberellins GA4 was stable to hydrolysis with no degradation observed in the corresponding dark control samples.

Levels of gibberellins GA7 declined steadily in both the irradiated and dark control samples, with less than 50% of the theoretical concentration remaining after 7 days. The rate of decline was slightly faster in the irradiated samples, but photolysis was limited and degradation was most likely due mainly to hydrolysis processes.

B. PHOTOLYSIS RATE

The first-order photolysis half-life for the components gibberellins GA4 and gibberellins GA7 are shown in Table 8.2.1.2-2.

Table 8.2.1.2-2: Photolysis of gibberellins GA4 and gibberellins GA7 in sterile aqueous buffer solutions

pH	Half-life (days)	Rate constant ($days^{-1}$)	Coefficient of determination (r^2)
GA4 and GA7 combined			
pH 5	104	0.00666	0.897
pH 7	123	0.00562	0.880
pH 9	267	0.00259	0.297
GA4			
pH 5	101	0.00683	0.973
pH 7	102	0.00677	0.919
pH 9	163	0.00426	0.437
GA7			
pH 5	114	0.00609	0.620
pH 7	145	0.00479	0.409
pH 9	57	0.01220	0.998

Results shown are corrected for degradation observed in dark control samples

The rate of photochemical degradation of gibberellins GA4/7 was determined in aqueous buffer solutions with artificial sunlight and the DT_{50} values ranged from 104 days at pH 5 to 267 days at pH 9. The rate of photolysis of the individual components gibberellins GA4 and gibberellins GA7 measured separately were 101 to 163 days and 57 to 145 days, respectively. The photolysis of gibberellins GA4 and gibberellins GA7 can therefore be regarded as a slow process. These results along with the UV-visible absorption data suggest that direct photolysis is a minor route of degradation in the environment.

C. TRANSFORMATION OF PARENT COMPOUND

Degradation products were not identified in the study, however the observed breakdown of gibberellins GA4 and gibberellins GA7 was due predominantly to hydrolytic degradation as evidenced by the degradation in dark control samples.

III. CONCLUSIONS

The molar absorption coefficient of the individual components gibberellins GA4 and gibberellins GA7 of the active substance gibberellins GA4/7 was less than 10 L/mol/cm and therefore a measurement of the photolytic half-life and quantum yield is not required.

Nevertheless, the rate of photolysis of non-radiolabelled gibberellins GA4/7 in aqueous buffer solutions at pH 5, 7 and 9 was investigated using artificial sunlight (Heraeus CPS+). The photochemical degradation of the active substance gibberellins GA4/7 was slow with a half-life in the range of 104 to 267 days. The photo-degradation half-life of gibberellins GA4 and gibberellins GA7 measured separately was 101 to 163 days and 57 to 145 days, respectively. Photolysis of gibberellins GA4 and gibberellins GA7 can therefore be regarded as a slow process and not a significant route of degradation in the environment. Degradation products were not identified in the study; however information obtained from dark control samples showed that breakdown resulted primarily from hydrolysis reactions.

RMS comments and conclusion:

Again, non-radiolabelled test material was used in the test, so transformation products and mass balance could not be determined. The molar absorption coefficient of gibberellins GA_4 and GA_7 was less than 10 $mol^{-1}cm^{-1}$ and therefore a measurement of the photolytic half-life and quantum yield is not required. Nevertheless, the rate of photolysis of gibberellins GA_4 and GA_7 in aqueous buffer solution at pH 5, 7 and 9 was investigated using artificial sunlight. The photochemical degradation of combined gibberellins GA_4 and GA_7 is expected to show less than 20% direct photolysis over the course of 30 days in natural water bodies. The GA_7 component of the test substance, however, did undergo hydrolysis during the 30-day test, by 84.4%, 86.0% and 100% for pH 5, 7 and 9, respectively.

The GA_4 component was hydrolytically stable and showed less than 20% photolysis. The half-life of the GA_4 and GA_7 combined gibberellins felt in the range of 104 days to 267 days.The given values at pH9 (DT_{50}:267 days, R^2: 0.297) indicated high uncertainity of the results.. The photo-degradation of gibberellins GA_4 and GA_7 measured separately was 101 to 163 days and 57 to 145 days, respectively. Photolysis of gibberellins GA_4 and GA_7 can therefore be regarded as a slow process and not a significant route of degradation in the environment. Degradation products were not identified in the study; however information obtained from dark control samples showed that breakdown resulted primarily from hydrolysis reactions. The study is considered acceptable.

Indirect photochemical degradation

Studies investigating the indirect photochemical degradation of the active substance are a higher tier option and are not currently triggered or considered necessary.

b) Evaluation of additional data for the purpose of renewal of Annex I inclusion

No new data were presented by applicant. RMS agree with applicant, that new studies are NOT necessary.

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

B.8.2.2.1. Biological degradation

Use of plant protection products containing the active substance gibberellins GA4/7 may result in contact with aquatic systems, therefore the route and rate of biological degradation in aquatic systems has been investigated in laboratory studies according to the data requirements laid down in EC Regulation 283/2013. In accordance with the data requirements defined by EC Regulation 283/2013, for studies investigating the route and rate of biological degradation in aquatic systems included under Point CA 7.2.2, metabolites are considered major if they exceed 10% AR or exceed 5% on consecutive sampling intervals or exceed 5% and are rising at the end of the study, otherwise metabolites are considered minor.

B.8.2.2.2. Ready biodegradability

a) Previous evaluation (2005-2011)

One existing study (CA 7.2.2.1/01) investigating the biodegradability of the active substance gibberellins GA4/7 is available and was included in the original DAR for Annex I inclusion. The study summary provided below is summarised in more detail than provided in the original DAR. The study is considered still adequate to address the data point. In addition, one "new" study (CA 7.2.2.1/02) which has been conducted since the original Annex I inclusion is also available and is included for completeness.

Data point addressed:	CA 7.2.2.1/01
Author(s) (year):	Barnes, S.P. (2005)
Title:	**Gibberellins A4 and A7 (Technical grade) Assessment of Ready Biodegradability – Modified Sturm Test**
Document No:	ZAB 049/043181
Testing facility:	Huntingdon Life Sciences Ltd., UK
Published:	Unpublished
Test guidelines used:	OECD Test Guideline 301B, EEC method C.4-C and OPPTS method 835.3110 (m)
Deviations:	/
GLP:	YES
Test material:	Gibberellins
Batch #/purity:	/
Status:	**Previously submitted**

Conclusion of the previous review (2005-2011): The study was considered as acceptable.

Executive Summary

The ready biodegradability of non-radiolabelled gibberellins GA4/7 was investigated using the carbon dioxide evolution (Modified Sturm) test. The mean cumulative level of carbon dioxide produced by mixtures containing gibberellins GA4/7 was equivalent to 10% of the theoretical value after *ca* 11 days, 65% after 19 days and 77% after 29 days. The results indicate that gibberellins GA4/7 is readily biodegradable under the conditions of the test. A reference material (sodium benzoate) was degraded by 68 and 69% after 7 days in the absence and presence of gibberellins GA4/7, respectively and gibberellins GA4/7 is therefore not considered inhibitory to microbial activity.

I. MATERIALS AND METHODS

A. TEST AND REFERENCE MATERIALS

1 Test material	Gibberellins GA4/7 (structures listed in Appendix 1)
Radiolabel position	Non-radiolabelled
Radiochemical purity	Not applicable (chemical purity 90.3% total gibberellins GA4 and gibberellins GA7)
Lot/batch no.	107-554-CD
Stability of test compound	Not stated
2 Reference material	
Chemical name	Sodium benzoate
-Purity	AR grade
-Lot/batch no.	0091837

3 Test System

Test water consisted of purified water with added minerals as specified in Table 8.2.2.2-1.

Table 8.2.2.2-1: Composition of water for ready biodegradability test

Minerals	**Amount of nutrient per litre deionised water (g)**
KH_2PO_4	8.5
K_2HPO_4	21.75
$Na_2HPO_4.2H_2O$	33.4
NH_4Cl	0.5
$MgSO_4.7H_2O$	22.5
$CaCl_2$	36.4
$FeCl_3.6H_2O$	0.25

The inoculum used was activated sewage sludge from a treatment plant treating predominantly domestic wastewater. The sewage sludge was diluted with test water to obtain a suspended solid concentration of 30 mg/L. Prior to use, the inoculated test water was aerated at room temperature.

B. STUDY DESIGN

1 Experimental conditions	Test vessels consisted of Dreschel bottles fitted with inlet and outlet tubing to permit a continuous flushing of air. The outlet air was passed through bottles containing 0.025N barium hydroxide solution (3 x 100 mL) to collect evolved carbon dioxide. Test samples were incubated in the dark at a temperature of 21 to 24°C over a period of 29 days. The pH of each solution was adjusted to 7.6 with 5N HCl prior to the test and was checked at the end of the incubation period. Test solutions were prepared as follows to a final total volume of 3 litres.
Control samples	Inoculated test water only (30 mg solids/L)
Reference	Inoculated test water plus sodium benzoate (10 mg C/L)
Test substance plus inoculated test water	10 mg C/L
Positive controls	Sodium benzoate (10 mg C/L) plus test substance (10 mg C/L) plus inoculated test water
2 Sampling	Barium hydroxide solutions were sampled at intervals up to 29 days. The cumulative production of carbon dioxide from the test vessels was measured by titrating the residual concentration of barium chloride with hydrochloric acid (0.05M) using a phenolphthalein indicator. The percentage biodegradation was calculated with reference to the theoretical carbon dioxide production (TCO_2) calculated from the chemical formula. The TCO_2 of the test substance and reference material was 110.1 mg CO_2.

II. RESULTS AND DISCUSSION

A. FINDINGS

The cumulative carbon dioxide production and percentage TCO_2 in the test vessels is summarised in Table 8.2.2.2-2.

Table 8.2.2.2-2: Cumulative carbon dioxide production and percent TCO_2

Interval (days)	Sodium benzoate		Sodium benzoate plus gibberellins GA4/7		Gibberellins GA4/7	
	CO_2 (mg)	% TCO_2	CO_2 (mg)	% TCO_2	CO_2 (mg)	% TCO_2
2	23.9	22	22.8	21	0	0
3	42.4	38	41.8	38	0	0
5	62.2	56	64.1	58	0.8	1
7	75.4	68	75.9	69	2.2	2
9	83.3	76	-	-	3.9	3
13	88.0	80	-	-	22.9	21
14	90.8	82	-	-	38.0	34
15	92.4	84	-	-	51.2	46
16	93.8	85	-	-	61.4	56
19	96.0	87	-	-	71.8	65
22	97.1	88	-	-	77.0	70
26	97.9	89	-	-	80.9	73
28	99.8	91	-	-	83.6	76
29	100.1	91	-	-	84.7	77

Gibberellins GA4/7 results are the mean of duplicate determinations
Cumulative CO_2 production in the controls (69.3 to 73.7 mg) was within the acceptable range for the test
All data shown above are corrected for the level of CO_2 present in the control samples

The mean cumulative level of CO_2 produced by solutions containing gibberellins GA4/7 was equivalent to 10% of the TCO_2 between 9 and 13 days. Degradation continued rapidly, reaching 21, 65 and 77% of the TCO_2 after 13, 19 and 29 days, respectively. The results show that gibberellins GA4/7 is readily biodegradable.

The reference substance was degraded by 68 and 69% of its TCO_2 in the absence and presence of gibberellins GA4/7, respectively after 7 days. The results confirm the suitability of the test medium and show that gibberellins GA4/7 had no inhibitory effect on microbial activity in the test medium.

Measurements taken at the end of the incubation period showed that the pH in the test vessels was maintained during the study.

III. CONCLUSIONS

Gibberellins GA4/7 is readily biodegradable under the conditions of the test and does not have an inhibitory effect on microbial activity in the test medium.

RMS comments and conclusion:

Cumulative levels of CO_2 production in the controls after 29 days were within the acceptable range for this assay system, although detailed data were not reported. These results confirm that the innoculum viable and the test result valid. Mean cumulative CO_2 production by mixtures containing gibberellins GA_4 and GA_7 was equivalent to 21% of the theoritical value after 13 days, 65% of the theoritical value after 19 days and 77% by the end of the test on day 29. Based on graphic estimation the evolved CO_2 was 10% on the day of 10 of test. Gibberellins GA_4 and GA_7 are readily biodegradable under the conditions of the test and do not have an inhibitory effect on microbial activity in the test medium. The study is considered acceptable.

b) Evaluation of additional data for the purpose of renewal of Annex I inclusion

The ready biodegradability of non-radiolabelled gibberellins GA4/7 was investigated using the carbon dioxide evolution (Modified Sturm) test. Gibberellins GA4/7 technical failed to meet the requirements for a pass in this test (≥60% degradation relative to the theoretical value), with a maximum of 6% recorded on day 14. Based on these results gibberellins GA4/7 cannot be classified as readily biodegradable under the conditions of this test. A reference material (sodium acetate) was degraded by 85 and 137% after 29 days in the absence and presence of gibberellins GA4/7, respectively and gibberellins GA4/7 is therefore not considered inhibitory to microbial activity.

Data point addressed:	CA 7.2.2.1/02
Author(s) (year):	Drake, R.M. (2009)
Title:	**An evaluation of the ready biodegradability of GA4/7 Technical using the OECD 301B CO_2 evolution test (Modified Sturm Test)**
Document No:	ENV8775/070905
Testing facility:	Chemex Environmental International Limited, UK
Published:	Unpublished
Test guidelines used:	OECD Guideline 308B
Deviations:	/
GLP:	YES
Test material:	Gibberellins
Batch #/purity:	see below
Status:	**New submission**

I. MATERIALS AND METHODS

A. TEST AND REFERENCE MATERIALS

1 Test material	Gibberellins GA4/7 (structures listed in Appendix 1) called GA4/7 Technical in the report
Radiolabel position	Non-radiolabelled
Radiochemical purity	Not applicable (chemical purity 65.1% gibberellins GA4 and 25.7% gibberellins GA7, i.e. 90.8% overall)
Lot/batch no.	081202 (supplied by Globachem NV)
Stability of test compound	Stable when stored as recommended
2 Reference material	
Chemical name	Sodium acetate
-Purity	Not specified
-Lot/batch no.	Not specified

3 Test System

The inoculum used was activated sewage sludge from a sewage treatment plant (Cambridge, UK), sieved to 850 µm, settled, decanted and re-suspended using mineral media. To determine dry sludge solids the media was centrifuged (4000 rpm, 10 mins), discarding the supernatant twice, and the resulting pellet weighed. Dry sludge solids content was 7.5% (0.03 g/L dry sludge solids in test).

B. STUDY DESIGN

1 Experimental conditions	Conical flasks of nominal volume 2000 mL were filled with inoculated mineral medium (1500 mL). The blanks, reference and test bottles were set up in duplicate, with a single toxic control. Test and reference materials were added to appropriate bottles to a final concentration of 20 mg C/L. Atmospheric air was pumped into the test system and scrubbed clean of carbon dioxide by passing over soda lime. The air continued into the test vessel where it collects and evolved carbon dioxide before moving in to the carbon dioxide traps, each containing 200 mL of 0.05 M sodium hydroxide solution. The test solutions were stirred for the duration of the study. Units were incubated in the dark at 22°C.
Control/blank samples	Inoculated mineral medium only (1500 mL)
Reference	Inoculated mineral medium plus sodium acetate (*ca* 102 mg in 1500 mL)
Test substance plus inoculated test water	Inoculated mineral medium plus GA4/7 technical, percentage carbon 62.38% (*ca* 48 mg in 1500 mL) i.e. concentration *ca* 20 mg C/L. GA4/7 technical was introduced into the test media weighed onto microscope cover slips and added directly.
Positive controls	Inoculated mineral medium plus GA4/7 technical, percentage carbon 62.38% (*ca* 48 mg in 1500 mL) i.e. concentration *ca* 20 mg C/L and sodium acetate, percentage carbon 29.3% (*ca* 102 mg in 1500 mL) i.e. concentration *ca* 20 mg C/L. Overall total concentration *ca* 40 mg C/L
2 Sampling	Trap solutions were sampled at intervals up to 29 days. Carbon dioxide in the traps is measured as Dissolved Inorganic Carbon (DIC) using a Tekmar-Dohrmann Phoenix 8000. The percentage biodegradation was calculated with reference to the theoretical carbon dioxide production ($ThCO_2$) calculated from the chemical formula. At the end of the test, concentrated hydrochloric acid was added to each bioreactor to drive off remaining carbon dioxide.

II. RESULTS AND DISCUSSION

A. FINDINGS

The cumulative percentage TCO_2 in the test vessels is summarised in Table 8.2.2.2-3.

Table 8.2.2.2-3: Percentage degradation

Time (days)	Reference			Test chemical			Toxicity control
	1	2	mean	1	2	mean	
0	0	0	0	0	0	0	0
2	28	32	30	3	3	3	35
5	45	51	48	4	3	3	52
7	51	58	55	4	4	4	58
9	55	61	58	5	4	4	62
14	62	67	64	6	5	6	75
19	66	72	69	6	6	6	94
23	68	73	71	6	6	6	102
28	72	78	75	4	5	4	120
29	77	84	80	3	4	3	128
29	84	85	85	5	3	4	137

Gibberellins GA4/7 technical failed to meet the requirements for a pass in this test (≥60% degradation relative to $ThCO_2$) with a maximum 6% recorded on day 14. The result shows that gibberellins GA4/7 cannot be classified as readily biodegradable on the basis of this test.

The reference substance was degraded by 85 and 137% of its TCO_2 in the absence and presence of gibberellins GA4/7, respectively after 29 days. The results confirm the suitability of the test medium and show that gibberellins GA4/7 had no inhibitory effect on microbial activity in the test medium.

It was noted that the degradation of the test material in the presence of the reference material was enhanced. Re-interpretation of the toxicity control data indicates that gibberellins GA4/7 technical degrades to *ca.* 50%. This was achieved after a lag phase of at least 10 days, suggesting that the presence of the reference material in the toxicity control made the inoculum more viable.

Measurements taken at the end of the incubation period showed that the pH in the test vessels was maintained during the study.

III. CONCLUSIONS

Gibberellins GA4/7 does not have an inhibitory effect on microbial activity in the test medium, but is not readily biodegradable under the conditions of the test.

RMS comments and conclusion:

On the basis of the conflicting results of the two ready biodegradability studies (Barnes 2005 and Drake 2009), it is concluded that the active substance gibberellins GA4/7 cannot be reliably classified as readily biodegradable. However, on the basis of the degree of biodegradation observed in the non-positive test (in conjunction with the rapid and extensive microbial degradation and ultimate complete mineralisation via other natural components observed in the soil and water/sediment studies), it is concluded that the active substance gibberellins GA4/7 can be considered as inherently biodegradable.

Both studies are considered acceptable.

B.8.2.2.3. Aerobic mineralisation in surface water

a) Previous evaluation (2005-2011)

No data were available during the Annex I inclusion.

b) Evaluation of additional data for the purpose of renewal of Annex I inclusion

The aerobic mineralisation of the active substance gibberellins GA4/7 is addressed by read across to a study conducted using the structurally related active substance gibberellic acid GA3. The study conducted using gibberellic acid GA3 is summarised below:

Data point addressed:	CA 7.2.2.2/01
Author(s) (year):	Lamond, P. (2017).
Title:	**[^{14}C] Gibberellic acid: Aerobic mineralisation in surface water**
Document No:	Study No. 3201604
Testing facility:	Smithers Viscient (ESG) Ltd
Published:	**Unp**ublished
Test guidelines used:	OECD Guideline 309 (Apr 2004) and Regulation (EC) 1107/2009 (Oct 2009)
Deviations:	/
GLP:	YES
Test material:	Gibberellic acid
Batch #/purity:	see below
Status:	**New submission**

Executive Summary

The route and rate of aerobic degradation of gibberellic acid GA3 was investigated in pelagic water at a concentration of 0.01 and 0.1 mg/L at 20°C in the dark.

Gibberellic acid degraded rapidly (DT_{50} values ≤17.2 days) at both low and high application rates under non-sterile conditions. Degradation was assumed to be abiotic as similar degradation was observed in samples from sterile conditions. Two metabolites were observed which were tentatively identified by LC-MS and LC-MS/MS as an isomerisation product of gibberellic acid GA3 (mean maximum observed level 51.7% AR by end of study) and a dicarboxylic acid degradant resulting from the opening of the lactone ring (mean maximum observed level 40.3% AR by end of study).

A. TEST AND REFERENCE MATERIALS

1 Test material	Radiolabelled gibberellic acid GA3 (structure listed in Appendix 1). Note: two batches of radiolabelled gibberellic acid GA3 were used in the study. The main study was conducted with Batch 2 (the first batch was used for method development work).
Radiolabel position	[methylene-^{14}C]-gibberellic acid GA3 (see Appendix 1)
Radiochemical purity	Batch 1: Details from Certificate of Analysis: >99.2% by HPLC. Batch 2: Details from Certificate of Analysis: >99.1% by HPLC. However, RCP details determined analytically at the laboratory were 86.5%. Following re-purification, the RCP was determined to be 95.5% (i.e. as used for study).
Lot/batch no.	Batch 1: 9504EKC042-3, specific activity 3.66 MBq/mg Batch 2: 9504EKC043-4, specific activity 3.96 MBq/mg

RCP analysis	HPLC – 10-50 µL (sample) / 5-10 µL (standards) injection volume, Waters HSS T3 C18 column (25 x 0.46 cm, 5 µm), mobile phase – gradient system a) 2 % formic acid in water, b) 2.0 % formic acid in acetonitrile, 0 mins 90% a), 5 mins 60% a), 15 mins 55% a) 30 mins 30% a), 35 mins 0% a), flow rate 1. mL/min, co chromatography with authentic reference standards, 14C detection – Radiomatic, Perkin Elmer Life Sciences UK Ltd. (FloLogic-U 1.5 mL/min or solid scintillant for offline counting (Topcount)).
2 Reference material (reference standard)	Non radiolabelled gibberellic acid GA3
Purity	99%
Lot/batch no.	A0366292 (source - ACROS Organics), retest date Nov-17 (certificate provided)
3 Control substance (reference substance))	Phenyl-U-^{14}C-Benzoic Acid
Purity	99%
Lot/batch no.	160811

Test System, natural pelagic water
A natural pelagic water system from The Lake at Studley Royal (North Yorkshire, UK) was used for the study. The lake is surrounded by parkland. The test system was named 'Fountains Abbey'. No pesticide had been applied in the immediate vicinity in the last 5 years. Water was sampled by bucket through a 100 µm sieve into a clean plastic container for transport and storage.

The surface water collection details and physico-chemical characteristics measured during sampling are presented in Table 8.2.2.3-1.

Table 8.2.2.3-1: Surface water collection details and physico-chemical characteristics measured during sampling

Surface water origination	Fountains Abbey
Sampling date	10th January 2017
Visual quality	clear, pale yellow
Temperature (°C)	4.0
Oxygen content (mg/L)	9.98
Conductivity/TDS (µS/cm)	49
pH	7.88
Water depth above sediment (cm)	*ca.* 30
Water depth sampled (cm)	*ca.* 0-10

The characterisation details of the sampled surface water are presented in Table 8.2.2.3-2.

Table 8.2.2.3-2: Surface water characterisations details

Surface water origination	Fountains Abbey
Total organic carbon (mg/L)	5.23
Dissolved organic carbon, DOC (mg/L)	4.63
Dissolved orthophosphate (mg/L PO_4^{3-} -P)	<0.05
Ammonium (mg/L NH_4^+ -N)	0.095
Total nitrogen (mg/L)	3.19
Suspended solids (mg/L)	3
Total phosphorus (mg/L PO_4^{3-} -P)	0.063
Nitrite (mg/L NO_2^- -N)	0.036
Nitrate (mg/L NO_3^- -N)	2.46

B. STUDY DESIGN

1 Conditions	The study was mainly to determine the biotic degradation of the test substance in natural pelagic water. Additional samples were treated (1) under sterile conditions to distinguish abiotic processes and (2) with a reference substance (known to degrade) to validate the conditions of the test. Biotic samples - Individual water samples (*ca* 100 mL) in borosilicate glass flasks (250 mL) continuously stirred with individual traps.Traps: 2 x 2 M sodium hydroxide (carbon dioxide). Abiotic samples – As above, using sterilised (autoclaved – 121°C, 15 min) dispensed in laminar flow cabinet. Reference substance – as for biotic samples. Control samples (non-radiolabelled) – as for biotic samples (used for measurement of redox potential and pH).
No. of concentrations	2 (biotic), 1 (abiotic), 1 (reference substance)
Incubation conditions	20 ± 2°C, dark
Main study, biotic conditions	2 replicate samples for analysis at 7 sampling interval plus 4 spares
Sterile controls, abiotic conditions	2 replicate samples for analysis at 1 sampling interval (end of study) plus 2 spares
Reference substance (positive controls)	2 replicate samples. Treated with [phenyl-U-^{14}C]-benzoic acid. Only traps quantified.
2 Test material application	Application in methanol (*ca* 90 µL)
Application rate Biotic samples Biotic and abiotic samples Reference substance Control samples	 10 and 100 µg/L (nominal) 100 µg/L (nominal) 100 µg/L (nominal). untreated
3 Sampling	Whole samples taken at each sampling interval
Sampling intervals, Biotic samples Abiotic samples Reference substance	 0, 3, 7, 14, 30, 45 and 59 DAT (each level) 58 DAT 0, 2, 6, 13, 30, 45, 58 and 59 DAT (traps only)
Monitoring of redox and pH	Readings were taken in the control samples at each sampling interval.
Collection of volatile trapping solutions	Volatile traps were replenished at each sampling interval. Sampled traps quantified by LSC.

Method of analysis

1 Method of analysis for water samples	
Extraction	Water samples (ca 80 mL) were amended with methanol (ca 10 mL) and glass vessels rinsed with further methanol (ca 20 mL).
Sample work-up/concentration	None performed.
Analytical method, primary	Various systems used: HPLC no. 1 (RCP and 0 – 7 DAT), HPLC no. 2 and 3 (14 – 58 DAT). HPLC (no. 1): Phenomenex Gemini C18 column (25 cm L x 4.6 mm id), mobile phase – gradient system a) 0.05% formic acid in water, b) 0.05% formic acid in acetonitrile, 0 mins 95% a), 1 min 95% a), 27 mins 40% a), 28 mins 0% a), 30 mins 0% a), 31 mins 95% a), 35 mins 95% a). Flow rate 1 mL/min. ^{14}C detection: β-ram, (Lablogic), liquid cell (Ultima-Flo M scintillant, 2 mL/min). UV detection (210 nm). Co-chromatography with non-radiolabelled reference standards. HPLC (no. 2): as above except 0 mins 95% a), 1 min 95% a), 23 mins 0% a), 25 mins 0% a), 26 mins 95% a), 30 mins 95% a). HPLC (no. 3): as per no. 1 except column Water Sunfire C18 (25 cm L x 4.6 mm id), solvent system a) 0.1% formic acid in water, b) 0.1% formic acid in methanol/acetonitrile (4/1 v/v). Column recoveries not reported. LOD assessed as 0.85% AR.
Analytical method, secondary/ confirmatory (qualitative)	LC-MS and LC-MS/MS was used for structural confirmation of gibberellic acid GA3 and tentative identification of observed metabolites: i) LC-MS MS HPLC method 1 – Phenomenex Gemini C18 column (25 cm L x 4.6 mm id), mobile phase – gradient system a) 0.05% formic acid in water, b) 0.05% formic acid in acetonitrile, 0 mins 95% a), 3 min 95% a), 23 mins 0% a), 25 mins 0% a), 26 mins 95% a), 30 mins 95% a). Flow rate 1 mL/min. ^{14}C detection: β-ram, (Lablogic), liquid cell (Ultima-Flo M scintillant, 2 mL/min). MS HPLC method 2 – Waters Sunfire C18 (25 cm L x 4.6 mm id, 5 µm), mobile phase – gradient system a) 0.1% formic acid in water, b) 0.1% formic acid in methanol/acetonitrile (4:1v/v), 0 mins 95% a), 3 min 95% a), 20 mins 60% a), 35 mins 0% a), 40 mins 0% a), 41 mins 95% a), 45 mins 95% a). Flow rate 1 mL/min (split ratio 3:1 RAD:MS) MS – Thermo Scientific Q-Exactive, ionisation mode: heated electrospray ionisation (HESI) –ve and +ve, scan range: 120-1250 m/z ii) LC-MS/MS MS HPLC method 2 – as above MS – as above, ionisation mode: HESI –ve.

2 Volatile components	Trapping solutions (sodium hydroxide) were collected at each sampling interval and quantified by LSC.
Confirmation of carbon dioxide	Minimal carbon dioxide was evolved (≤2% AR), confirmation was therefore not conducted.

Degradation kinetics

1 Procedure followed	DT_{50} and DT_{90} values for the degradation of gibberellic acid GA3 in soil were determined in accordance with the recommendations of the FOCUS work group on kinetics FOCUS 2006[3] and FOCUS 2014[4] (determination of persistence endpoints only i.e. degradation endpoints for use as triggers for additional work).
Software used	CAKE v2.0
Input data used	Input data for the degradation of gibberellic acid GA3 was taken from the individual data presented in Tables 8.2.2.3-6 and 8.2.2.3-7, with zero time values corrected to the total recovery at this time point multiplied by the radiochemical purity. All data points were equally weighted.

II. RESULTS AND DISCUSSION

A. DISTRIBUTION OF APPLIED RADIOACTIVITY

The recovery and distribution of the applied radioactivity from the main study biotic samples (10 µg/L and 100 µg/L dose levels) is summarised in Tables 8.2.2.3-3 and 8.2.2.3-4.

The recovery and distribution of the applied radioactivity from the sterile samples (100 µg/L dose level) is summarised in Table 8.2.2.3-5.

[3] FOCUS (2006) "Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration" Report of the FOCUS Work Group on Degradation Kinetics, EC Document Reference Sanco/10058/2005 version 2.0, 434 pp

[4] FOCUS 2014. Generic guidance for Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration, version 1.1, 18 December 2014.

Table 8.2.2.3-3: Recovery and distribution of applied radioactivity from low dose level (10 µg/L)

Sampling Interval (Days)	0			3			7			14		
Unit Code	A1	A2	Mean	A3	A4	Mean	A5	A6	Mean	A7	A8	Mean
Solution (Extract 1)	100.4	101.2	100.8	100.2	98.7	99.5	99.3	96.2	97.8	97.5	98.1	97.8
Vessel rinse (Extract 2)	0.4	ND	0.2	1.4	0.3	0.9	0.6	0.5	0.6	0.9	ND	0.5
Sodium Hydroxide Trap 1	NA	NA	NA	4.0	ND	2.0	ND	0.2	0.1	0.2	0.3	0.3
Sodium Hydroxide Trap 2	NA	NA	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
Mass Balance	100.8	101.2	101.0	105.6	99.0	102.3	99.9	96.9	98.4	98.6	98.4	98.5

NA = not applicable

ND = not detected (or <0.1% AR)

Sampling Interval (Days)	30			45			59*		
Unit Code	A9	A10	Mean	A11	A12	Mean	A13	A14	Mean
Solution (Extract 1)	99.5	98.0	98.8	99.3	98.1	98.7	97.5	101.2	99.4
Vessel rinse (Extract 2)	0.9	1.0	1.0	ND	ND	ND	0.7	0.5	0.6
Sodium Hydroxide Trap 1	0.3	0.3	0.3	0.4	1.4	0.9	0.7	0.4	0.6
Sodium Hydroxide Trap 2	ND	ND	ND	ND	ND	ND	ND	ND	ND
Mass Balance	100.7	99.3	100.0	99.7	99.5	99.6	98.9	102.1	100.5

ND = not detected (or <0.1% AR)

* = sum of subsamples at 58 DAT and 59 DAT

Table 8.2.2.3-4: Recovery and distribution of applied radioactivity from high dose level (100 µg/L)

Sampling Interval (Days)	0			3			7			14		
Unit Code	B1	B2	Mean	B3	B4	Mean	B5	B6	Mean	B7	B8	Mean
Solution (Ext 1)	99.8	99.4	99.6	98.7	100.4	99.6	97.4	97.5	97.5	98.8	98.8	98.8
Vessel wash (Ext 2)	0.2	0.3	0.3	0.5	0.5	0.5	0.7	0.5	0.6	0.7	0.5	0.6
Sodium Hydroxide Trap 1	NA	NA	NA	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.2
Sodium Hydroxide Trap 2	NA	NA	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
Mass Balance	100.0	99.7	99.9	99.3	101.0	100.2	98.2	98.1	98.2	99.7	99.5	99.6

NA = not applicable

ND = not detected (or <0.1% AR)

Sampling Interval (Days)	30			45			59*		
Unit Code	B9	B10	Mean	B11	B12	Mean	B13	B14	Mean
Solution (Ext 1)	98.4	98.5	98.5	99.4	100.0	99.7	98.3	98.7	98.5
Vessel wash (Ext 2)	0.7	0.7	0.7	0.2	0.2	0.2	0.9	0.8	0.9
Sodium Hydroxide Trap 1	0.2	0.3	0.3	0.2	0.2	0.2	0.3	0.3	0.3
Sodium Hydroxide Trap 2	ND	ND	ND	ND	ND	ND	ND	ND	ND
Mass Balance	99.3	99.5	99.4	99.8	100.4	100.1	99.5	99.8	99.7

ND = not detected (or <0.1% AR)

* = sum of subsamples at 58 DAT and 59 DAT

Table 8.2.2.3-5: Recovery and distribution of applied radioactivity from sterile samples

Sampling Interval (Days)	59*		
Unit Code	C1	C2	Mean
Solution (Ext 1)	98.2	100.3	99.3
Vessel wash (Ext 2)	0.4	0.4	0.4
Sodium Hydroxide Trap 1	ND	ND	ND
Sodium Hydroxide Trap 2	ND	ND	ND
Mass Balance	98.6	100.7	99.7

ND = not detected (or <0.1% AR)

* = sum of subsamples at 58 DAT and 59 DAT

The applied radioactivity was mainly recovered from the water (≥97.4% AR based on mean values), with very little evolved volatile radioactivity observed (mainly <1% AR with the exception of 2 samples, max individual sample 4.0% AR). No evolved volatile radioactivity was observed in the sterile controls (abiotic samples).

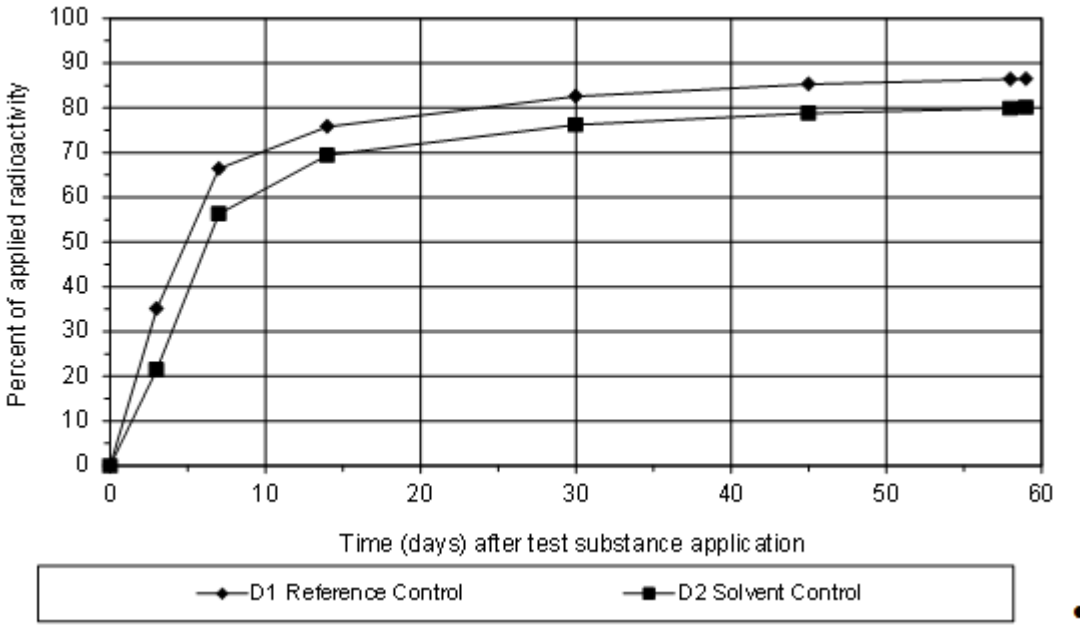
B. MASS BALANCE

The mean recovery of applied radioactivity from the samples ranged from 98.4 to 102.3 %AR at a dose level of 10 µg/L and from 98.2 to 100.2 %AR at a dose level of 100 µg/L, indicating a complete mass balance.

C. REFERENCE CONTROLS

Mineralisation rates of sodium [^{14}C] benzoate in the reference and solvent controls are shown in Figure 8.2.2.3-1. Profiles were similar showing that the acetonitrile did not inhibit the microbial degradation of sodium [^{14}C] benzoate in the natural water. Mineralisation exceeded 50% AR within 7 days and therefore the natural water used was microbially active and the study was valid.

Figure 8.2.2.3-1: The mineralisation of sodium [^{14}C] benzoate in the reference and solvent control vessels



D. PROFILE OF COMPONENTS

The profile of components analysed in the water samples is summarised in Tables 8.2.2.3-6 to 8.2.2.3-8.

Gibberellic acid levels decreased rapidly during the incubation period, forming two main compounds. The relative compositions of the different compounds were similar for the low and high application rates at each of the time points, demonstrating that the rates of degradation were not related to concentration. The relative compositions of the different compounds were similar in samples from sterile and non-sterile conditions. This demonstrated that the rate of degradation was not associated with microbial mineralisation but was likely to be due to chemical reactions/rearrangements.

Table 8.2.2.3-6: Percent of applied radioactivity present as Gibberellic acid and degradation products in natural water treated with [methylene-^{14}C] Gibberellic acid at the low application rate

Sampling Interval (Days)	0			3			7			14		
Unit Code	A1	A2	Mean	A3	A4	Mean	A5	A6	Mean	A7	A8	Mean
Gibberellic acid	99.9	101.2	100.5	82.7	83.7	83.2	68.5	68.0	68.2	52.8	50.0	51.4
Unknown AM1	ND	ND	ND	11.9	9.4	10.7	13.6	18.6	16.1	29.2	24.7	26.9
Unknown AM2	ND	ND	ND	4.5	5.4	5.0	10.9	9.3	10.1	14.0	22.6	18.3
Other unknowns	ND	ND	ND	ND	ND	ND	5.5	ND	2.7	ND	ND	ND
Unresolved background	0.6	0.0	0.3	1.1	0.2	0.6	0.9	0.3	0.6	1.6	0.8	1.2
Total	100.4	101.2	100.8	100.2	98.7	99.5	99.3	96.2	97.8	97.5	98.1	97.8

ND = not detected (or <0.1% AR)

Sampling Interval (Days)	30			45			58		
Unit Code	A9	A10	Mean	A11	A12	Mean	A13*	A14*	Mean
Gibberellic acid	31.8	31.2	31.5	19.9	20.1	20.0	12.6	15.2	13.9
Unknown AM1	42.0	39.0	40.5	48.8	47.8	48.3	56.7	46.7	51.7
Unknown AM2	25.1	27.3	26.2	29.3	29.4	29.4	27.3	38.7	33.0
Other unknowns	ND	ND	ND	ND	ND	ND	ND	ND	ND
Unresolved background	0.6	0.5	0.5	1.4	0.8	1.1	0.9	0.6	0.8
Total	99.5	98.0	98.8	99.3	98.1	98.7	97.5	101.2	99.3

ND = not detected (or <0.1% AR)

* = subsample

Table 8.2.2.3-7: Percent of applied radioactivity present as Gibberellic acid and degradation products in natural water treated with [methylene-^{14}C] Gibberellic acid at the high application rate

Sampling Interval (Days)	0			3			7			14		
Unit Code	B1	B2	Mean	B3	B4	Mean	B5	B6	Mean	B7	B8	Mean
Gibberellic acid	94.8	95.4	95.1	85.4	85.9	85.6	69.9	74.0	72.0	52.1	49.7	50.9
Unknown AM1	3.0	2.5	2.8	9.7	11.3	10.5	16.8	16.2	16.5	27.7	26.2	26.9
Unknown AM2	ND	ND	ND	3.2	2.9	3.0	8.4	7.0	7.7	18.6	20.1	19.4
Other unknowns	1.8	1.4	1.6	ND	ND	ND	1.9	ND	0.9	ND	ND	ND
Unresolved background	0.2	0.1	0.2	0.4	0.3	0.4	0.5	0.2	0.3	0.4	1.0	0.7
Total	99.8	99.4	99.6	98.7	100.4	99.6	97.4	97.5	97.4	98.8	97.0	97.9

ND = not detected (or <0.1% AR)

Sampling Interval (Days)	30			45			58		
Unit Code	B9	B10	Mean	B11	B12	Mean	B13*	B14*	Mean
Gibberellic acid	31.2	32.6	31.9	20.4	20.0	20.2	9.6	15.1	12.3
Unknown AM1	38.2	34.5	36.3	47.0	47.3	47.1	49.8	40.4	45.1
Unknown AM2	28.8	27.5	28.1	31.6	31.6	31.6	38.2	42.4	40.3
Other unknowns	ND	3.4	1.7	ND	0.9	0.4	ND	ND	ND
Unresolved background	0.2	0.6	0.4	0.4	0.4	0.4	0.7	0.8	0.7
Total	98.4	98.5	98.5	99.4	100.0	99.7	98.3	98.7	98.5

ND = not detected (or <0.1% AR)

* = subsample

Table 8.2.2.3-8: Percent of applied radioactivity present as Gibberellic acid and degradation products in sterilised natural water treated with [methylene-^{14}C] Gibberellic acid at the high application rate

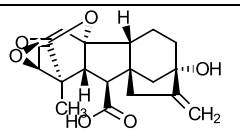
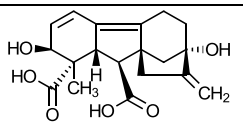
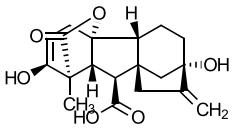
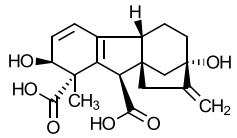
Sampling Interval (Days)	58*		
Unit Code	C1	C2	Mean
Gibberellic acid	9.6	9.9	9.7
Unknown AM1	50.4	49.8	50.1
Unknown AM2	35.7	37.9	36.8
Other unknowns	2.1	1.2	1.6
Unresolved background	0.5	1.5	1.0
Total	**98.2**	**100.3**	**99.3**

* = subsample

Gibberellic acid GA3 was identified using HPLC co-chromatography with the non-radiolabelled reference standard. The identity of Gibberellic acid GA3 was also confirmed by LC-MS in selected samples.

Two peaks were observed in the HPLC chromatograms at the 3 DAT time point. The quantity of these compounds, 'Unknown AM1' (max 51.7% AR, 58 DAT) and 'Unknown AM2' (max 40.3% AR, 58 DAT), increased at a steady rate at each time point throughout the study. Selected samples were sent for LC-MS analysis to establish structural information on the products. Unknown AM1 at *ca* 22.4 minutes was postulated to be an isomerisation product of gibberellic acid GA3. Unknown AM2 at *ca* 23.3 minutes was postulated to be a dicarboxylic acid degradant resulting from the opening of the lactone ring. No significant degradation of the metabolites was observed.

Table 8.2.2.3-9: Tentative identities of metabolites of gibberellic acid GA3 in natural water

Metabolite AM1 and AM2	
	
	

The extent of degradation and the profile and observed levels of the metabolites was the same in the sterile samples indicating that the degradation rate and pathway was not associated with microbial mineralisation.

E. DEGRADATION RATE

Degradation rates for gibberellic acid GA3 in natural water were calculated based on the declining concentrations observed in the water samples and were fitted to SFO kinetics using CAKE (version 2) software. The DT_{50} and DT_{90} values are summarised in Table 8.2.2.3-10. Gibberellic acid GA3 declined rapidly in natural water with best-fit persistence half-lives of ≤17.2 days.

Table 8.2.2.3-10: Degradation rate of gibberellic acid GA3 in natural water

Soil	DT_{50}	DT_{90}	Chi2err (%)	Correlation coefficient, r^2
Low appln rate (10 µg/L)	16.9	56.0	6.77	0.9884
High appln rate (100 µg/L)	17.2	57.2	4.85	0.9914

III. CONCLUSIONS

Gibberellic acid GA3 (at nominal concentrations of 10 and 100 µg/L) was found to be rapidly degraded (FOCUS persistence DT_{50} value of ≤17.2 days) in natural pelagic water when incubated in the dark at 20°C. Two metabolites were observed which were tentatively identified by LC-MS and LC-MS/MS as an isomerisation product of gibberellic acid GA3 (maximum mean observed level 51.7% AR by end of study) and a dicarboxylic acid degradant resulting from the opening of the lactone ring (maximum mean observed level 40.3% AR by end of study). The degradation rate and pathway was not associated with microbial mineralisation.

The aerobic mineralisation of the active substance gibberellins GA4/7 is therefore expected to proceed rapidly with the formation of metabolites via isomerisation and opening of the lactone ring. Any metabolites formed are also likely to be found naturally due to the natural occurrence of gibberellins GA4/7 and are therefore not considered to be of environmental concern.

RMS comments and conclusion:

No significant deviations from the guideline (OECD 309, 2004) was noted. The test was performed using a radiolabelled form of [methylene - ^{14}C]Gibberellic acid at two concentrations levels (*ca* 10 µg/L and *ca* 100 µg/L). Sterile samples were tested at the higher concentration. The recovery of applied radioactivity was within the acceptable range of 90-110%. Two metabolite compounds, named 'Unknown AM1' and 'Unknown AM2' were detected at 3 DAT using HPLC. At the lower application rate (10 µg/L), the quantity of these metabolite compounds continued to increase during the study to mean values of 51.7% AR (Unknown AM1) and 33.0% AR (Unknown AM2). At the higher application rate (100 µg/L), the quantity of these metabolite compounds also continued to increase during the study to (maximum) mean values of 47.1% AR (Unknown AM1) and 40.3% AR (Unknown AM2). Degradation was assumed to be not microbial as similar degradation was observed in samples from sterile conditions.
In case of determination of degradation parameters the percent of applied radioactivity present as test substance was plotted against the time after treatment in days.
Curves were constructed through appropriate data points using non-linear regression analysis to give lines of best fit. The degradation rates of the test substance, the parameters used to calculate them and parameter statistics were determined using CAKE version 2 software.

The single first-order (SFO) model used was as follows:

$$M = M_0 e^{-kt}$$

Where M was the percent of test substance at time t days, M_o was the computed initial concentration and k was the rate constant (slope).

The data was not fitted to the two compartment models (FOMC, DFOP or hockey step) as there was insufficient degradation for kinetic model comparisons to be made.

Concentrations of Gibberellic acid derived by summing the amounts in vessel washes and in solution were fitted to SFO kinetics. A summary of the results obtained is presented in the following table and the full results are presented in pages below this table.

Table 8.2.2.3-111: Detailed degradation kinetics analyses for gibberellic acid GA3 in natural water

Group	DT-50 (days)	DT-90 (days)	χ^2 Error (%)	r^2
Gibberellic acid low application (A)	16.9	56.0	6.77	0.9884
Unknown AM1 low application	497	1.66E+03	6.04	0.9792
Unknown AM2 low application	500	1.66E+03	7.88	0.9436
Gibberellic acid high application (B)	17.2	57.2	4.85	0.9871
Unknown AM1 high application	213	706	9.13	0.9496
Unknown AM2 high application	2.63E+183	8.75E+183	9.81	0.9704

Gibberellic acid degraded rapidly (DT-50 values ≤ 17.2 days) at both low and high application rates under non-sterile conditions with a DT-90 of ≤ 57.2 days. Two degradation products, 'Unknown AM1' and 'Unknown AM2' had formed at detectable levels by the 3 DAT timepoint and increased in amount at each subsequent timepoint. The degradation products were included in the kinetics. There was no discernible lag phase. A full description of the kinetic analyses performed is presented in further pages, together with detailed output from the software used.

a.) [Methylene - ^{14}C] Gibberellic acid at the low application rate, Parent,

Model Setup:

Topology: Parent, A1, B1
Optimiser: IRLS (IRLS Its. 10, IRLS Tol. 1E-05, Max. Its. 100, Tol. 1E-05)
SANN Max Iterations: 10000
Use Extra Solver: False

Initial Values of Sequence Parameters:

Parameter	Initial Value	Bounds	Fixed
Parent_0	100	0 to (unbounded)	No
k_Parent	0.1	0 to (unbounded)	No
f_Parent_to_A1	0.3333	0 to 1	No
f_Parent_to_B1	0.3333	0 to 1	No
k_A1	0.1	0 to (unbounded)	No
k_B1	0.1	0 to (unbounded)	No

Fit step: Final

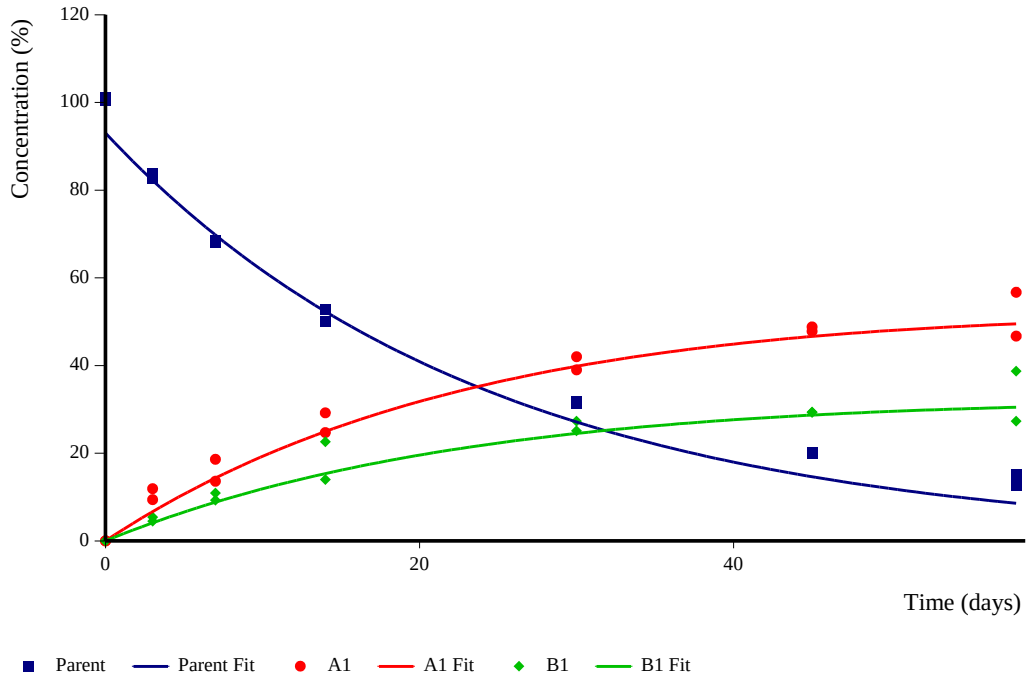
Reference Table:

Compartment	Name
Parent	Parent

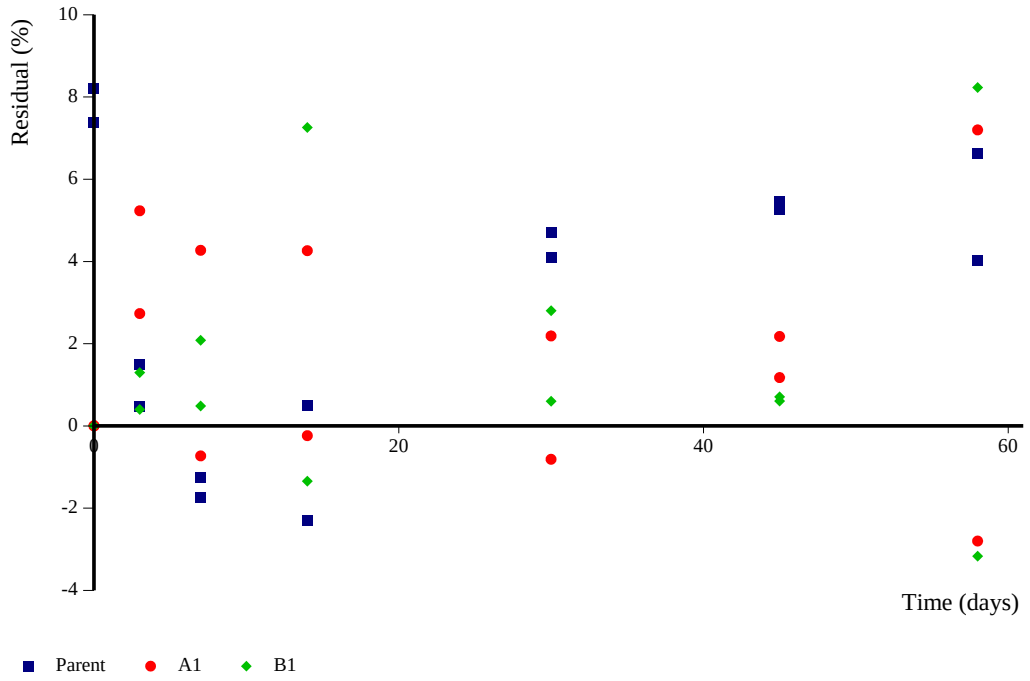
A1	A1
B1	B1

Graphical Summary:

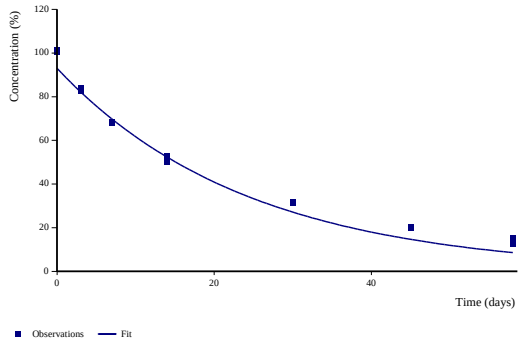
Observations and Fitted Model:

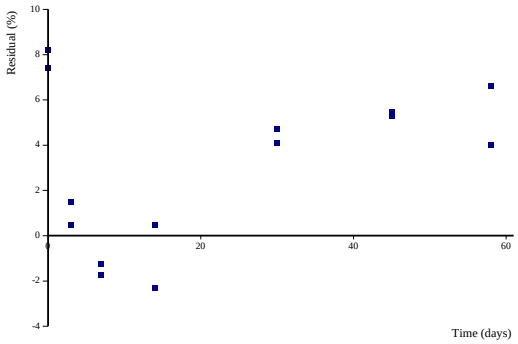


Residuals:

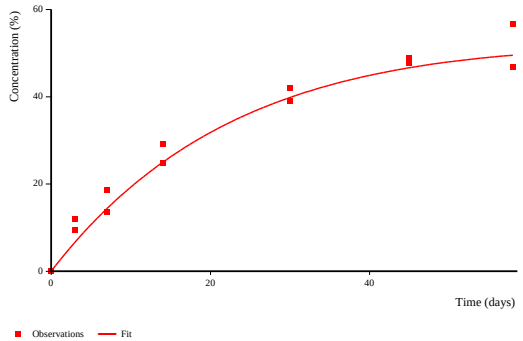


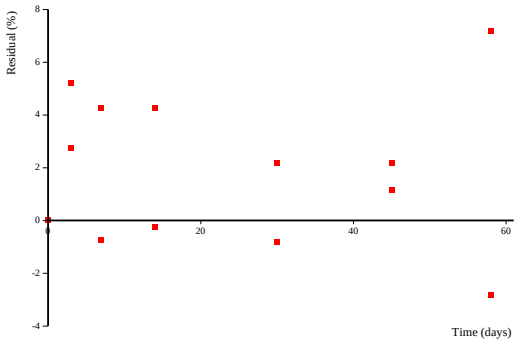
Compartment Parent:



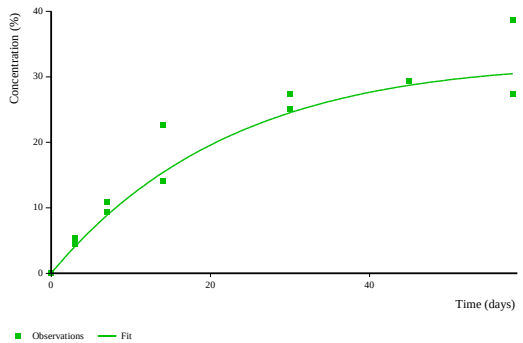


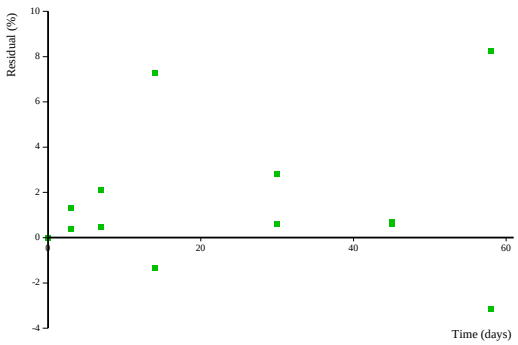
Compartment A1:





Compartment B1:





Initial Values for This Step:

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f_Parent_to_B1	0.3548	0 to 1	No
k_A1	0.00362	0 to (unbounded)	No
k_B1	5.196E-25	0 to (unbounded)	No

Estimated Values:

Parameter	Value	σ	Prob. > t	Lower (90%) CI	Upper (90%) CI	Lower (95%) CI	Upper (95%) CI
Parent_0	93.01	2.433		88.9	97.12	88.07	97.94
k_Parent	0.0411	0.001668	1.97E-024				
f_Parent_to_A1	0.6194	0.02877		0.5709	0.668	0.5611	0.678
k_A1	0.001396	0.001736	0.2133				
f_Parent_to_B1	0.3811	0.02875		0.3326	0.4296	0.3228	0.439
k_B1	0.001386	0.00295	0.3207				

WARNING: Flow fractions for Parent sum to more than 1 (by 0.55%). Consider rerunning the fit with the sink disabled for Parent.

χ^2

Parameter	Error %	Degrees of Freedom
All data	8.52	15
Parent	6.77	5
A1	6.04	5
B1	7.88	5

Decay Times:

Compartment	DT50 (days)	DT90 (days)
Parent	16.9	56
A1	497	1.65E+03
B1	500	1.66E+03

Additional Statistics:

Parameter	r^2 (Obs v Pred)	Efficiency
All data	0.9863	0.98
Parent	0.9884	0.9777
A1	0.9792	0.9702
B1	0.9436	0.9282

Parameter Correlation:

	Parent_0	k_Parent	f_Parent_to_A1	k_A1	f_Parent_to_B1	k_B1
Parent_0	1	0.2077	-0.1446	0.1867	0.1389	0.2108
k_Parent	0.2077	1	-0.1706	0.1789	0.1704	0.3223
f_Parent_to_A1	-0.1446	-0.1706	1	0.7583	-1	-0.8426
k_A1	0.1867	0.1789	0.7583	1	-0.7586	-0.525
f_Parent_to_B1	0.1389	0.1704	-1	-0.7586	1	0.8434
k_B1	0.2108	0.3223	-0.8426	-0.525	0.8434	1

Observed v. Predicted:

Compartment Parent

Time (days)	Value (%)	Predicted Value	Residual
0	100.4	93.01	7.391
0	101.2	93.01	8.191
3	82.7	82.22	0.4806
3	83.7	82.22	1.481
7	68.5	69.76	-1.255
7	68	69.76	-1.755
14	52.8	52.32	0.4849
14	50	52.32	-2.315
30	31.8	27.1	4.696
30	31.2	27.1	4.096
45	19.9	14.63	5.268
45	20.1	14.63	5.468
58	12.6	8.575	4.025
58	15.2	8.575	6.625

Compartment A1

Time (days)	Value (%)	Predicted Value	Residual
0	0	0	0
0	0	0	0
3	11.9	6.669	5.231
3	9.4	6.669	2.731
7	13.6	14.33	-0.7308
7	18.6	14.33	4.269
14	29.2	24.94	4.261
14	24.7	24.94	-0.2394
30	42	39.81	2.187
30	39	39.81	-0.8134
45	48.8	46.63	2.174
45	47.8	46.63	1.174
58	56.7	49.5	7.198
58	46.7	49.5	-2.802

Compartment B1

Time (days)	Value (%)	Predicted Value	Residual
0	0	0	0
0	0	0	0
3	4.5	4.103	0.3969
3	5.4	4.103	1.297

7	10.9	8.817	2.083
7	9.3	8.817	0.4828
14	14	15.35	-1.345
14	22.6	15.35	7.255
30	25.1	24.5	0.6005
30	27.3	24.5	2.801
45	29.3	28.7	0.6052
45	29.4	28.7	0.7052
58	27.3	30.47	-3.168
58	38.7	30.47	8.232

Sequence Creation Information:
Fit generated by CAKE version 2.0 (Release)
running on R version 3.0.0 (2013-04-03)

Report Information:
Report generated by CAKE version 2.0 (Release)
CAKE developed by Tessella Ltd, Abingdon, Oxfordshire, UK for Syngenta
Running on .Net version 4.0.30319.18444

b.) [Methylene - ^{14}C] Gibberellic acid at the high application rate, Parent,

Model Setup:

Topology: Parent, A1, B1
Optimiser: IRLS (IRLS Its. 10, IRLS Tol. 1E-05, Max. Its. 100, Tol. 1E-05)
SANN Max Iterations: 10000
Use Extra Solver: False

Initial Values of Sequence Parameters:

Parameter	Initial Value	Bounds	Fixed
Parent_0	100	0 to (unbounded)	No
k_Parent	0.1	0 to (unbounded)	No
f_Parent_to_A1	0.3333	0 to 1	No
f_Parent_to_B1	0.3333	0 to 1	No
k_A1	0.1	0 to (unbounded)	No
k_B1	0.1	0 to (unbounded)	No

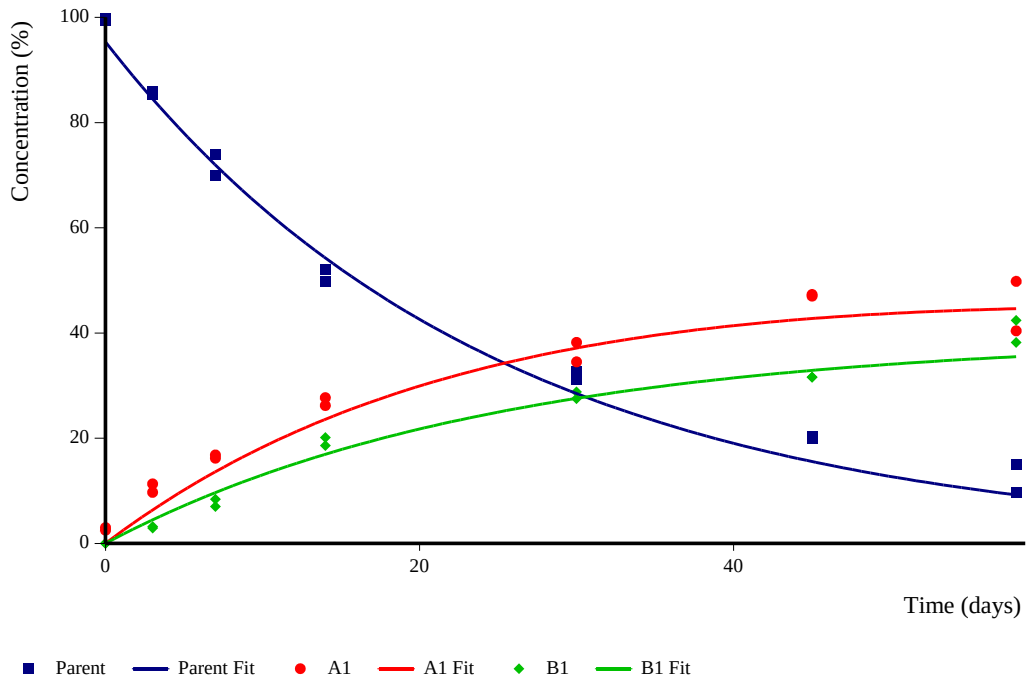
Fit step: Final

Reference Table:

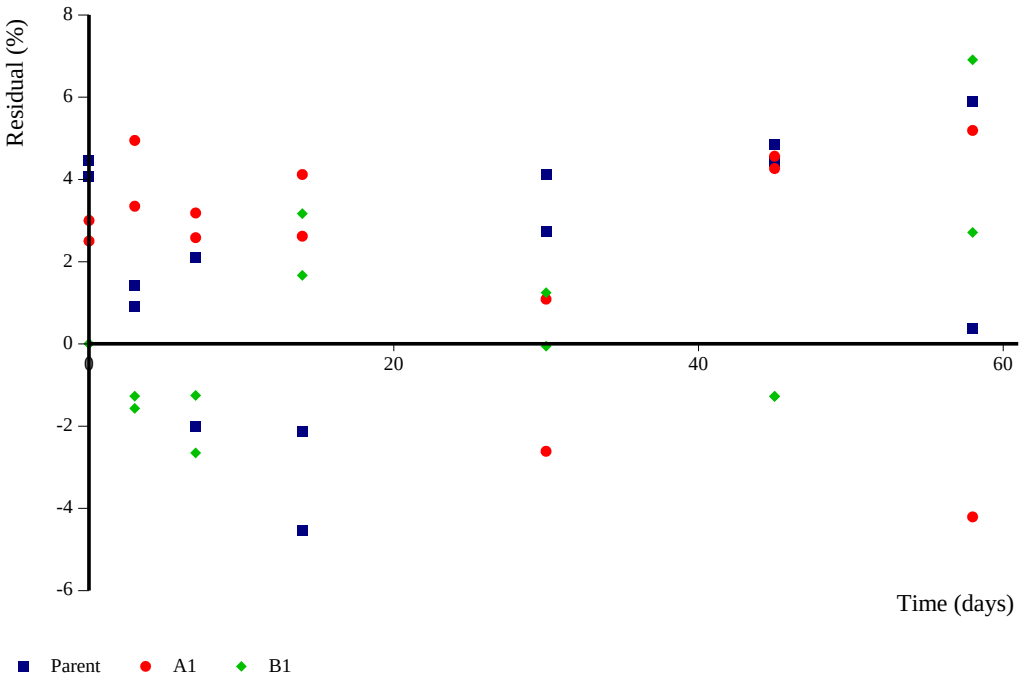
Compartment	Name
Parent	Parent
A1	A1
B1	B1

Graphical Summary:

Observations and Fitted Model:



Residuals:



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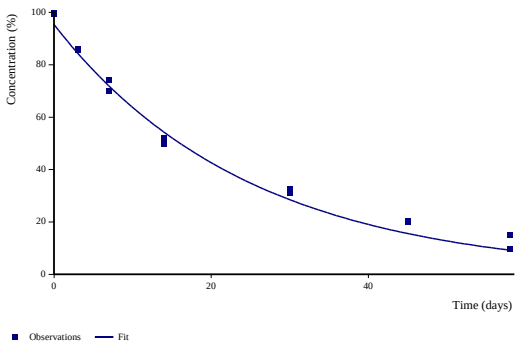
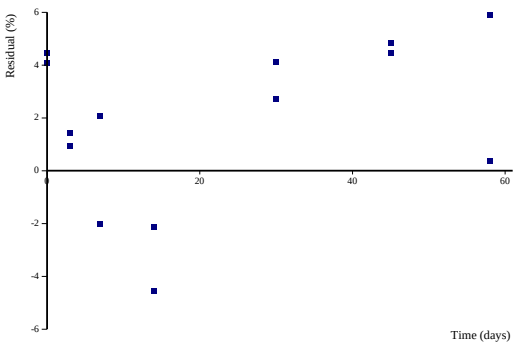
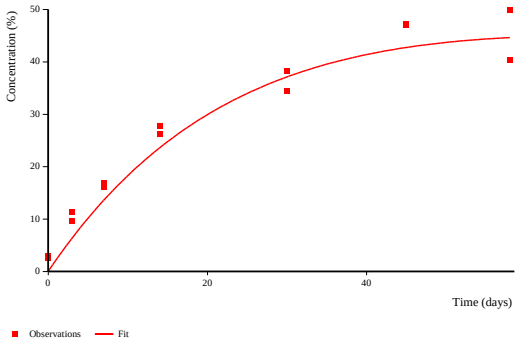
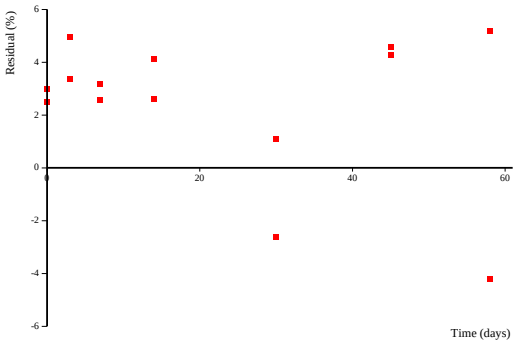


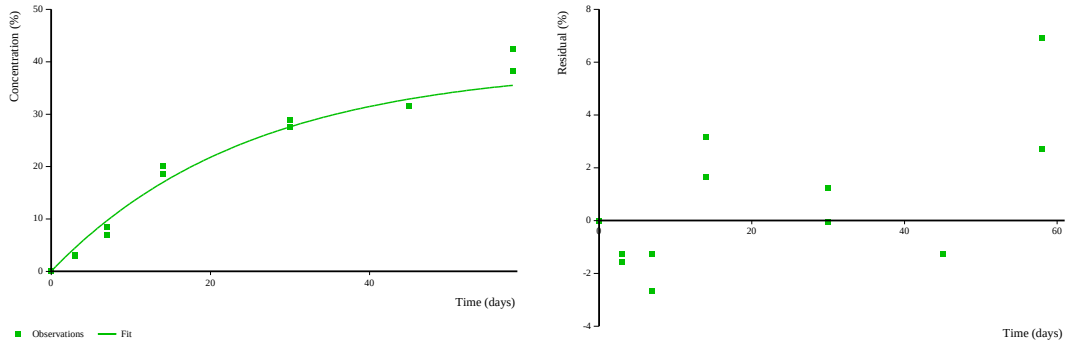


Compartment A1:





Compartment B1:



Initial Values for This Step:

Parameter	Initial Value	Bounds	Fixed
Parent_0	96.42	0 to (unbounded)	No
k_Parent	0.03862	0 to (unbounded)	No
f_Parent_to_A1	0.5924	0 to 1	No
f_Parent_to_B1	0.4081	0 to 1	No
k_A1	0.006907	0 to (unbounded)	No
k_B1	3.064E-33	0 to (unbounded)	No

Estimated Values:

Parameter	Value	σ	Prob. > t	Lower (90%) CI	Upper (90%) CI	Lower (95%) CI	Upper (95%) CI
Parent_0	95.33	2.001		91.95	98.71	91.27	99.39
k_Parent	0.04028	0.001444	2.84E-026				
f_Parent_to_A1	0.5883	0.0268		0.5431	0.6336	0.534	0.643
k_A1	0.003262	0.002079	0.06268				
f_Parent_to_B1	0.4121	0.02677		0.3669	0.4573	0.3578	0.466
k_B1	2.63E-184	0.002235	0.5				

WARNING: Flow fractions for Parent sum to more than 1 (by 0.47%). Consider rerunning the fit with the sink disabled for Parent.

χ^2

Parameter	Error %	Degrees of Freedom
All data	8.08	15
Parent	4.85	5
A1	9.13	5
B1	9.81	5

Decay Times:

Compartment	DT50 (days)	DT90 (days)
Parent	17.2	57.2
A1	213	706
B1	2.63E+183	8.75E+183

Additional Statistics:

Parameter	r² (Obs v Pred)	Efficiency
All data	0.9886	0.9849
Parent	0.9914	0.9871
A1	0.9753	0.9496
B1	0.9776	0.9704

Parameter Correlation:

	Parent_0	k_Parent	f_Parent_to_A1	k_A1	f_Parent_to_B1	k_B1
Parent_0	1	0.2576	0.009125	0.153	-0.01529	0.1452
k_Parent	0.2576	1	0.1478	0.3671	-0.1479	0.1101
f_Parent_to_A1	0.009125	0.1478	1	0.7695	-1	-0.8484
k_A1	0.153	0.3671	0.7695	1	-0.7685	-0.5392
f_Parent_to_B1	-0.01529	-0.1479	-1	-0.7685	1	0.849
k_B1	0.1452	0.1101	-0.8484	-0.5392	0.849	1

Observed v. Predicted:

Compartment Parent

Time (days)	Value (%)	Predicted Value	Residual
0	99.8	95.33	4.468
0	99.4	95.33	4.068
3	85.4	84.48	0.92
3	85.9	84.48	1.42
7	69.9	71.91	-2.008
7	74	71.91	2.092
14	52.1	54.24	-2.139
14	49.7	54.24	-4.539
30	31.2	28.47	2.729
30	32.6	28.47	4.129
45	20.4	15.56	4.841
45	20	15.56	4.441
58	9.6	9.216	0.3835
58	15.1	9.216	5.884

Compartment A1

Time (days)	Value (%)	Predicted Value	Residual
0	3	0	3
0	2.5	0	2.5
3	9.7	6.353	3.347
3	11.3	6.353	4.947
7	16.8	13.62	3.182
7	16.2	13.62	2.582

14	27.7	23.58	4.118
14	26.2	23.58	2.618
30	38.2	37.11	1.087
30	34.5	37.11	-2.613
45	47	42.74	4.264
45	47.3	42.74	4.564
58	49.8	44.61	5.191
58	40.4	44.61	-4.209

Compartment B1

Time (days)	Value (%)	Predicted Value	Residual
0	0	0	0
0	0	0	0
3	3.2	4.472	-1.272
3	2.9	4.472	-1.572
7	8.4	9.654	-1.254
7	7	9.654	-2.654
14	18.6	16.94	1.665
14	20.1	16.94	3.165
30	28.8	27.56	1.245
30	27.5	27.56	-0.0551
45	31.6	32.88	-1.277
45	31.6	32.88	-1.277
58	38.2	35.49	2.709
58	42.4	35.49	6.909

Sequence Creation Information:
Fit generated by CAKE version 2.0 (Release)
running on R version 3.0.0 (2013-04-03)

Report Information:
Report generated by CAKE version 2.0 (Release)
CAKE developed by Tessella Ltd, Abingdon, Oxfordshire, UK for Syngenta
Running on .Net version 4.0.30319.18444

Study done it with gibberellic acid (GA3) is valid and acceptable and can be used for use in risk assessment for gibberellins (GA4/7).

B.8.2.3. Degradation in the saturated zone

No data on the degradation of GA4/7 in the saturated zone is available. However as the compound degrades rapidly in soil it is not expected to reach the saturated zone.

B.8.2.4. Degradation in water sediment system

a) Previous evaluation (2005-2011)

No data on the degradation of GA4/7 in natural water/sediment systems were available during the Annex I inclusion. However as the compound degrades rapidly by chemical hydrolysis and has a very low soil sorption constant, it can be inferred that it will rapidly degrade in natural waters (at least at the same rate as by hydrolysis) and will not partition to sediment.

b) Evaluation of additional data for the purpose of renewal of Annex I inclusion

The degradation in water/sediment systems of the active substance gibberellins GA4/7 is addressed by read across to a study conducted using the structurally related active substance gibberellic acid GA3. The study conducted using gibberellic acid GA3 is summarised below:

Data point addressed	CA 7.2.2.3/01
Author(s) (year):	Cooper, T. (2017).
Title:	**[^{14}C]Gibberellic acid: Degradation in water-sediment systems under aerobic conditions**
Document No:	Study No. 3201605
Testing facility:	Smithers Viscient (ESG) Ltd.
Published:	Unpublished
Test guidelines used:	OECD Guideline 308 (Apr 2002) and OPPTS 835.4300 (Oct 2008)
Deviations:	/
GLP:	YES
Test material:	Gibberellic acid
Batch #/purity:	see below
Status:	**New submission**

Executive Summary

The route and rate of aerobic degradation of gibberellic acid GA3 in water/sediment systems was investigated at a rate equivalent to a nominal treatment rate of 212 g as/ha (assuming a depth of 30 cm) at 20°C in the dark.

Gibberellic acid GA3 was shown to degrade quickly in two water/sediment systems (<2% AR after 59 days), with calculated DT_{50} values of 10.9-11.2 days (SFO). Five major metabolites were observed, with ultimate degradation leading to the formation of carbon dioxide and un-extractable sediment bound residues. Tentative structures for the metabolites were proposed on the basis of extensive LC-MS/MS analysis as follows: WS1a – GA6 an isomer of GA3 or re-arrangement of the double bond; WS1b - opening of the lactone ring; WS2 - loss of H_2 from the parent molecule; WS3 and WS4 - open lactone-dihydroxy-GA3 and positional isomers (actual structures provided).

I. MATERIALS AND METHODS

A. TEST AND REFERENCE MATERIALS

1 Test material	Radiolabelled gibberellic acid GA3 (structure listed in Appendix 1). Note: two batches of radiolabelled gibberellic acid GA3 were used in the study. The main study was conducted with Batch 2 (the first batch was used for method development work)
Radiolabel position	[methylene-^{14}C]-gibberellic acid GA3 (see Appendix 1)
Radiochemical purity	Batch 1: Details from Certificate of Analysis: >99.2% by HPLC. Batch 2: Details from Certificate of Analysis: >99.1% by HPLC. However, RCP details determined analytically at the laboratory for Batch 1 were 86.5%, however this batch was only used for method development work. The RCP of Batch 2 was determined to be 99% (i.e. as used for study).
Lot/batch no.	Batch 1: 9504EKC042-3, specific activity 3.66 MBq/mg Batch 2: 9504EKC043-6, specific activity 4.55 MBq/mg
2 Reference material 1	Non radiolabelled gibberellic acid GA3
Purity	99%
Lot/batch no.	A0366292 (source - ACROS Organics), retest date Nov-17 i.e. after the end of experimental work (certificate provided)

Test system, water/sediment systems

Two natural aquatic water/sediment systems, collected from sites in the UK were used as test systems for the study. The test systems were selected to provide contrasting properties with respect to particle size distribution, organic carbon content and pH. Sediments were sieved to 2 mm and the water samples passed through a 212 µm sieve during sampling (Calwich Abbey: 10-Jan-17 and Emperor lake: 9-Jan-17). Details on pesticide history from the vicinity indicate no pesticide use in the last 5 years. After arrival at the laboratory (12-Jan-17), the water/sediment systems were stored for 21 days before dispensing into the incubation units (2-Feb-17) for use in the study (biomass determinations at the start of the study confirmed the viability of the sediments with values generally similar to those after 4 weeks on similar batches). The water/sediment systems were acclimatised for 19 days prior to being treated with test material (21-Feb-17).

The characterisation details of the water/sediment systems are summarised in Table 8.2.4-1.

Table 8.2.4-1: Water-sediment characterisation details

Water/sediment system name	Calwich Abbey	Emperor Lake
Sediment characteristics-		
Particle size distribution	-	-
% Sand	40	74
% Silt	52	15
% Clay	8	11
Textural classification (USDA)	Silt Loam	sandy loam
pH (0.01M $CaCl_2$)	7.4	5.8
pH (water)	7.7	6.5
Organic carbon content (%)	5.1	2.1
Cation exchange capacity (meq/100g)	18.9	13.3

Water/sediment system name	Calwich Abbey	Emperor Lake
Microbial biomass (µg C/g sediment)		
Pre-study	1990	1060
0 DAT	1859	958
End of study	1152	507
Water characteristics-		
pH	7.8	7.0
Total organic carbon (mg/L)	4.79	7.28
Suspended solids (mg/L)	9	17

B. STUDY DESIGN

1 Conditions	Samples of each water/sediment system were incubated in individual glass flasks with straight sides (ca 4.5 cm id). Sediment layer (3 cm) and associated water layer (9 cm), i.e. water: sediment ratio of 3:1. Traps: 2 with 2M sodium hydroxide (carbon dioxide). Flasks attached to an enclosed incubation system with moistened air bubbled through, controlled by flow restrictors. The water/sediment systems were maintained on an orbital shaker to aid aerobic conditions.
No. of water sediment systems	2
Incubation conditions	Aerobic, 20±2°C, dark
2 Pre-treatment acclimatisation	19 days
Parameters monitored	Complete phase separation, redox potential (water and sediment layer), pH (water), oxygen content (water)
3 Test material application, radiolabelled	
Application rate	14C- gibberellic acid GA3 (10.01 µg per flask) was added in methanol (88 µL, <1% by volume of the water layer). Equivalent to a treatment rate of 212 g a.s./ha. Note: the nominal treatment rate required was 360 g as/ha, however, a problem with the quoted specific radioactivity only came to light after the water/sediment samples were treated with the test material. Based on the corrected specific radioactivity, the actual treatment rate was 212 g as/ha.
Application procedure	Treated by adding the solution drop-wise to the surface.
3 Sampling	Duplicate flasks removed with associated traps
Sampling intervals	0, 3, 7, 14, 30, 59, and 100 days
Control flasks	For each water/sediment system, samples were set up for determination of microbial biomass (pre-study and at end of study) and for monitoring of redox, pH and oxygen content.
Collection of volatile trapping solutions	Volatile traps were replenished at each sampling interval. Sampled traps quantified by LSC.

Method of analysis

1 Method of analysis for sediment and aqueous samples	
Extraction	Water layer separated from sediment by transfer into methanol and radioactivity quantified by LSC. Sediment was extracted with 0.01 M phosphate buffer (100 mL), shaken (30 mins), centrifuged (2500, 10 mins) and the supernatant was decanted. The process was repeated twice with methanol (100 mL) and once with acetone (100 mL). Sediment extracts were combined and quantified by LSC. Additional extractions were performed on selected samples (sample codes A11 and B11 from 59 DAT containing the highest levels of un-extracted soil residue) extracting twice by shaking with 1% formic acid in methanol (100 mL) and once with the same solvent by Soxhlet extraction (100 mL *ca* 5 hrs). Additional extracts were combined and quantified by LSC.
Sample work-up/concentration	Sediment extracts were concentrated by centrifugal evaporation (*ca* 1 mL) and reconstituted with methanol:water (*ca* 6 mL, 1:1 v/v). 59 DAT sediment extracts were further concentrated (*ca* 1 mL) by evaporation under nitrogen. Procedural recoveries were 86-105% (on occasions where recovery of radioactivity was less than 90%, the actual loss of applied radioactivity was ≤2.6%).
Analytical method, primary	Used for RCP (injection volume 10 µL) and study samples (injection volume 150-1300 µL): HPLC –- Phenomenex Gemini C18 column (25 cm x 4.6 mm), mobile phase – gradient solvent a) 0.05% formic acid in water, b) 0.05% formic acid in acetonitrile; 0 mins 95% a), 1 min 95% a), 27 mins 40% a), 28 mins 0% a), 30 mins 0% a), 31 mins 95% a), 35 mins 95% a). Flow rate 1 mL/min. Co chromatography with authentic reference standard (GA3). 14C detection – LabLogic β-Ram radiodetector, liquid cell (0.5 mL) using liquid scintillant (3 mL/min). Column recoveries (92-120% AR).
Analytical method, secondary	Used for RCP and representative study samples: TLC – Merck silica gel plates developed in methanol/ chloroform: acetic acid (8:1.5:0.5 v/v/v). Detection by linear analyser, co-chromatography with authentic reference standards.

Analytical method, secondary / confirmation and structural elucidation	LC-MS and LC-MS/MS was used for structural confirmation of gibberellic acid GA3 and tentative identification of observed metabolites: i) LC-MS MS HPLC method 2 – as primary method except 0 mins 95% a), 3 min 95% a), 27 mins 40% a), 28 mins 0% a), 30 mins 0% a), 31 mins 95% a), 35 mins 95% a). MS HPLC method 3 – Waters Sunfire C18 (25 cm L x 4.6 mm id, 5 µm), mobile phase – gradient system a) 0.1% formic acid in water, b) 0.1% formic acid in methanol/acetonitrile (4:1v/v), 0 mins 95% a), 3 min 95% a), 20 mins 60% a), 35 mins 0% a), 40 mins 0% a), 41 mins 95% a), 45 mins 95% a). Flow rate 1 mL/min (split ratio 3:1 RAD:MS) MS – Thermo Scientific Q-Exactive, ionisation mode: heated electrospray ionisation (HESI) –ve and +ve, scan range: 120-1250 m/z ii) LC-MS/MS MS HPLC method 3 – as above MS – as above, ionisation mode: HESI –ve.
2 Method of analysis for extracted sediment	After extraction, sediment samples were air-dried, weighed and ground to a fine powder. Triple aliquots (*ca* 0.2 g) were combusted. Combustion products were quantified by LSC.
Unextracted residue fractionation	Performed on selected samples only (same as for additional extractions). Bound residue fractionation was performed by precipitation with acidic and basic conditions into fulvic and humic acid fractions.
3 Volatile components	Volume of each trap solution was measured and radioactivity determined by LSC.
Confirmation of carbon dioxide	Radioactivity collected in sodium hydroxide traps confirmed to be $^{14}CO_2$ by barium carbonate precipitation.

Degradation kinetics

1 Procedure followed	DT_{50} and DT_{90} values for the degradation of gibberellic acid GA3 in soil were determined in accordance with the recommendations of the FOCUS work group on kinetics FOCUS 2006[5] and FOCUS 2014[6] (determination of persistence endpoints only i.e. degradation endpoints for use as triggers for additional work).
Software used	CAKE v2.0
Input data used	Input data for the degradation of gibberellic acid GA3 were taken from the individual data presented in Tables 8.2.4-4 and 8.2.4-9, with zero time values corrected to the total recovery at this time point multiplied by the radiochemical purity. All data points were equally weighted.

[5] FOCUS (2006) "Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration" Report of the FOCUS Work Group on Degradation Kinetics, EC Document Reference Sanco/10058/2005 version 2.0, 434 pp

[6] FOCUS 2014. Generic guidance for Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration, version 1.1, 18 December 2014.

II. RESULTS AND DISCUSSION

A. DISTRIBUTION OF APPLIED RADIOACTIVITY

The recovery and distribution of the applied radioactivity in the water/sediment systems is summarised in Tables 8.2.4-2 and 8.2.4-3.

The distribution of radioactivity in the two water-sediment systems was similar. Radioactivity in surface water decreased steadily to 59 DAT in both water-sediment systems then decreased more slowly. At the start of the incubation all the applied radioactivity was present in the surface water and at the end of the incubation period 24 or 40% AR remained in surface water in the Calwich Abbey and Emperor Lake systems, respectively.
Radioactivity extracted from sediment reached a maximum value before decreasing. In the Calwich Abbey system the maximum was at 30 DAT (24% AR) and in the Emperor Lake system it was at 59 DAT (20% AR).

Table 8.2.4-2: Percent recovery of applied radioactivity from the Calwich Abbey water-sediment system treated with [^{14}C]gibberellic acid

Sampling Interval (Days)	0			3			7			14		
Vessel Code	A1	A2	Mean	A3	A4	Mean	A5	A6	Mean	A7	A8	Mean
Overlying Water	100.1	100.3	100.2	89.5	90.6	90.1	84.9	84.1	84.5	78.0	75.9	77.0
Sediment Extract	ND	ND	ND	8.8	9.2	9.0	14.4	15.8	15.1	17.3	19.3	18.3
Un-extracted from Sediment	ND	ND	ND	0.2	0.2	0.2	0.7	0.7	0.7	4.9	4.5	4.7
Total in Vessel	100.1	100.3	100.2	98.5	100.0	99.3	100.0	100.6	100.3	100.2	99.7	100.0
Sodium Hydroxide Traps	NA	NA	NA	ND	ND	ND	ND	ND	ND	0.1	0.1	0.1
Mass Balance	**100.1**	**100.3**	**100.2**	**98.5**	**100.0**	**99.3**	**100.0**	**100.6**	**100.3**	**100.3**	**99.8**	**100.1**

NA = not applicable, ND = not detected (or <0.1% AR)

Sampling Interval (Days)	30			59			100		
Vessel Code	A9	A10	Mean	A11	A12	Mean	A13	A14	Mean
Overlying Water	63.3	60.7	62.0	24.1	25.6	24.9	16.6	32.2	24.4
Sediment Extract	22.9	24.3	23.6	15.6	19.6	17.6	10.9	14.7	12.8
Un-extracted from Sediment	12.4	12.7	12.6	37.1	34.9	36.0	26.5	25.0	25.8
Total in Vessel	98.6	97.7	98.2	76.8	80.1	78.5	54.0	71.9	63.0
Sodium Hydroxide Traps	0.2	0.8	0.5	12.9	10.1	11.5	31.9	18.9	25.4
Mass Balance	**98.8**	**98.5**	**98.7**	**89.7**	**90.2**	**90.0**	**85.9**	**90.8**	**88.4**

Table 8.2.4-3: Percent recovery of applied radioactivity from the Emperor Lake water-sediment system treated with [^{14}C]gibberellic acid

Sampling Interval (Days)	0			3			7			14		
Vessel Code	B1	B2	Mean	B3	B4	Mean	B5	B6	Mean	B7	B8	Mean
Overlying Water	99.6	100.7	100.2	85.4	90.0	87.7	83.8	83.7	83.8	82.5	75.8	79.2
Sediment Extract	ND	ND	ND	12.7	8.8	10.8	13.6	13.8	13.7	15.7	18.6	17.2
Un-extracted from Sediment	ND	ND	ND	0.9	0.8	0.9	2.0	2.1	2.1	2.4	6.3	4.4
Total in Vessel	99.6	100.7	100.2	99.0	99.6	99.3	99.4	99.6	99.5	100.6	100.7	100.7
Sodium Hydroxide Traps	NA	NA	NA	ND	ND	ND	0.1	ND	0.1	0.1	0.1	0.1
Mass Balance	**99.6**	**100.7**	**100.2**	**99.0**	**99.6**	**99.3**	**99.5**	**99.6**	**99.6**	**100.7**	**100.8**	**100.8**

NA = not applicable, ND = not detected (or <0.1% AR)

Sampling Interval (Days)	30			59			100		
Vessel Code	B9	B10	Mean	B11	B12	Mean	B13	B14	Mean
Overlying Water	65.9	70.1	68.0	39.7	57.8	48.8	52.4	27.8	40.1
Sediment Extract	18.1	16.9	17.5	16.7	22.5	19.6	19.8	12.4	16.1
Un-extracted from Sediment	15.2	12.7	14.0	36.0	16.7	26.4	20.4	28.6	24.5
Total in Vessel	99.2	99.7	99.5	92.4	97.0	94.7	92.6	68.8	80.7
Sodium Hydroxide Traps	0.1	0.1	0.1	6.6	1.8	4.2	3.0	21.5	12.3
Mass Balance	**99.3**	**99.8**	**99.6**	**99.0**	**98.8**	**98.9**	**95.6**	**90.3**	**93.0**

Table 8.2.4-4: Percent of applied radioactivity present as gibberellic acid and degradation products in Calwich Abbey surface water

Sampling Interval (Days)	0			3			7			14		
Vessel Code	A1	A2	Mean	A3	A4	Mean	A5	A6	Mean	A7	A8	Mean
Gibberellic acid	100.1	99.9	100.0	77.3	79.4	78.3	67.1	65.0	66.1	27.2	32.8	30.0
Metabolite WS1	ND	ND	ND	11.6	10.8	11.2	17.8	18.7	18.3	33.5	30.8	32.1
Metabolite WS2	ND	ND	ND	ND	ND	ND	ND	ND	ND	10.3	7.9	9.1
Metabolite WS3	ND	ND	ND	ND	ND	ND	ND	ND	ND	3.5	2.9	3.2
Metabolite WS4	ND	ND	ND	ND	ND	ND	ND	ND	ND	3.2	1.5	2.4
Other Minor Unknowns	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Unresolved Background	ND	0.4	0.2	0.6	0.4	0.5	0.1	0.3	0.2	0.3	0.1	0.2
Total	**100.1**	**100.3**	**100.2**	**89.5**	**90.6**	**90.1**	**84.9**	**84.1**	**84.5**	**78.0**	**75.9**	**77.0**
Largest Other Unknown	ND	ND		ND	ND		ND	ND		ND	ND	

ND = not detected (or <0.1%)

Sampling Interval (Days)	30			59			100		
Vessel Code	A9	A10	Mean	A11	A12	Mean	A13	A14	Mean
Gibberellic acid	6.8	6.4	6.6	1.2	0.8	1.0	0.4	0.5	0.4
Metabolite WS1	35.6	34.1	34.8	2.0	2.6	2.3	3.4	0.9	2.2
Metabolite WS2	ND	1.4	0.7	1.3	2.7	2.0	1.8	2.2	2.0
Metabolite WS3	4.8	6.1	5.5	3.0	4.7	3.8	2.1	17.9	10.0
Metabolite WS4	11.3	9.2	10.2	1.9	2.9	2.4	2.2	ND	1.1
Other Minor Unknowns	4.4	3.4	3.9	14.3	11.9	13.1	6.6	10.7	8.6
Unresolved Background	0.4	0.3	0.3	0.5	ND	0.2	0.1	ND	0.1
Total	**63.3**	**60.7**	**62.0**	**24.1**	**25.6**	**24.8**	**16.6**	**32.2**	**24.4**
Largest Other Unknown	2.9	3.4		3.1	4.0		2.1	3.2	

ND = not detected (or <0.1%)

Table 8.2.4-5: Percent of applied radioactivity present as gibberellic acid and degradation products in Emperor Lake surface water

Sampling Interval (Days)	0			3			7			14		
Vessel Code	B1	B2	Mean	B3	B4	Mean	B5	B6	Mean	B7	B8	Mean
Gibberellic acid	99.1	98.0	98.6	73.1	76.5	74.8	61.6	62.3	62.0	45.9	32.7	39.3
Metabolite WS1	ND	2.6	1.3	9.4	11.3	10.4	17.6	17.5	17.6	32.0	30.1	31.1
Metabolite WS2	ND	ND	ND	2.8	2.0	2.4	1.3	1.5	1.4	1.5	6.3	3.9
Metabolite WS3	ND	ND	ND	ND	ND	ND	2.1	2.1	2.1	1.5	6.6	4.0
Metabolite WS4	ND	ND	ND	ND	ND	ND	ND	ND	ND	1.6	ND	0.8
Other Minor Unknowns	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Unresolved Background	0.5	0.1	0.3	0.1	0.1	0.1	1.1	0.4	0.8	ND	0.2	0.1
Total	**99.6**	**100.7**	**100.2**	**85.4**	**90.0**	**87.7**	**83.8**	**83.7**	**83.8**	**82.5**	**75.8**	**79.2**
Largest Other Unknown	ND	ND		ND	ND		ND	ND		ND	ND	

ND = not detected (or <0.1%)

Sampling Interval (Days)	30			59			100		
Vessel Code	B9	B10	Mean	B11	B12	Mean	B13	B14	Mean
Gibberellic acid	4.6	4.6	4.6	ND	ND	ND	2.7	0.3	1.5
Metabolite WS1	31.0	37.2	34.1	9.2	9.3	9.2	6.2	1.6	3.9
Metabolite WS2	1.9	2.4	2.1	1.3	3.5	2.4	8.4	1.6	5.0
Metabolite WS3	7.9	7.6	7.7	19.9	34.1	27.0	25.3	16.5	20.9
Metabolite WS4	12.1	10.4	11.3	ND	0.5	0.3	ND	ND	ND
Other Minor Unknowns	8.3	7.6	8.0	8.7	9.3	9.0	9.7	7.5	8.6
Unresolved Background	0.2	0.4	0.3	0.6	1.1	0.9	0.1	0.3	0.2
Total	**65.9**	**70.1**	**68.0**	**39.7**	**57.8**	**48.7**	**52.4**	**27.8**	**40.1**
Largest Other Unknown	3.4	3.4		3.5	2.3		2.3	2.3	

ND = not detected (or <0.1%)

Table 8.2.4-6: Percent of applied radioactivity present as gibberellic acid and degradation products in Calwich Abbey sediment extract

Sampling Interval (Days)	0			3			7			14		
Vessel Code	A1	A2	Mean	A3	A4	Mean	A5	A6	Mean	A7	A8	Mean
Gibberellic acid	NA	NA	NA	8.8	9.2	9.0	10.5	10.2	10.3	9.1	10.0	9.5
Metabolite WS1	NA	NA	NA	ND	ND	ND	3.9	5.5	4.7	8.0	9.1	8.5
Metabolite WS2	NA	NA	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
Metabolite WS3	NA	NA	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
Metabolite WS4	NA	NA	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
Other Minor Unknowns	NA	NA	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
Unresolved Background	NA	NA	NA	ND	ND	ND	ND	0.1	0.1	0.3	0.3	0.3
Total	**NA**	**NA**	**NA**	**8.8**	**9.2**	**9.0**	**14.4**	**15.8**	**15.1**	**17.3**	**19.3**	**18.3**
Largest Other Unknown	NA	NA		ND	ND		ND	ND		ND	ND	

NA = not applicable, ND = not detected (or <0.1%)

Sampling Interval (Days)	30			59			100		
Vessel Code	A9	A10	Mean	A11	A12	Mean	A13	A14	Mean
Gibberellic acid	4.0	3.9	4.0	0.6	0.4	0.5	0.2	0.2	0.2
Metabolite WS1	13.3	10.0	11.6	0.7	1.6	1.2	1.5	1.3	1.4
Metabolite WS2	ND	ND	ND	0.6	0.5	0.6	0.5	0.5	0.5
Metabolite WS3	3.3	1.6	2.4	4.4	6.0	5.2	1.5	4.7	3.1
Metabolite WS4	2.1	3.4	2.8	1.9	2.2	2.1	2.2	2.4	2.3
Other Minor Unknowns	ND	5.2	2.6	7.2	8.8	8.0	5.1	5.5	5.3
Unresolved Background	0.3	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.1
Total	**22.9**	**24.3**	**23.6**	**15.6**	**19.6**	**17.6**	**10.9**	**14.7**	**12.8**
Largest Other Unknown	ND	5.1		1.0	1.4		0.9	0.7	

ND = not detected (or <0.1%)

Table 8.2.4-7: Percent of applied radioactivity present as gibberellic acid and degradation products in Emperor Lake sediment extract

Sampling Interval (Days)	0			3			7			14		
Vessel Code	B1	B2	Mean	B3	B4	Mean	B5	B6	Mean	B7	B8	Mean
Gibberellic acid	NA	NA	NA	10.1	8.8	9.5	7.6	10.0	8.8	8.2	11.5	9.8
Metabolite WS1	NA	NA	NA	2.5	ND	1.3	3.1	3.8	3.5	7.5	7.1	7.3
Metabolite WS2	NA	NA	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
Metabolite WS3	NA	NA	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
Metabolite WS4	NA	NA	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
Other Minor Unknowns	NA	NA	NA	ND	ND	ND	2.9	ND	1.4	ND	ND	ND
Unresolved Background	NA	NA	NA	0.1	ND	ND	ND	ND	ND	0.1	ND	0.1
Total	**NA**	**NA**	**NA**	**12.7**	**8.8**	**10.8**	**13.6**	**13.8**	**13.7**	**15.7**	**18.6**	**17.1**
Largest Other Unknown	NA	NA		ND	ND		2.9	ND		ND	ND	

NA = not applicable, ND = not detected (or <0.1%)

Sampling Interval (Days)	30			59			100		
Vessel Code	B9	B10	Mean	B11	B12	Mean	B13	B14	Mean
Gibberellic acid	1.4	1.1	1.3	0.5	1.1	0.8	0.3	0.5	0.4
Metabolite WS1	7.8	12.8	10.3	6.0	3.8	4.9	1.8	0.7	1.3
Metabolite WS2	ND	ND	ND	0.6	0.9	0.7	2.7	0.5	1.6
Metabolite WS3	3.1	1.2	2.1	2.1	3.0	2.6	2.5	5.4	3.9
Metabolite WS4	2.7	1.5	2.1	1.0	1.6	1.3	8.1	ND	4.0
Other Minor Unknowns	3.1	ND	1.6	6.4	12.1	9.2	4.3	5.1	4.7
Unresolved Background	0.1	0.3	0.2	0.2	0.1	0.1	0.2	0.1	0.1
Total	**18.1**	**16.9**	**17.5**	**16.7**	**22.5**	**19.6**	**19.8**	**12.4**	**16.1**
Largest Other Unknown	3.1	ND		1.7	2.1		0.8	1.0	

ND = not detected (or <0.1%)

Table 8.2.4-8: Percent of applied radioactivity present as gibberellic acid and degradation products in Calwich Abbey water-sediment system

Sampling Interval (Days)	0			3			7			14		
Vessel Code	A1	A2	Mean	A3	A4	Mean	A5	A6	Mean	A7	A8	Mean
Gibberellic acid	100.1	99.9	100.0	86.1	88.6	87.3	77.5	75.2	76.4	36.3	42.8	39.5
Metabolite WS1	ND	ND	ND	11.6	10.8	11.2	21.7	24.2	23.0	41.5	39.8	40.7
Metabolite WS2	ND	ND	ND	ND	ND	ND	ND	ND	ND	10.3	7.9	9.1
Metabolite WS3	ND	ND	ND	ND	ND	ND	ND	ND	ND	3.5	2.9	3.2
Metabolite WS4	ND	ND	ND	ND	ND	ND	ND	ND	ND	3.2	1.5	2.4
Other Minor Unknowns*	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Unresolved Background	ND	0.4	0.2	0.6	0.5	0.5	0.1	0.4	0.3	0.5	0.4	0.5
Total	**100.1**	**100.3**	**100.2**	**98.3**	**99.8**	**99.1**	**99.3**	**99.9**	**99.6**	**95.3**	**95.2**	**95.3**

ND = not detected (or <0.1%), * = each of which did not exceed an average of 4.5% AR

Sampling Interval (Days)	30			59			100		
Vessel Code	A9	A10	Mean	A11	A12	Mean	A13	A14	Mean
Gibberellic acid	10.8	10.3	10.5	1.8	1.2	1.5	0.6	0.6	0.6
Metabolite WS1	48.9	44.1	46.5	2.7	4.2	3.5	4.9	2.3	3.6
Metabolite WS2	ND	1.4	0.7	1.9	3.2	2.5	2.3	2.7	2.5
Metabolite WS3	8.1	7.7	7.9	7.4	10.7	9.0	3.6	22.6	13.1
Metabolite WS4	13.4	12.6	13.0	3.8	5.2	4.5	4.3	2.4	3.3
Other Minor Unknowns*	4.4	8.5	6.5	21.5	20.7	21.1	11.7	16.2	14.0
Unresolved Background	0.6	0.5	0.6	0.6	0.1	0.4	0.2	0.2	0.2
Total	**86.2**	**85.0**	**85.6**	**39.7**	**45.2**	**42.4**	**27.5**	**46.9**	**37.2**

ND = not detected (or <0.1%), * = each of which did not exceed an average of 4.5% AR

Table 8.2.4-9: Percent of applied radioactivity present as gibberellic acid and degradation products in Emperor Lake water-sediment system

Sampling Interval (Days)	0			3			7			14		
Vessel Code	B1	B2	Mean	B3	B4	Mean	B5	B6	Mean	B7	B8	Mean
Gibberellic acid	99.1	98.0	98.6	83.2	85.3	84.3	69.2	72.2	70.7	54.1	44.1	49.1
Metabolite WS1	ND	2.6	1.3	12.0	11.3	11.6	20.7	21.3	21.0	39.5	37.2	38.4
Metabolite WS2	ND	ND	ND	2.8	2.0	2.4	1.3	1.5	1.4	1.5	6.3	3.9
Metabolite WS3	ND	ND	ND	ND	ND	ND	2.1	2.1	2.1	1.5	6.6	4.0
Metabolite WS4	ND	ND	ND	ND	ND	ND	ND	ND	ND	1.6	ND	0.8
Other Minor Unknowns*	ND	ND	ND	ND	ND	ND	2.9	ND	1.4	ND	ND	ND
Unresolved Background	0.5	0.1	0.3	0.2	0.2	0.2	1.2	0.4	0.8	0.1	0.2	0.1
Total	**99.6**	**100.7**	**100.2**	**98.1**	**98.8**	**98.5**	**97.4**	**97.5**	**97.5**	**98.2**	**94.4**	**96.3**

ND = not detected (or <0.1%), * = each of which did not exceed an average of 4.4% AR

Sampling Interval (Days)	30			59			100		
Vessel Code	B9	B10	Mean	B11	B12	Mean	B13	B14	Mean
Gibberellic acid	6.0	5.7	5.8	0.5	1.1	0.8	3.0	0.8	1.9
Metabolite WS1	38.8	50.0	44.4	15.2	13.0	14.1	8.0	2.4	5.2
Metabolite WS2	1.9	2.4	2.1	1.9	4.4	3.1	11.1	2.1	6.6
Metabolite WS3	10.9	8.8	9.9	22.0	37.1	29.5	27.7	22.0	24.8
Metabolite WS4	14.8	11.9	13.3	1.0	2.1	1.6	8.1	ND	4.0
Other Minor Unknowns*	11.4	7.6	9.5	15.1	21.3	18.2	14.0	12.6	13.3
Unresolved Background	0.2	0.7	0.5	0.8	1.2	1.0	0.3	0.4	0.3
Total	**84.0**	**87.0**	**85.5**	**56.4**	**80.3**	**68.3**	**72.2**	**40.2**	**56.2**

ND = not detected (or <0.1%), * = each of which did not exceed an average of 4.4% AR

Volatile radioactivity, shown to be carbon dioxide by precipitation with barium chloride, increased to 25% or 12% AR in the Calwich Abbey and Emperor Lake systems, respectively. Mineralisation was highest in the system with the higher organic carbon content and microbial biomass.
Un-extracted radioactivity (sediment residue) increased to maximum values at 59 DAT before decreasing. In the Calwich Abbey system mean levels were 36% AR at 59 DAT decreasing to 26% AR at 100 DAT. In the Emperor Lake system mean levels were 26% at 59 DAT decreasing to 25% at 100 DAT.
Amounts in the additional sediment extractions were <1% AR.

B. MASS BALANCE

Total recovery of applied radioactivity was 88.4 to 100.8% (mean values) over all sampling intervals.

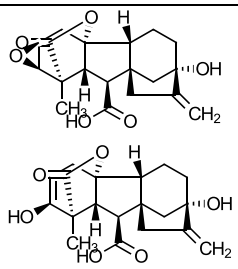
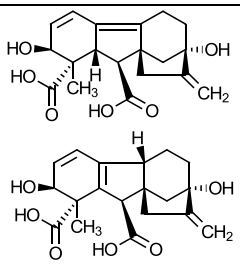
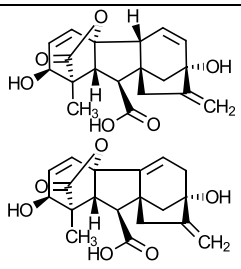
C. PROFILE OF COMPONENTS

The profile of components from the Calwich Abbey and Emperor Lake systems in the water and sediment layers is summarised in Tables 8.2.4-4 and 8.2.4-5 and 8.2.4-6 and 8.2.4-7, respectively.

The profile of components from the total Calwich Abbey and Emperor Lake systems is summarised in Tables 8.2.4-8 and 8.2.4-9.

Concentrations of gibberellic acid GA3 in the total systems decreased steadily during the study, reaching 39.5-49.1% AR after 14 DAT and <2% AR after 59 DAT. Degradation of gibberellic acid GA3 led to the formation of 4 significant metabolites (i.e. observed at levels >10%, or >5% on consecutive sampling intervals or >5% and increasing at the end of the study). Prior to identification, the metabolites were labelled WS1 (maximum mean observed level 46.5% AR after 30 DAT), WS2 (max mean 9.1% AR, 14 DAT), WS3 (max mean 29.5% AR, 59 DAT) and WS4 (max mean 13.3% AR, 30 DAT).
TLC was used in an attempt to confirm levels of gibberellic acid GA3 and metabolites in selected samples. The samples used were representative 14 DAT surface water and sediment samples. Unfortunately, the TLC method did not separate gibberellic acid GA3 from its metabolites, and therefore the results are inconclusive.
The identity of gibberellic acid GA3 was confirmed by LC-MS/MS on the basis of retention time, molecular ion/full spectrum and MS/MS spectra of characteristic ions in comparison with an authentic reference standard. Authentic reference standards of the metabolites were not available, however, sufficient supporting MS/MS information was obtained to propose reasonable tentative structures for each of the metabolites, as follows:
Metabolite WS1 was found to be composed of 2 components i.e. WS1a and WS1b (each >10% AR), each with the same molecular mass as gibberellic acid GA3 i.e. 346 g/mol. The tentative structures for the 2 components are shown in Table 8.2.4-10 i.e. WS1a is GA6 an isomer of GA3 or re-arrangement of the double bond and WS1b is formed through opening of the lactone ring. The product ion spectra were a good match for the 2 metabolites observed in the aerobic mineralisation study (see CA 7.2.2.2/01).
Metabolite WS2 was found to have a molecular mass of 344 g/mol i.e. consistent with the loss of H_2 from the parent molecule. Based on the product ion spectra, two tentative structures were proposed. The product ion spectra were a good match for metabolite AS1 observed in the aerobic soil degradation study (see CA 7.1.1.1/01) and two tentative structures were proposed i.e. 9,11-dehydro-GA3 or 11,12- dehydro-GA3.
Metabolites WS3 and WS4 were found to have molecular mass 380 g/mol. Based on the product ion spectra, tentative structures were proposed i.e. open lactone-dihydroxy-GA3 and positional isomers. The product ion spectra were a good match for metabolite AS7 observed in the aerobic soil degradation study (see CA 7.1.1.1/01).
The tentative structures are proposed on the basis of product ion spectra and matching of fragments with the ions observed. Full details are provided in an appendix in the study report.

Table 8.2.4-10: Tentative identities of metabolites of gibberellic acid GA3 in water/sediment systems

Metabolite WS1*	Metabolite WS2	Metabolite WS3 and Metabolite WS4
		

*: actually a mixture of two metabolites (WS1a and WS1b) which co-eluted

D. ORGANIC MATTER FRACTIONATION

The non-extractable radioactivity present in the soil residue was further characterised by organic matter fractionation and the results are presented in Table 8.2.4-11.

Table 8.2.4-11: Bound residue fractionation

Sample code	Amount of applied radioactivity (% AR)			
	Fulvic acids	**Humic acids**	**Humin**	**Recovery (%)**
A11 (Calwich Abbey)	9.1	7.8	21.3	106
B11 (Emperor Lake)	7.9	4.9	19.8	93

Radioactivity was present in all three fractions showing assimilation into sediment organic matter.

E. DEGRADATION RATE

Degradation rates for gibberellic acid GA3 in water/sediment systems were calculated based on the declining concentrations observed in the total water/sediment systems and fitted to SFO kinetics. DT_{50} and DT_{90} values are summarised in Table 8.2.4-12. Gibberellic acid GA3 declined rapidly in natural water with best-fit persistence DT_{50} values of 10.9 to 11.2 days.

Table 8.2.4-122: Degradation rate of gibberellic acid GA3 in combined layers of water/sediment systems

Soil	DT_{50}	DT_{90}	Chi2err (%)	Correlation coefficient, r^2
Calwich Abbey	10.9	36.3	8.03	0.9858
Emperor Lake	11.2	37.3	8.78	0.9817

Some attempts were made to determine DT_{50} values of the metabolites but these were inconclusive and not conducted to FOCUS guidelines and therefore are not discussed further.

III. CONCLUSIONS

Gibberellic acid GA3 was shown to degrade quickly in two water/sediment systems (<2% AR after 59 days), with calculated DT_{50} values of 10.9-11.2 days (SFO). Five major metabolites were observed, with ultimate degradation leading to the formation of carbon dioxide and un-extractable sediment bound residues. Tentative structures for the metabolites were proposed on the basis of extensive LC-MS/MS analysis as follows: WS1a – GA6 an isomer of

GA3 or re-arrangement of the double bond; WS1b - opening of the lactone ring; WS2 - loss of H_2 from the parent molecule; WS3 and WS4 - open lactone-dihydroxy-GA3 and positional isomers (actual structures are tabulated above).

RMS comments and conclusion:

The route and rate of degradation of gibberellic acid in aerobic water-sediment were studied at 20 ± 2°C in the dark using one radiolabelled form of the test substance, [methylene-^{14}C]gibberellic acid. The application rate was 10.1 µg/vessel, equivalent to a field application rate of 360 g a.i./ha (assuming a depth of 51 cm) or 212 g a.i./ha (assuming a depth of 30 cm)[7]. Microbial biomass of the sediment was determined on samples removed from incubation at application and at the end of the incubation period. Duplicate samples were removed for analysis immediately after application of [^{14}C]gibberellic acid and at 3, 7, 14, 30, 59 and 100 DAT (days after treatment). The total recovery of applied radioactivity (AR), for samples treated with [^{14}C]gibberellic acid, ranged from 88.4[8] to 100.8% (mean values). Volatile radioactivity increased to a maximum 25.4% AR at 100 DAT (Calwich Abbey system). This was confirmed to be carbon dioxide by precipitation with barium chloride.

The maximum levels of un-extracted radioactivity at any sampling interval were in the Calwich Abbey system and were 36.0% AR (59 DAT). Concentrations of gibberellic acid decreased steadily during the study and five major metabolites were found. The metabolites were present in both the water and sediment phases, although greater proportions were observed in the water phase. Up to 30 DAT, although gibberellic acid had dissipated steadily, a large percentage of applied radioactivity was present in the water and sediment phases as either gibberellic acid (6 – 11% AR at 30 DAT) or metabolites (total 68 – 70% AR at 30 DAT). After 30 DAT significant degradation of the remaining gibberellic acid and metabolites was observed, to un-extracted residues and to volatile radioactivity. Metabolite formation and degradation varied between vessels and was not always consistent.

DT-50 values for gibberellic acid were determined by FOCUS kinetics. SFO kinetics provided the best fit. Metabolites were also modelled using SFO kinetics in the total water-sediment system, but for Metabolites WS2, WS3 and WS4 it was not possible to determine a true degradation rate as variability between vessels prevented accurate modelling of the results. For Metabolite WS1, the sum of the two components was modelled. Tentative assignment of metabolite identities was made after analysis with LC-MS/MS. Metabolite WS1a and WS1b could each have one of four potential isomeric structures (including alternate gibberellic acid structure GA6), Metabolite WS2 was postulated to be dehydro-GA3, and Metabolite WS3 and Metabolite WS4 were proposed to be isomers of open lactone dihydroxy-GA3.
Gibberellic acid degraded steadily in the two water-sediment systems used, with DT-50 values in the total system of 10.9 and 11.2 days by SFO kinetics at 20 ± 2°C. Degradation occurred to five major metabolites, un-extracted sediment residues (maximum 36.0% AR) and carbon dioxide (maximum 25.4% AR). Of the major metabolites; Metabolite WS1 was a mixture of two components, (WS1a and WS1b), the sum of which reached a maximum of 46.5% AR, Metabolite WS2 (a maximum 9.1% AR), Metabolite WS3 (a maximum 29.5% AR), and Metabolite WS4 (a maximum 13.3% AR). Metabolites were tentatively identified by LC-MS/MS. Metabolites WS1a and WS1b could each have one of four potential isomeric structures (including alternate gibberellic acid structure GA6), Metabolite WS2 was postulated to be dehydro-GA3, and Metabolite WS3 and Metabolite WS4 were proposed to be isomers of open lactone dihydroxy-GA3.

The study was conducted according to OECD Guideline 308 (April 2002) and OPPTS 835.4000 (October 2008) and is considered acceptable.

[7] The rate of application had to be re-calculated due to an error in the specific activity of the radiolabel.
[8] The vessel with mass balance of < 90% AR contained the highest amount of volatile radioactivity in the trapping solutions.

Determination of DT-50 and DT-90

CAKE version 2 software was used to plot the percent of applied radioactivity present as test substance against time of incubation in days (with the exception of 0 DAT, where the mass balance corrected for radiochemical purity was used, as per the FOCUS guidelines). Curves were constructed through appropriate data points using non-linear regression analysis to give lines of best fit. The degradation rates of the test substance, the parameters used to calculate them and parameter statistics were determined.

For both systems parent was derived individually in the water and sediment phases, and as the sum of parent in the water and sediment extract. This was then fitted to an SFO model. There was a good visual fit of the data.

Other models were also tested but SFO provided the best fit to the data; detailed results are contained in following pages. The significant metabolites were also fitted to an SFO model, taking into consideration the limit of detection (as per the FOCUS guidelines).

Study done it with gibberellic acid (GA3) is valid and acceptable and can be used for use in risk assessment for gibberellins (GA4/7).
All and detailed degradation kinetics analyses for this study can be seen in Appendix 4.

Irradiated water/sediment study

Studies investigating the degradation of the active substance in water/sediment systems under irradiated conditions are a higher tier option and are not currently triggered or considered necessary.

Degradation in the Saturated Zone

Based on the information presented following use of the formulated product, the active substance is not expected to leach through the soil profile to the saturated zone in significant quantities. Therefore, no further studies have been carried out.

Summary of degradation in aquatic systems

The active substance gibberellins GA4/7, comprises two components i.e. gibberellins GA4 and gibberellins GA7. The degradation of the active substance in aquatic systems has been investigated by conducting studies on the individual components, where necessary.
Gibberellins GA4 was stable to hydrolysis at pH 4, 7 and 9 (i.e. half-life values of > 1 year at 20°C). Gibberellins GA7 was stable to hydrolysis at pH 7 but was hydrolysed at pH 4 and pH 9 at 50°C. However, the rate of hydrolysis at 20°C is expected to be slow. Degradation products were not identified, but may include the diol derivative of gibberellins GA7 following hydroxylation at the double bond position in the gibberellins GA7 unsaturated ring. Potential degradation products are not considered to be of any environmental concern and are not considered further. The molar absorption coefficients of gibberellins GA4 and gibberellins GA7 are <10 L/mol/cm and therefore a measurement of the photolytic half-life and quantum yield is not required. Nevertheless, the rate of photolysis of gibberellins GA4 and gibberellins GA7 in aqueous buffer solutions at pH 5, 7 and 9 was investigated using artificial sunlight. The photochemical degradation of combined gibberellins GA4 and gibberellins GA7 was slow with a half-life in the range of 104 to 267 days. The photo-degradation of gibberellins GA4 and gibberellins GA7 measured separately was 101 to 163 days and 57 to 145 days, respectively. Photolysis of gibberellins GA4 and gibberellins GA7 can therefore be regarded as a slow process and not a significant route of degradation in the environment.
The active substance gibberellins GA4/7 was determined to be readily biodegradable in a modified Sturm test (OECD 301B). However, a second study, which was also conducted using the carbon dioxide evolution (Modified Sturm) test, provided a conflicting result (i.e. not readily biodegradable). On the basis of the conflicting results of the two ready biodegradability studies (Barnes 2005 and Drake 2009), it is concluded that the active substance gibberellins GA4/7 cannot be reliably concluded as being classified as readily biodegradable. However, on the basis of the degree of biodegradation observed in the non-positive test (in conjunction with the rapid and extensive microbial degradation

and ultimate complete mineralisation via other natural components observed in the soil and aquatic metabolism studies), it is concluded that the active substance gibberellins GA4/7 can be considered inherently biodegradable.
Degradation of the active substance gibberellins GA4/7 in aquatic systems (pelagic water and water/sediments systems) was assessed by read across to studies conducted using the structurally related active substance gibberellins GA3. On this basis degradation of the active substance gibberellins GA4/7 in these systems is assumed to proceed via isomerisation and/or opening of the lactone ring to form metabolites which are not likely to be environmentally important and would also be formed by degradation of naturally occurring gibberellins GA4 and gibberellins GA7.
The available data indicate that gibberellins GA4/7 and its individual components gibberellins GA4 and gibberellins GA7 are not persistent in pelagic water or water-sediment systems. Therefore, in accordance with Regulation (EC) No. 1272/2008, gibberellins GA4/7 fulfils the criteria for consideration as a low-risk active substance in this regard.

B.8.2.5. Predicted environmental concentrations in surface water and in ground water (PECsw, PECgw)

See separate Annex B.8 for product related data.

B.8.3. FATE AND BEHAVIOUR IN AIR

B.8.3.1. Route and rate of degradation in air

a) Previous evaluation during the first review (DAR 2005 - 2011)

Based on vapour pressure values of 0.160 and 0.067 Pa (22°C) for the components gibberellins GA4 and gibberellins GA7, respectively, the components are potentially volatile from plant and soil surfaces. However, no volatility of the individual components gibberellins GA4 or gibberellins GA7 was observed in any of the environmental fate studies and therefore volatility under the conditions of use is not expected. Furthermore, the estimated photochemical oxidative degradation half-lives in air of the components gibberellins GA4 and gibberellins GA7 (calculated using the Atkinson equation), are 1.67 and 0.99 hours, respectively (EFSA LoEP, page 39/50). Therefore, these components will not persist in the atmosphere, if present.
The available data clearly indicate that gibberellins GA4/7 and its individual components gibberellins GA4 and gibberellins GA7 are not persistent in air. Therefore, in accordance with Regulation (EC) No. 1272/2008, gibberellins GA4/7 fulfils the criteria for consideration as a low-risk active substance in this regard.

b) Evaluation of additional data for the purpose of renewal of Annex I inclusion

No new data were available by applicant.

RMS comments and conclusion:

No special experimental data are available on the active substance (GA4/7) or product in air. However, GA4/7 is naturally occurring and has low volatility (Vapour pressure: 7.68 x 10^{-6} Pa at 25ºC; calculated Henry's law constant: 2-6.5 x 10^{-5} Pa.m^3/mol) and hence it is not considered to pose any significant concern in air.

B.8.3.2. Transport via air

a) Previous evaluation during the first review (DAR 2005 - 2011)
Gibberellins GA4 and gibberellins GA7 are not expected to undergo significant volatilisation from soil or plant surfaces in the environment or to persist in the atmosphere, consequently transport in air is not expected to be of significant concern.

b) Evaluation of additional data for the purpose of renewal of Annex I inclusion

No new data were available by applicant.

B.8.3.3. Local and global effects

a) Previous evaluation during the first review (DAR 2005 - 2011)
Gibberellins GA4 and gibberellins GA7 are not expected to undergo significant volatilisation from soil or plant surfaces in the environment or to persist in the atmosphere, consequently transport in air is not expected to be of significant concern.

b) Evaluation of additional data for the purpose of renewal of Annex I inclusion

No new data were available by applicant.

B.8.3.4. Predicted environmental concentrations in air

Not applicable. No volatility of the individual components gibberellins GA4 or gibberellins GA7 was observed in any of the environmental fate studies and therefore volatility under the conditions of use is not expected. Furthermore, the estimated photochemical oxidative degradation half-lives in air of the components gibberellins GA4 and gibberellins GA7 (calculated using the Atkinson equation), are 1.67 and 0.99 hours, respectively (EFSA LoEP, page 39/50). Therefore, these components will not persist in the atmosphere, if present.
The available data clearly indicate that gibberellins GA4/7 and its individual components gibberellins GA4 and gibberellins GA7 are not persistent in air. Therefore, in accordance with Regulation (EC) No. 1272/2008, gibberellins GA4/7 fulfils the criteria for consideration as a low-risk active substance in this regard.

B.8.4. MONITORING DATA CONCERNING FATE AND BEHAVIOUR OF THE ACTIVE SUBSTANCE, METABOLITES, DEGRADATION AND REACTION PRODUCTS

No monitoring studies for the active substance gibberellic acid GA3 are available.

Additional information on natural occurrence

It was noted in the EFSA Conclusion for gibberellins (EFSA Journal 2012;10(1):2502) that a quantitative assessment of naturally occurring levels of GA4 and GA7 in soil and aquatic systems was not provided in support of the original application for Annex I inclusion. To address this, a thorough and detailed review of available published literature on levels of naturally occurring gibberellins in the environment has been conducted. Modern guideline compliant studies on the aerobic soil metabolism and degradation and adsorption desorption characteristics of gibberellins GA4 and GA7 in the active substance GA4/7 are also provided, along with studies on the route and rate of degradation in surface water of the structurally similar active substance gibberellic acid GA3, which provide robust data on the fate and behaviour of the active substance in the environment. Published scientific data on quantified levels of gibberellins in soil and surface water resulting from exposure to naturally occurring levels in plant materials are scarce. However, a wealth of published information exists on the presence of endogenous gibberellins in both higher plants and in algae, mosses and lichens and on the biosynthesis of gibberellins in the natural environment by soil bacteria and fungi. These data are summarised in Table 7.6-1 below and provide a weight of evidence on the natural occurrence of gibberellins in the environment.

Table 8.11-1: **Summary of literature papers**

Dossier location	Author and Year	Crop or matrix	Analyte(s)	Methodology	Details
CA 7.6/01	Acheampong *et al*., (2015)	Vines	GA1, GA4 (+ various precursors and deactivation products of GA1 & GA4)	Triplicate samples of freeze-dried organs/tissues were solvent extracted and analysed by LC-MS and UHPLC. Endogenous GA contents were calculated from the peak area ratios of the endogenous GA to internal standards spiked during the extraction process.	The 13-hydroxylation pathway, which leads to the biosynthesis of GA1 was characterized by high levels of GA19 in all organs (0.1 ± 0.0 to 28.8 ± 1.7 ng g^{-1} fw). Levels of GA1 ranged from (0.4 ± 0.1 to 2.9 ± 1.2 ng g^{-1} fw and reduced by 4-fold at fruit set and further decreased as berries developed. GA8, the deactivation product of GA1 was detected in most organs (0.2 ± 0.0 to 14.8 ± 0.8 ng g^{-1} fw), and showed a similar temporal profile to GA1. GA8 levels were elevated during carpel-berry transition, but declined during berry development. The non-13-hydroxylation pathway that produces GA4 was characterized by relatively lower quantities of GA intermediates. Levels of GA4 ranged from 0.1 ± 0.0 to 2.1 ± 0.5 ng g^{-1} fw and levels in carpels decreased towards fruit set. Generally, the levels of the different GA species either decreased or remained constant as tissues / organs developed.
CA 7.6/02	Allen *et al*., (1982)	Blue grama grass (leaf and root tissue of mycorrhizal and non-mycorrhizal infected seedlings)	Gibberellins	Ground samples were solvent extracted, separated using silica gel TLC plates, developed using ethyl acetate:chloroform:acetic acid (5:15:1) and analysed by bioassay (barley half-seed bioassay). GAs were quantified using a standard curve obtained from incubation of known concentrations of GA3 and expressed as GA3 equivalents.	Measured concentrations of GAs (expressed as GA3 equivalents) in 50-day old mycorrhizal (M) and non-mycorrhizal (NM) infected seedlings, (n=3) were as follows: i) Leaves = 15.0 to 87.3 µg kg^{-1} fw (NM) 34.0 to 347.1 µg kg^{-1} fw (M) ii) Roots = 2.6 to 21.5 µg kg^{-1} fw (NM) 1.9 to 4.6 µg kg^{-1} fw (M) Infection by mycorrhizal fungi resulted in significantly increased GA activity in the leaves and a tendency for decreased activity in the roots.

Dossier location	Author and Year	Crop or matrix	Analyte(s)	Methodology	Details
CA 7.6/03	Barea *et al*., (1976)	Rhizosphere bacteria	Gibberellins	The production of gibberellins by selected phosphate dissolving bacteria isolated from the rhizosphere soils of several crop plants was investigated. Following incubation, bacterial cells were removed by centrifugation and the supernatant fluid was solvent extracted. Separation was achieved by descending chromatography and chromatograms were cut into a sequence of 10 R_f values and analysed by bioassay (lettuce hypocotyls method). The total amount of gibberellin-like substances produced by the micro-organisms was calculated from a standard dose response curve and presented as gibberellic acid (GA3) equivalents.	29 of the 50 bacterial cultures tested produced gibberellins (58%). Reported levels of gibberellins in the bacterial cultures expressed as GA3 equivalents ranged from 0.008 to 0.2 µg mL^{-1}.
CA 7.6/04	Barendse *et al*., (2012)	*Pharbitis nil* (seeds)	Gibberellins	Endogenous levels of gibberellin-like substances from the developing seeds of Japanese Morning Glory (*Pharbitis nil* cv. Violet) were determined using HPLC and the dwarf maize bioassay.	Three GA fractions were obtained, their retention times being equivalent to GA3, GA5 or GA20 and GA19 and/or GA44. The bioactivity of all three fractions increased during early seed development, followed by a decline during seed maturation. The total endogenous levels (expressed as GA3 equivalents), reached a maximum of *ca*. 0.92 µg/ seed at 19 days after anthesis and pollination, just prior to the maximal fresh weight of seeds, which was reached approximately 23 days after anthesis.
CA 7.6/05	Baydar and Harmankaya (2005)	Grapes	GA3	Solvent extracted, separated using silica gel TLC plates (recovered by plate scraping), methylated using diazomethane and quantified using GC analysis (calibration with authentic GA3 reference standard).	Grapes (seedless varieties, n=3) i) 0.0045 mg kg^{-1} fw at 16 DAA to 0.00014 mg kg^{-1} fw at ripening (factor *ca* 32) ii) 0.0072 mg kg^{-1} fw at 25 DAA to 0.0005 mg kg^{-1} fw at ripening (factor *ca* 14) iii) 0.0022 mg/kg fw at 20 DAA to 0.00026 mg kg^{-1} fw at ripening (factor *ca* 8)
CA 7.6/06	Böttcher *et al*., (2013)	Grapes (Shiraz berries)	GA1, GA3, GA4, GA7, GA8, GA9, GA9, GA20, GA24, GA29, GA34, GA44, GA51, GA53	Frozen tissue was ground to a powder, solvent extracted, partitioned and analysed by UPLC-ESI-MS/MS (comparison with deuterated internal standards).	Only five GAs were reliably detected early in berry development and none were reliably detected in post-veraison fruit. GA4 and GA7 were detected at low concentrations in berries at 4, 6 and 8 weeks post-flowering, but not thereafter. The most abundant species was GA24 (*ca*. 130-180 ng g^{-1} dw), which is a precursor for GA4 and GA7 in the non-hydroxylation pathway, indicating that GA4 and GA7 were actively synthesized during this period. GA34, which is a catabolite of GA4, was present in berries at 4, 6 and 8

Dossier location	Author and Year	Crop or matrix	Analyte(s)	Methodology	Details
					weeks post-flowering, indicating that both synthesis and breakdown of GA4 is occurring at these stages. The results of this study indicated that gibberellic acids were present in Shiraz grape berries before veraison but not after, which is consistent with a role in promoting cell division and enlargement in grape berries.
CA 7.6/07	Bottini *et al*., (2004)	Soil bacteria	GA1 to GA126 inclusive	A review of published studies on bacteria for which biosynthesis/production of gibberellins have been demonstrated.	The review reports that the total amount of gibberellins produced in pure bacteria cultures of *ca.* 10^8 CFU mL^{-1}, as determined by biological assays, ranges from 20 pg mL^{-1} to 400 pg mL^{-1}.
CA 7.6/08	Boyaci *et al*., (2011)	Aubergine	GA3	Endogenous GA3 levels were determined in nine aubergine genotypes (2 parthenocarpic and 7 non–parthenocarpic genotypes) at five crop developmental stages (small bud, middle bud, huge bud, flower and small fruit). Crop samples were collected at each developmental stage and stored at -20°C. Samples were solvent extracted and partitioned, separated using silica gel TLC plates (recovered by plate scraping) and analysed using HPLC (calibration with authentic GA3 reference standard).	Endogenous GA3 levels varied in time for both parthenocarpic and non-parthenocarpic genotypes and the floral development stages. The highest GA3 level in the parthenocarpic genotypes was 1.71 µg g^{-1} for Parthenone F_1 at the huge bud stage in January and the lowest level was 0.01 µg g^{-1} in March. In the non-parthenocarpic genotypes the highest GA3 levels were for Faselis F_1 in February (2.97 µg g^{-1}) and Karadaylak F_1 in January (2.79 µg g^{-1}). GA3 levels on non-parthenocarpic genotypes were higher than those of parthenocarpic genotypes.
CA 7.6/09	Chudasama and Thaker (2007)	Pigeon pea (seed and pod)	GA3	Seeds and pods collected from two varieties of pigeon pea were crushed with liquid nitrogen and solvent extracted. Pooled supernatant was evaporated and the final volume of the samples (10 mL) was prepared with phosphate buffer saline (pH 7.2) and used for the estimation of GA content. Endogenous levels of GA in the samples, expressed as GA3 equivalents were quantified by indirect ELISA using antibodies against GA3 raised in rabbits.	Measured levels of endogenous GA (expressed as GA3 equivalents) in seeds (n=3) were as follows: i) Variety 1 – Negligible levels were recorded during early stages of seed development (up to 18 DAA). From 24 DAA levels increased gradually to a maximum of 40.3 µg $seed^{-1}$ at 45 DAA followed by a decrease at later periods of seed development (11.1 µg $seed^{-1}$ at 54 DAA). ii) Variety 2 – Low levels were recorded during the early stages of seed development (2.27 µg $seed^{-1}$ up to 18 DAA. From 24 DAA levels increased gradually to a maximum of 15.8 µg $seed^{-1}$ at 45 DAA, followed by a gradual decline at later stages. Measured levels of endogenous GA (expressed as GA3 equivalents) in pods (n=3) were as follows: i) Variety 1 – Levels gradually increased with age reaching a maximum of 153.1 µg at 30 DAA and then gradually declined to 35.3 µg at 54 DAA. ii) Variety 2 – Levels gradually increased with age reaching a maximum of 43.6 µg at 39 DAA and then gradually declined thereafter.

Dossier location	Author and Year	Crop or matrix	Analyte(s)	Methodology	Details
CA 7.6/10	Csukasi *et al*., (2011)	Strawberry	GA1, GA3, GA4	Frozen tissue samples were ground to a fine powder, solvent extracted and analysed by UPLC (calibration with internal non-labelled standards).	Levels of GA1, GA3 and GA4 were measured in strawberry fruit tissue at three stages of development (green stage, white stage and red stage). Levels of GA1 and GA3 ranged from 3 to 12 ng g^{-1} fw and levels of GA4 ranged from 14 to 151 ng g^{-1} fw, reaching a clear maximum at the white stage of fruit development (151 ng g^{-1} fw).
CA 7.6/11	Deng *et al*., (2017)	Rice and *Arabidopsis thaliana* (single leaf)	GA1, GA3, GA4, GA7, GA8, GA9, GA19, GA20	A spatial-resolved analysis method for profiling of GAs in a single leaf was developed on the basis of microscale sample preparation and pre-column derivatization coupled with UPLC-MS-MS. The microscale sample preparation was based on a modified matrix solid-phase dispersion (MSPD) method in which the plant sample and C18 sorbent were ground together in one microcentrifuge tube. A new derivatization agent (BPTAB) was also used to enhance the signal intensities of GAs on MS by 3-4 orders of magnitude.	Measured concentrations of endogenous GAs in 0.50 mg fw *Arabidopsis thaliana* and rice leaves were as follows: GA1 = 0.72 ± 0.07 ng g^{-1} fw GA3 = 2.06 ± 0.12 ng g^{-1} fw GA4 = 0.28 ± 0.02 to 0.88 ± 0.04 ng g^{-1} fw GA7 = 12.93 ± 1.20 ng g^{-1} fw GA8 = 0.13 ± 0.01 to 2.05 ± 0.24 ng g^{-1} fw GA9 = not detected GA19 = 2.78 ± 0.27 to 26.86 ± 3.01 ng g^{-1} fw GA20 = 2.97 ± 0.23 ng g^{-1} fw
CA 7.6/12	Dobbelaere *et al*., (2003)	Diazotrophic bacteria	Gibberellins	-	This review reports that gibberellins are produced by diazotrophic bacteria. GA production has been demonstrated in *Azotobacter* spp., *P. polymyxa*, *Rhizobium leguminosarum*, *A. brasilense*, *A. lipoferum*, *Acetobacter diazotrophicus*, *Herbaspirillum seropedicae*, *Bacillus pumilus* and *Bacillus licheniformis*. Quantified levels are not included but this published review is provided as supporting information.
CA 7.6/13	Ergün *et al*., (2002)	Mosses and lichens	GA3	Levels of GA3 were determined in 10 species of mosses and 9 species of lichens. Either 1 g fresh weight of each moss sample or 1 g dry weight of each lichen sample was taken and combined with 60 mL of methanol:chloroform:2N ammonium hydroxide (12:5:3 v/v/v). Combined extracts (60 mL) were stored at -20°C prior to further analysis. The extracts were solvent extracted, separated using silica gel TLC plates and quantified using UV-VIS spectroscopy.	The highest total GA3 levels in the moss samples were observed in *H. sericeum* (58977.07 µg mL^{-1}, equivalent to *ca.* 118 mg g^{-1} fw, n=3) and the lowest levels were observed in *H. lustescens* (10581.11 µg mL^{-1}, equivalent to *ca.* 21 mg g^{-1} fw, n=3). The highest total GA3 levels in the lichen samples were observed in *P. furfuracea* (102678.16 µg mL^{-1}, equivalent to *ca.* 205 mg g^{-1} dw, n=3) and the lowest levels were observed in *X. polycarpa* (41941.40 µg mL^{-1}, equivalent to *ca.* 84 mg g^{-1} dw, n=3).

Dossier location	Author and Year	Crop or matrix	Analyte(s)	Methodology	Details
CA 7.6/14	Good (1974)	Leaf washings	Gibberellins	Leaf washings were collected during and immediately after rainfall (natural) from Sitka Spruce and Silver Birch trees. Washings were also collected following intermittent spray application of a fine water mist (artificial) to the needles of Sitka Spruce saplings under glasshouse conditions. Sterile washings were solvent extracted, partitioned against ethyl acetate, separated and analysed by bioassay (lettuce hypocotyl test).	The author reports that stimulatory gibberellin-like substances were detected in the washings from foliage of Sitka Spruce and Silver Birch. However, these were not quantified or characterised.
CA 7.6/15	Hamayun *et al*., (2009)	Endophytic fungi	GA1, GA3, GA4, GA7, GA5, GA19, GA9,GA24, GA15	Fungal endophytes were isolated from the roots of drought stressed soybean and the culture filtrates were bioassayed to determine the presence of plant growth promoting metabolites. The fungal isolate D-2-1 (identified as *Chrysosporium pseudomerdarium*), which provided the best result for plant height and biomass was then analysed for the presence of GAs. The culture filtrate was solvent extracted, separated by reverse-phase HPLC and analysed by GC-MS-SIM. Retention times were determined using hydrocarbon standards to calculate KRIs and GA quantification was based on the peak area ratios of non-deuterated to deuterated GAs.	Analysis of the culture filtrate of *C. pseudomerdarium* showed the presence of bioactive GA1 (0.24 ng mL^{-1}), GA3 (8.99 ng mL^{-1}), GA4 (2.58 ng mL^{-1}) and GA7 (1.39 ng mL^{-1}) in conjunction with physiologically inactive GA5 (*ca.* 0.24 ng mL^{-1}), GA9 (*ca.* 1.0 ng mL^{-1}), GA15 (*ca.* 0.24 ng mL^{-1}), GA19 (*ca.* 1.3 ng mL^{-1}) and GA24 (*ca.* 1.0 ng mL^{-1}). The fungal isolate contained higher levels of GA3 than wild type *G.fujikuroi* (3.12 ng mL^{-1}), but levels of the other bioactive GAs were not significantly different.
CA 7.6/16	Han *et al*., 2012	Grapes	GA3	A high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) method was developed and validated for the simultaneous determination of five acid/alkaline phytohormones (including GA3) in grapes.	Measured concentrations of GA3 in grape samples collected from local markets in China ranged from 1.9 to 24.1 µg kg^{-1}.
CA 7.6/17	Hasan (2002)	Rhizosphere and rhizoplane mycoflora of crop plants	Gibberellins	Fourteen species belonging to seven genera were isolated as the fungal flora from fababean, melochia, sesame and soyabean rhizosphere and rhizoplane. Twenty isolates of filamentous fungi isolated from the	The production of GA by different species of fungi as reported in this study is shown below: Fungal species | Source of isolation | GA (mg/ 50 mL)

<table>
<tr><th>Dossier location</th><th>Author and Year</th><th>Crop or matrix</th><th>Analyte(s)</th><th>Methodology</th><th>Details</th></tr>
<tr><td></td><td></td><td></td><td></td><td>rhizosphere and rhizoplane of the plants were tested to determine their GA production. Culture filtrates were solvent extracted, separated on TLC plates using isopropanol:ammonia:water (10:1:1, v/v/v) and quantified using spectrophotometric assay.</td><td>
<table>
<tr><td>A. carneus</td><td>Melochia</td><td>10</td></tr>
<tr><td>A. flavipes</td><td>Sesame</td><td>10</td></tr>
<tr><td>A. flavus</td><td>Fababean</td><td>5</td></tr>
<tr><td>A. flavus</td><td>Melochia</td><td>6</td></tr>
<tr><td>A. flavus</td><td>Sesame</td><td>3</td></tr>
<tr><td>A. flavus</td><td>Soyabean</td><td>3</td></tr>
<tr><td>A. niger</td><td>Fababean</td><td>11</td></tr>
<tr><td>A. niger</td><td>Melochia</td><td>12</td></tr>
<tr><td>A. niger</td><td>Sesame</td><td>6</td></tr>
<tr><td>A. niger</td><td>Soyabean</td><td>9</td></tr>
<tr><td>A. tamarii</td><td>Melochia</td><td>5</td></tr>
<tr><td>Emericella nidulans</td><td>Soyabean</td><td>1</td></tr>
<tr><td>F. oxysporum</td><td>Melochia</td><td>10</td></tr>
<tr><td>F. oxysporum</td><td>Sesame</td><td>12</td></tr>
<tr><td>F. oxysporum</td><td>Soyabean</td><td>13</td></tr>
<tr><td>P. corylophilum</td><td>Melochia</td><td>10</td></tr>
<tr><td>P. corylophilum</td><td>Soyabean</td><td>9</td></tr>
<tr><td>P. corylophilum</td><td>Melochia</td><td>1</td></tr>
<tr><td>P. corylophilum</td><td>Soyabean</td><td>7</td></tr>
<tr><td>R. stolonifer</td><td>sesame</td><td>10</td></tr>
</table>

Whilst all cultures were observed to produce GA in variable amounts, F. oxysporum produced the highest levels. Isolates of A. carneus, A. flavipes, A. niger, P. corylophilum and R. stolonifer also have the potential for production of GA in reasonable amounts.</td></tr>
<tr><td>CA 7.6/18</td><td>Hayashi et al., (1968)</td><td>Apple</td><td>GA3</td><td>GA3 was isolated from frost-induced parthenocarpic apple fruit and its identity was determined by biological response, thin layer, paper and gas liquid chromatography.</td><td>A GA3 concentration in apple equivalent to ca. 143 µg kg^{-1} of fresh tissue is reported.</td></tr>
<tr><td>CA 7.6/19</td><td>Hofman (1990)</td><td>Orange</td><td>Gibberellins</td><td>Fruitlet and leaf samples collected from ‘Valencia’ orange trees were frozen in liquid nitrogen, freeze dried and stored at -20°C. The samples were milled just prior to analysis. Samples were solvent extracted, partitioned and purified. The final eluant was evaporated to dryness and the residue dissolved in phosphate buffered saline for</td><td>Measured GA concentrations in fruitlets (mainly expected to be GA3, but assay also showed a response to GA1 and GA20) ranged from ca. 20 to 40 ng g^{-1} dw. Concentrations in immature and mature leaves ranged from ca. 20 to 40 ng g^{-1} dw and from ca. 35 to 50 ng g^{-1} dw, respectively.</td></tr>
</table>

Dossier location	Author and Year	Crop or matrix	Analyte(s)	Methodology	Details
				radioimmunoassay. Gibberellin immunogen was prepared by coupling GA3 to bovine serum albumin. The suitability of the extraction procedure was tested by extract dilution, internal standards and immunohistogram techniques and no specific inhibition was suggested.	
CA 7.6/20	Hu *et al*., (2017)	Oilseed Camellia (*C. oleifera*)	Gibberellins	Tissue samples were collected from the leaves of trees of different ages at two developmental stages in early May and late August. The samples were frozen in liquid nitrogen and stored at -80°C prior to analysis. Freeze-dried powdered tissues were stirred and solvent extracted. The pooled supernatant was evaporated and the residue was re-dissolved in a buffer solution. GA content was determined by ELISA using antigens and antibodies purchased from the Chinese Agricultural University.	Measured GA concentrations (n=3) in the leaves of *C. oleifera* trees at different ages in May and August ranged from *ca.* 5 to 18 ng g^{-1} fw. The concentration of GA was lower in August than in May for trees of all ages (2-70 years). A marked decrease in GA levels was observed in trees between 2- and 4-years of age, which coincides with the transition from seedling to sapling stage. Levels increased in trees between 6- and 10-years of age and then decreased again in older trees.
CA 7.6/21	Jennings (1968)	Green and brown algae	GA3	Gibberellin-like activity was determined in samples of green and brown algae (*Enteromorpha prolifera* and *Ecklonia radiata*). Plant material was homogenized and filtered and the resulting aqueous residue was solvent extracted. Separation was achieved by descending chromatography and chromatograms were cut into a sequence of 10 R_f units and analysed by bioassay with dwarf maize seedlings.	It was clear from the extraction experiments that compounds with gibberellin-like activity were present in the algae investigated. Although the methodology used did not permit the definitive identification of the chemicals responsible for this activity, the author assumes the active material to be GA3 and reports a concentration in algal material of *ca.* 100 μg kg^{-1} fw, which is comparable to levels of GA3 extracted from tissues of higher plants.

Dossier location	Author and Year	Crop or matrix	Analyte(s)	Methodology	Details
CA 7.6/22	Jennings and McComb (1967)	Red algae	Gibberellins	Gibberellin-like activity was determined in samples of red algae (*Hypnea musciformis*). Plant material was homogenized and filtered and the resulting aqueous residue was solvent extracted. Separation was achieved by descending chromatography and chromatograms were cut into a sequence of 10 R_f units and analysed by bioassay using dwarf maize.	It was clear from the extraction experiment that compounds with gibberellin-like activity were present in the algae investigated. However, it was not possible to estimate the total level of gibberellin activity in the extract, as dose-response curves were not available for comparison with known GA3. The authors speculate that the levels of activity detected are likely to be of the same order as those found in extracts of shoots of higher plants.
CA 7.6/23	Joo *et al*., (2004)	Rhizobacteria	GA1, GA3, GA4, GA5, GA7, GA8, GA9, GA12, GA15, GA19, GA20, GA24, GA34, GA36, GA44, GA53	*Bacillus cereus* (MJ-1), *Bacillus. macroides* (CJ-29) and *Bacillus. pumilus* (CJ-69) were isolated from rhizosphere soils and analysed to determine GA levels. After incubation the culture broth of each bacterial isolate was acidified and deuterated internal standards were added. Samples were solvent extracted and GAs were separated by HPLC and analysed by GC-MS. GAs were identified by comparison of their mass spectra and KRIs with authentic standards and quantified based on the peak area ratios of non-deuterated to deuterated GAs	The range of measured GA concentrations in the culture broths of the three bacterial isolates were as follows: GA1 = 2.2 ± 0.1 to 3.5 ± 0.1 ng/100 mL^{-1} GA3 = 2.7 ± 0.6 to 14.5 ± 1.8 ng/100 mL^{-1} GA4 = 4.2 ± 0.3 to 7.8 ± 1.6 ng/100 mL^{-1} GA5 = 6.1 ± 1.0 ng/100 mL^{-1} GA7 = 1.7 ± 0.1 to 8.7 ± 0.7 ng/100 mL^{-1} GA8 = 3.3 ± 0.1 ng/100 mL^{-1} GA9 = 0.5 ± 0.0 to 6.0 ± 0.1 ng/100 mL^{-1} GA12 = 0.1 ± 0.0 to 0.4 ± 0.1 ng/100 mL^{-1} GA15 = Not detected GA19 = 0.5 ± 0.0 to 1.6 ± 0.1 ng/100 mL^{-1} GA20 = 0.2 ± 0.0 to 0.3 ± 0.1 ng/100 mL^{-1} GA24 = 1.3 ± 0.1 to 1.6 ± 0.1 ng/100 mL^{-1} GA34 = 1.1 ± 0.1 to 6.8 ± 0.3 ng/100 mL^{-1} GA36 = 0.7 ± 0.0 to 4.6 ± 0.1 ng/100 mL^{-1} GA44 = 0.3 ± 0.1 to 15.5 ± 0.9 ng/100 mL^{-1} GA53 = 0.1 ± 0.0 to 0.3 ± 0.0 ng/100 mL^{-1} Comparatively high concentrations of GA1, GA3, GA4 and GA7 were observed in all culture broth tested.
CA 7.6/24	Joo *et al*., (2005)	Red pepper (seedling shoots)	GA1, GA3, GA4, GA5, GA7, GA9, GA12, GA15, GA19, GA20, GA24, GA44, GA53	Red pepper seedlings cultivated in a greenhouse were treated with rhizobacteria (1 mL of bacteria diluted with tap water to an estimated cell density of 10^8 c.f.u./mL). Endogenous GAs in the shoots were measured at 67 days after inoculation. Lyophilized tissue samples were weighed,	Mean measured concentrations of endogenous GAs (n=3) following single and co-inoculation of *Bacillus cereus (*MJ-1), *B. macroides* (CJ-29) and *B. pumilus* (CJ-69) in the shoots of red pepper seedlings at 67 days after initial treatment were as follows: | | Calculated amounts (ng g^{-1} of fresh weight) |

<table>
<tr><th>Dossier location</th><th>Author and Year</th><th>Crop or matrix</th><th>Analyte(s)</th><th>Methodology</th><th>Details</th></tr>
<tr><td></td><td></td><td></td><td></td><td>ground to a fine powder and solvent extracted. GAs were separated by HPLC and analysed by GC-MS (comparison of mass spectra and KRIs with authentic standards).</td><td>
<table>
<tr><td>GA1</td><td>2.2 ± 0.01 – 3.1 ± 0.19</td></tr>
<tr><td>GA3</td><td>1.5 ± 0.14 – 3.0 ± 0.21</td></tr>
<tr><td>GA4</td><td>10.5 ± 0.28 – 11.9 ± 0.07</td></tr>
<tr><td>GA5</td><td>2.1 ± 0.14 – 5.2 ± 0.11</td></tr>
<tr><td>GA7</td><td>2.2 ± 0.10 – 4.5 ± 0.16</td></tr>
<tr><td>GA9</td><td>8.0 ± 0.09 – 10.0 ± 0.12</td></tr>
<tr><td>GA12</td><td>2.1 ± 0.17 – 4.3 ± 0.11</td></tr>
<tr><td>GA15</td><td>3.5 ± 0.16 – 12.1 ± 0.18</td></tr>
<tr><td>GA19</td><td>2.1 ± 0.14 – 3.4 ± 0.01</td></tr>
<tr><td>GA20</td><td>1.3 ± 0.07 – 2.9 ± 0.16</td></tr>
<tr><td>GA24</td><td>5.3 ± 0.01 – 8.9 ± 0.18</td></tr>
<tr><td>GA44</td><td>0.5 ± 0.13 – 1.7 ± 0.129</td></tr>
<tr><td>GA53</td><td>0.2 ± 0.12 – 1.7 ± 0.15</td></tr>
</table>
Total endogenous GAs content was 1.31 to 1.63 times higher in the inoculated samples than in the non-inoculated controls, indicating that commonly occurring soil bacteria have a positive impact on the production of gibberellins in red pepper plants.</td></tr>
<tr><td>CA 7.6/25</td><td>Juntilla (2012)</td><td>Woody plants</td><td>Gibberellins</td><td>-</td><td>This review reports that a number of gibberellins have been conclusively identified in the vegetative tissues of deciduous and coniferous woody plants (GA1, GA3, GA4, GA7, GA8, GA9, GA15, GA17, GA19, GA20, GA29, GA44, GA53.). However, levels were not quantified in this review.</td></tr>
<tr><td>CA 7.6/26</td><td>Kampert et al., (1975)</td><td>Bacteria and fungi isolated from the roots of pine seedlings</td><td>Gibberellins</td><td>Bacteria and fungi were isolated from the roots of pine seedlings and forest soil and tested to determine their synthesis of gibberellin-like substances. Bacteria and fungi were removed from the culture medium by centrifugation and filtration, respectively. The samples were solvent extracted, separated using silica gel TLC plates (solvent system benzene:acetic acid (10:3 v/v)) and analysed by bioassay using the lettuce hypocotyl test. The total amount of gibberellin-like substances produced by the micro-organisms was calculated from a standard dose response curve and presented as gibberellic acid (GA3) equivalents.</td><td>Measured concentrations of gibberellin-like substances (expressed as GA3 equivalents) ranged from 0.00007 to 0.00065 µg mL^{-1} in the soil bacterial isolates and from 0.00014 to 0.00118 µg mL^{-1} in the fungal isolates. A considerable number of the micro-organisms tested produced detectable amounts of gibberellin-like substances (55% and 86% of the bacterial and fungal isolates, respectively). The study therefore concludes that a large number of the micro-organisms tested produced GA3 and that the production of gibberellin-like substances is quite common among micro-organisms inhabiting the root zone of pine seedlings.</td></tr>
</table>

Dossier location	Author and Year	Crop or matrix	Analyte(s)	Methodology	Details
CA 7.6/27	Karadeniz *et al*., (2006)	Bacteria	GA3	The aim of the study was to determine levels of GA3 in the culture media of the bacteria *P. mirabilis*, *P. vulgaris*, *B. megaterium*, *B. cereus*, *K. pneumoniae* and *E. coli*. Following incubation, culture filtrates were solvent extracted, and separated by TLC. Bands detected were scraped from the TLC plate, dissolved in methanol and analysed by HPLC (comparison with authentic standards).	Total GA3 content in the bacterial culture medium samples ranged from 0.84 ± 0.03 to 70.52 ± 1.87 µg 100 mL^{-1}. The results indicate that the bacteria used in this study synthesized gibberellin.
CA 7.6/28	Katznelson and Cole (1965)	Bacteria and actinomycetes	Gibberellins	The production of gibberellin-like substances by a range of bacteria and actinomycetes was investigated. Following incubation, bacterial cells were removed by centrifugation and the supernatant fluid was solvent extracted. Separation of active constituents was achieved by descending chromatography in a solvent system composed of isopropanol:ammonium hydroxide:water (8:1:1 v/v/v) with reference standards of indoleacetic acid (IAA), gibberellic acid (A3) and gibberellin A9. The portion of the chromatogram containing the test material was then cut into segments corresponding to each R_f unit and analysed by bioassay (*Avena* coleoptile method).	Most of the bacterial cultures tested produced a gibberellic acid-like substance (A3) in amounts varying from 1 to 14 µg L^{-1}. The production of a gibberellin-A9 like substance was more limited, with 6 out of 15 cultures yielding small amounts (<1 to 6 µg L^{-1}). Six out of the eleven actinomycetes cultures tested showed evidence of A3 synthesis (<1 to 6 µg L^{-1}) and two produced A9 (1.5 to 5 µg L^{-1}).
CA 7.6/29	Kobayashi *et al*., (1995)	Barley (seeds)	GA1, GA3	Harvested seeds were blended, solvent extracted, separated by HPLC and analysed by full scan GC-MS for identification and GC-SIM for quantification. (comparison with deuterated internal standards).	Measured concentrations of GA1 in barley seeds ranged from *ca*. 0.5 to 3 ng g^{-1} fw, reaching a maximum at day 2. Measured concentrations of GA3 in barley seeds ranged from *ca*. 0.25 to 0.5 ng g^{-1} fw, reaching a maximum at day 4.

Dossier location	Author and Year	Crop or matrix	Analyte(s)	Methodology	Details
CA 7.6/30	Letter to CTB - Annex I (Date not reported)	-	Gibberellins	In a letter to the CTB to support an application for registration of the product BERELEX GA4A7, 96-358TV, the natural occurrence of gibberellins is discussed (Annex I to the letter). This is provided as supporting information.	The review highlights that 35 GAs have been isolated between the vegetative and fruiting tissues of apples. Therefore, in seeds and fruits GA metabolism is high and would likely reduce any imbalance caused by exogenous GAs. The review reports endogenous levels of GA4 and GA7 in apple seeds of 4.19 and 4.4 ng g^{-1} fw, respectively. Endogenous levels of GA4 in the seeds of corn and *Arabidopsis thaliana* of 0.4 and 6.1 ng g^{-1} fw, respectively are also reported.
CA 7.6/31	Lin *et al*., (1991)	Apple seeds	Gibberellins	The endogenous GAs in immature apple seeds were identified by HPLC and GC-SIM.	Various GAs were identified (GA8, GA3, GA1, GA35, GA20, GA68, GA63, GA44, GA19, GA54, GA34, GA62, GA61, GA51, GA17, GA7, GA4, GA53, GA24, GA9, GA15). However levels were not quantified in the report.
CA 7.6/32	MacMillan (2002)	Vascular plants	GA1 to GA126 inclusive	-	No quantified levels, included as supporting information only
CA 7.6/33	Motosugi *et al*., (1996)	Apple xylem exudate	Gibberellins	Xylem exudate was extracted from the stems of apple trees. Extracts were fractionated, re-purified and chromatographed by reverse-phase HPLC. The GA-like activity of the HPLC fraction was tested using modified micro-drop bioassay on dwarf rice and the biologically active HPLC fractions were finally analysed by GC-MS (comparison with KRIs).	From the biologically active fractions, 7 gibberellins were identified in the xylem exudate of apple trees (GA15, GA17, GA18, GA19, GA23, GA44 and GA53). However, levels are not quantified in the report. The authors conclude that the presence of GA53, GA44 and GA19 suggested the early-13-hydroxylation pathway to be operating in the rootstock of apple trees so that these gibberellins may be converted to an active form in the growing tissues. The results suggest that apple rootstock translocates the less-active GAs in xylem, which may be converted into biologically active GAs in the shoot tip or in other growing organs.

Dossier location	Author and Year	Crop or matrix	Analyte(s)	Methodology	Details
CA 7.6/34	Nadeem *et al*., (2010)	Endophytic fungi	GA3, GA4, GA7, GA9, GA12, GA24	Fungal endophytes were isolated from *M. vaginalis* and the culture filtrates were bioassayed to determine plant growth promotion capacity. Two isolates (identified as *Penicillium* and *Aspergillus*), which were observed to significantly promote plant height and shoot length were then analysed for the presence of GAs. The culture filtrates were extracted, separated and analysed by GC-MS-SIM. Retention times were determined using hydrocarbon standards to calculate KRI indices and GA quantification was based on the peak area ratios of non-deuterated (extracted) to deuterated GAs.	Analysis of the culture filtrate of *Penicillium* showed the presence of bioactive GA3 (2.8 ng mL^{-1}), GA4 (2.6 ng mL^{-1}) and GA7 (6.68 ng mL^{-1}) in conjunction with physiologically inactive GA9 (1.61 ng mL^{-1}) and GA24 (0.18 ng mL^{-1}). The culture filtrates of *Aspergillus* showed the presence of bioactive GA3 (1.64 ng mL^{-1}), GA4 (1.37 ng mL^{-1}) and GA7 (6.29 ng mL^{-1}), along with physiologically inactive GA9 (3.44 ng mL^{-1}), GA12 (0.3 ng mL^{-1}) and GA24 (0.59 ng mL^{-1}). Both fungal isolates tested contained higher levels of GA7 and GA9 than wild type *G.fujikuroi* during the study.
CA 7.6/35	Nehela *et al*., (2016)	Orange (leaves and roots)	GA3, GA4, GA7	Citrus tissues (leaves, roots and root tips) were ground using liquid nitrogen, solvent extracted and derivatized with *N*-Methyl-*N*-(trimethylsilyl) trifluoroacetamide (MSTFA). Samples were analysed by GC-MS in the full scan mode and GC-SIM (comparison with authentic standards).	Mean measured concentrations in citrus tissues (n=10) were as follows: GA3: *ca.* 25 ng g^{-1} fw (leaves), *ca.* 48 ng g^{-1} fw (roots), *ca.* 66 ng g^{-1} fw (root tips) GA4: *ca.* 12 ng g^{-1} fw (leaves), *ca.* 30 ng g^{-1} fw (roots), *ca.* 36 ng g^{-1} fw (root tips) GA7: *ca.* 100 ng g^{-1} fw (leaves), *ca.* 240 ng g^{-1} fw (roots), *ca.* 420 ng g^{-1} fw (root tips) Measured concentrations of GAs were higher in root tips than leaves and roots.
CA 7.6/36	Perez *et al*., (2000)	Grapes (berries and seeds)	GA1, GA3, GA17, GA19, GA20	Homogenised samples of berries and seeds were solvent extracted, separated by HPLC and analysed by GC-MS (comparison of mass spectra and KRIs with authentic standards). Levels of GAs in the extracts were determined by α-amylase bioassay and expressed as equivalents of GA3/g.	Levels of active gibberellins in berries during fruit development expressed as GA3 equivalents reached a maximum of 5.94 ng g^{-1} fw at 21 days and 2.87 ng g^{-1} fw at 16 days in the seeded and seedless grape varieties, respectively. Levels of active gibberellins in seeds during seed growth expressed as GA3 equivalents reached a maximum of 456 ng g^{-1} fw at 56 days, representing an increase of *ca*. 77-fold with respect to the maximum level determined in berries. However, levels of active GAs in seeds decreased dramatically at the end of the growing period suggesting that GAs are related to seed growth.
CA 7.6/37	Radley (1989)	Kelp (*Macrocystis integrifolia*)	Gibberellins	Samples of kelp harvested in the spring (May) were analysed to determine the	The measured concentration of endogenous GAs in seaweed (expressed as GA3 equivalents) is reported as 10 µg /100 g fw.

Dossier location	Author and Year	Crop or matrix	Analyte(s)	Methodology	Details
				presence of endogenous gibberellins. Frozen samples were solvent extracted and partitioned against ethyl acetate and n-butanol. Fractions expected to contain gibberellin-like substances were then separated by descending chromatography and analysed by bioassay (comparison with the mobility of authentic GA3 standard).	
CA 7.6/38	Ramírez *et al*., (2004)	Apple (seeds and xylem sap)	Gibberellins	The presence of endogenous GAs in immature seeds and xylem sap from apples (Red Delicious) was investigated. Samples were frozen, freeze dried and ground. and gibberellin content was determined by the lettuce hypocotyls bioassay. Additional seed samples were also collected for the identification of gibberellins. These samples were frozen, freeze dried and ground prior to solvent extraction and purification, followed by GC-MS analysis (comparison of KRI and MS spectra with authentic standards).	The amount of endogenous gibberellins in seeds was reported to be higher than in xylem sap samples during the experiment. However, quantified levels are not reported in the text and it is not possible to ascertain the values from Figure 1 in the publication as the text is illegible. GA1, GA4, iso-GA7, GA15, GA20, GA44 and GA53 were identified in the immature seed samples.
CA 7.6/39	Scienza *et al*., (1978)	Grapes (berries)	Gibberellins	Berries were collected at 8-10 day intervals from fruit set to ripening. Berries containing 1-3 seeds were analysed to determine levels of gibberellin-like substance activity by barley endosperm bioassay.	Levels of gibberellin-like substances in extracted berries reached a maximum of 0.14 to 1.15 µg/100 g fw according to seed number at 45 days after anthesis. Three-seeded berries contained higher levels of gibberellin-like substances than one or two-seeded berries.

Dossier location	Author and Year	Crop or matrix	Analyte(s)	Methodology	Details
CA 7.6/40	Sciuto *et al*., (1980)	Marine algae	Gibberellins	A screening test was conducted to determine gibberellin-like activity in 21 species of marine algae belonging to the phyla Phaeophyta and Rhodophyta. Samples were freeze-dried and finely ground prior to solvent extraction and partitioning with ethyl acetate and butanol successively at different pH values. Extracts were bioassayed using the Amaranthus assay. Concentrations of GA3 in the range 0-1 µg/mL were used to plot the variation of amaranthin inhibition versus the increase of GA3 concentration and levels of gibberellin-like activity in the test samples were expressed as GA3 equivalents.	Reported levels of gibberellin-like activity in the test samples (expressed as GA3 equivalents) ranged from 8 to 42 µg kg^{-1} frond. Gibberellin-like activity was detected more frequently in red algae (Rhodophyta) than brown algae (Phaeophyta).
CA 7.6/41	Serrani *et al*., (2007)	Tomato fruit	GA1, GA8, GA19, GA20, GA29, GA44, GA53	Tomato fruits were collected 10 days after pollination and analysed to determine endogenous levels of GA1, GA8, GA19, GA20, GA29, GA44 and GA53. Aliquots of frozen fruit were solvent extracted, partitioned against ethyl acetate and purified. The GAs were then separated by reverse-phase HPLC and quantified by GC-SIM using internal standards.	Mean measured concentrations of endogenous GAs in tomato fruits (n=3) were as follows: GA1 = 2.7 ± 0.8 ng g^{-1} fw GA8 = 31.4 ± 0.3 ng g^{-1} fw GA19 = 8.7 ± 0.4 ng g^{-1} fw GA20 = 23.5 ± 0.6 ng g^{-1} fw GA29 = 18.5 ± 2.6 ng g^{-1} fw GA44 = 2.7 ± 0.1 ng g^{-1} fw GA53 = <0.1 ng g^{-1} fw
CA 7.6/42	Skene (1967)	Vines (root exudate and plant tissue)	GA3	Sap extracted by partition. Plant tissue samples solvent extracted. Separated by silica gel TLC (against GA3 reference standard) and quantified by barley endosperm or dwarf corn assay.	Levels produced per plant per day 0.007-0.02 µg/day. Amount per leaf 0.005 µg.
CA 7.6/43	Sponsel and Hedden (2004)	Various plant species	Gibberellins	-	This review reports that gibberellins are now known to be regulators of many phases of higher plant development, including seed germination, stem growth, induction of flowering, pollen development and fruit growth. The concentration of bioactive GAs in plants is in the range of 10^{-11} to 10^{-9} g g^{-1} fw depending on the tissue and species.

Dossier location	Author and Year	Crop or matrix	Analyte(s)	Methodology	Details
CA 7.6/44	Stephan *et a*l., (1999)	Apple	GA1, GA3, GA4, GA7, GA20, GA34	Immature apple fruits including pedicels were collected from a variety of apple cultivars. The pedicel ends of the fruit were incubated in Agar gel for 20 hours. The fruits were then removed and the plates were stored at -20°C prior to analysis. The plates were lyophilized, powdered and solvent extracted. Extracts were separated by HPLC and analysed by LC-ESI-MS (calibration with internal reference standards).	Reported levels of endogenous GAs in the fruit exudates of different cultivars of *Malus domestica* harvested at 4-7 weeks after full bloom were as follows: GA1 = 0.1 to 0.4 ng fruit^{-1} GA3 = 0.7 to 3.5 ng fruit^{-1} GA4 = 0.1 to 33.3 ng fruit^{-1} GA7 = 0.8 ng fruit^{-1} GA20 = 0.2 to 0.7 ng fruit^{-1} GA34 = 0.1 to 0.3 ng fruit^{-1}

Dossier location	Author and Year	Crop or matrix	Analyte(s)	Methodology	Details
CA 7.6/45	Stirk *et al*., (2013)	Microalgae	GA1, GA3, GA4, GA5, GA6, GA7, GA8, GA9, GA12, GA12^{ald}, GA13, GA15, GA19, GA20, GA24, GA29, GA34, GA44, GA51, GA53	Levels of endogenous GAs were quantified in 24 micro-algal strains after 4 days growth in culture. Samples of algae culture were extracted with acetonitrile containing 5% formic acid and internal standards. Extracts were centrifuged, purified using ion exchange cartridges and analysed by UPLC-MS-MS. The standard isotope dilution method was used to quantify GA levels.	Between 18 and 20 GAs were detected in the 24 micro-algal strains analysed after 4 days growth in culture. This is similar to higher order plants. The range of measured concentrations (n=3) were as follows: GA1 = 2.0 ± 0.2 to 21.2 ± 1.3 pg mg^{-1} dw GA3 = 0.2 ± 0.0 to 4.1 ± 0.1 pg mg^{-1} dw GA4 = 3.4 ± 0.0 to 20.2 ± 0.2 pg mg^{-1} dw GA5 = 4.3 ± 0.2 to 34.4 ± 1.9 pg mg^{-1} dw GA6 = 87.1 ± 2.5 to 383.3 ± 5.5 pg mg^{-1} dw GA7 = 0.6 ± 0.0 to 3.4 ± 0.1 pg mg^{-1} dw GA8 = 1.6 ± 0.4 to 25.5 ± 2.3 pg mg^{-1} dw GA9 = 4.2 ± 0.1 to 86.3 ± 2.0 pg mg^{-1} dw GA12 = <LOD to 443.5 ± 27.4 pg mg^{-1} dw GA12^{ald} = <LOD to 494.4 ± 16.5 pg mg^{-1} dw GA13 = 6.3 ± 0.2 to 348.7 ± 5.9 pg mg^{-1} dw GA15 = 14.7 ± 0.2 to 3452.9 ± 178.1 pg mg^{-1} dw GA19 = 0.3 ± 0.0 to 5.8 ± 0.5 pg mg^{-1} dw GA20 = 0.6 ± 0.0 to 15.6 ± 1.2 pg mg^{-1} dw GA24 = 2.5 ± 0.3 to 22.3 ± 2.7 pg mg^{-1} dw GA29 = 1.4 ± 0.0 to 17.9 ± 0.7 pg mg^{-1} dw GA34 = 0.5 ± 0.0 to 3.2 ± 0.1 pg mg^{-1} dw GA44 = 3.6 ± 0.1 to 81.9 ± 3.9 pg mg^{-1} dw GA51 = 23.1 ± 0.5 to 609.8 ± 5.6 pg mg^{-1} dw GA53 = 1.5 ± 0.1 to 138.5 ± 1.8 pg mg^{-1} dw Biologically active GAs (GA1, GA3, GA4, GA5, GA6 and GA7) contributed 5 to 33% of the total GA content, the metabolic end products (GA13 and GA51) contributed 7 to 40% and the intermediates contributed 36 to 91% of the total GA content. GA6 was detected at the highest concentration in all strains.

Dossier location	Author and Year	Crop or matrix	Analyte(s)	Methodology	Details
CA 7.6/46	Takahashi and Kobayashi (2012)	Rice	GA1, GA8, GA17, GA19, GA20, GA29, GA44, GA53, GA4, GA9, GA12, GA24, GA34, GA51	Plant tissues (leaves, shoots, roots and ears) were harvested from rice cultivars at various growth stages. Plant materials were solvent extracted and analysed by GC-MS with a full mass scan and GC-SIM using internal standards.	Reported levels of endogenous GAs in various plant tissues were as follows: GA1 = 0.05 to 10 ng g^{-1} fw GA8 = not detected GA17 = not detected GA19 = 0.43 to 42 ng g^{-1} fw GA20 = 0.02 to 1.1 ng g^{-1} fw GA29 = 0.1 to 0.4 ng g^{-1} fw GA44 = not detected GA53 = not detected GA4 = 0.3 to 3700 ng g^{-1} fw GA9 = 0.6 to 32 ng g^{-1} fw GA12 = not detected GA24 = 4.3 to 150 ng g^{-1} fw GA34 = 0.4 to 9 ng g^{-1} fw GA51 = 1.0 to 29 ng g^{-1} fw 13-hydroxy-GAs such as GA1, GA17, GA19, GA20, GA29, GA44 and GA53 were identified in most types of plant tissue. However, non-13-hydroxy GAs such as GA4, GA9, GA12, GA24, GA34 and GA51 were mainly identified in the reproductive tissues.
	Phinney *et al*., (2012)	Maize	GA53, GA44, GA19, GA29, GA20, GA5, GA3, GA8, GA1	Tissue samples from maize plants were solvent extracted and analysed by GC-SIM using internal standards.	Reported levels of endogenous GAs in maize stem tissues at various growth stages were as follows: GA53 = 75 to 1700 ng/100 g fw GA44 = 155 to 500 ng/100 g fw GA19 = 270 to 875 ng/100 g fw GA20 = 36 to 94 ng/100 g fw GA29 = 4 to 10 ng/100 g fw GA1 = 13 to 50 ng/100 g fw GA8 = 12 to 106 ng/100 g fw GA5 = 2 to 3 ng/100 g fw GA3 = 2 to 16 ng/100 g fw
	Murofushi *et al*., (2012)	Maize	GA1, GA3 GA4, GA9, GA20	Seeds of dwarf and tall maize genotypes were harvested at 1, 2, 3 and 4 weeks after pollination. The seeds were extracted, purified and analysed for the presence of GA1, GA4, GA9 and GA20 by	Reported concentrations of endogenous GAs in the seed of dwarf and tall maize harvested at 1, 2, 3 and 4 weeks after pollination ranged as follows: GA1 = 0.04-0.1 ng g^{-1} fw (wk 1) to 0.02-0.07 ng g^{-1} fw (wk 2) (not detected thereafter)

Dossier location	Author and Year	Crop or matrix	Analyte(s)	Methodology	Details
				immunoassay using anti-GA1-antiserum and anti-GA20-antiserum. In a separate experiment, purified extracts from the tassels, ears and silk of maize (hybrid Ko-No. 7) were analysed for the presence of GA1, GA3 and GA4 by immunoassay using anti-GA1-antiserum. GA3 in the silk extracts was identified by full-scan GC-MS.	GA4 = 0.02-0.2 ng g^{-1} fw (wk 1) to 0.04 ng g^{-1} fw (wk 4) GA9 = 0.4 ng g^{-1} fw (wk 1) to 3 ng g^{-1} fw (wk 4) GA20 = 2-4 ng g^{-1} fw (wk 1) to 10 ng g^{-1} fw (wk 4) Reported concentrations of GA1, GA3 and GA4 in maize ear and silk extracts ranged from 0.06 to 0.2 ng g^{-1} fw, 1 to 4 ng g^{-1} fw and 0.4 to 0.6 ng g^{-1} fw, respectively. In the tassel extracts, levels of GA1 and GA4 were not quantified and GA3 was not detected.
CA 7.6/47	Tukey (1970)	-	-	This is a review of published literature on the leaching of substances from plants by the action of aqueous solutions such as rain, dew mist and fog.	The review reports that GAs1-3 and GA5 have been shown to leach from non-flowering vegetative chrysanthemum plants and that GAs1-3 and GA6 have been shown to leach from flowering plants. No quantified levels are reported, included as supporting information only.

Dossier location	Author and Year	Crop or matrix	Analyte(s)	Methodology	Details
CA 7.6/48	White *et al*., (2000)	Maize kernels	GA1, GA3, GA8, GA19, GA20, GA29, GA44	Concentrations of GA1, GA3, GA8, GA19, GA20, GA29 and GA44 were determined in developing maize kernels harvested at intervals between 15 and 27 days after pollination (embryo stages 2-4). Kernels were flash-frozen, solvent extracted, separated by reverse-phase HPLC and analysed by GC-MS-SIM using internal standards.	Measured concentrations of endogenous GAs in maize kernels were as follows: GA1 = ca. 0.03 to 0.07 ng g^{-1} fw GA3 = *ca.* 0.3 to 0.75 ng g^{-1} fw GA8 = *ca.* 0.1 to 0.55 ng g^{-1} fw GA19 = *ca.* 5 to 10 ng g^{-1} fw GA20 = *ca.* 4 to 12 ng g^{-1} fw GA29 = *ca.* 0.6 to 1.5 ng g^{-1} fw GA44 = *ca.* 3 to 8 ng g^{-1} fw Concentrations of the two biologically active gibberellins GA1 and GA3 were highest in the earliest samples (day 15, embryo stage 2) and declined markedly as embryos matured. GA8 declined similarly. GA19, GA29 and GA44 were highest in the 21 day samples (embryo stage 3) and GA20 was highest at 27 days (embryo stage 4).
CA 7.6/49	Wolf and Loubser (1992)	Grapes	GA1,GA3, GA20	Solvent extracted and analysed by radio-immunoassay using antiserum which cross-reacts with GA1,3,20.	Measured concentrations of GA3 in grapes (seedless variety, n=2) were as follows: i) max *ca.* 0.1 mg kg^{-1} fw, immature fruit to *ca.* 0.025 mg kg^{-1} fw at harvest (decline factor 4) ii) max *ca.* 0.1 mg kg^{-1} fw, 10 mm berry stage to *ca.* 0.06 mg kg^{-1} fw at harvest (decline factor 1.7)
CA 7.6/50	Zhang *et al*., (2007)	Japanese Pear	GA1, GA3, GA4	Endogenous levels of GA1, GA3 and GA4 were determined in the fruit of two Japanese pear cultivars. Frozen fruit samples were homogenized, solvent extracted, separated by reverse-phase HPLC and analysed by GC-MS-SIM with deuterated internal standards.	Maximum observed concentrations of GA1, GA3 and GA4 in the fruit of both cultivars were *ca.* 10 to 70 ng g^{-1} fw, *ca.* 40 to 135 ng g^{-1} fw and *ca.* 8 to 30 ng g^{-1} fw, respectively.

Dossier location	Author and Year	Crop or matrix	Analyte(s)	Methodology	Details
CA 7.6/51	Zhang *et al*., (2010)	Japanese Pear	GA1, GA3, GA4	GA levels of fruitlets in natural conditions and of the male gametophyte (pollen grain and tube) were quantified *in vitro* using GC-MS-SIM with deuterated internal standards.	Levels of total gibberellins in immature fruits (fruitlets) ranged from *ca.* 5 to 60 ng g^{-1} fw. Maximum observed concentrations for GA1, GA3 and GA4 in fruitlets were *ca.* 21, 27 and 12 ng g^{-1} fw respectively, in the control samples and *ca.* 20, 5 and 2.5 ng g^{-1} fw respectively, in the pollen diluted samples. Levels of total gibberellins in the pollen control samples ranged from *ca.* 160 to 320 ng g^{-1} fw. Maximum observed concentrations for GA1, GA3 and GA4 in pollen were *ca.* 170, 170 and 15 ng g^{-1} fw respectively in the control samples and *ca.* 40, 20 and 4 ng g^{-1} fw respectively, in the diluted pollen samples.

fw = fresh weight
dw = dry weight

There is evidence of the widespread natural occurrence of GAs in plants, fungi and bacteria across many different species and also within different plant parts of the same species.

Naturally occurring levels of gibberellins GA4 and GA7 may enter the soil from a variety of higher plants, including agricultural crops, as well as from mosses and lichens as vegetation is degraded in soil. Gibberellins may also leach naturally from plants. For example, Tukey (1970), reports that gibberellins (GAs1-3 and GA5) have been shown to leach from non-flowering vegetative chrysanthemum plants and that GAs1-3 and GA6 have been shown to leach from flowering plants. Multiple soil organisms are known to produce gibberellins. There is a very substantial body of evidence demonstrating that micro-organisms, including bacteria, fungi, yeasts, actinomycetes and algae are capable of producing plant growth hormones and plant growth regulators such as auxins, gibberellins, cytokinins, ethylene and abscisic acid in appreciable quantities. Many of the micro-organisms that are common in the rhizospheres of plants can produce such plant growth-regulating substances. For example, Barea *et al*., (1976), reported that, of 50 bacterial isolates obtained from the rhizosphere of various plants, 86% could produce auxins, 58% gibberellins and 90% kinetin-like substances. The rhizosphere and rhizoplane of faba bean *(Vicia faba)* melochia *(Corchorus olitorius)*, sesame *(Sesamum indicum)*, and soya bean *(Glycine max)* plants are inhabited with fungi, mostly *Aspergillus flavus, A. niger, Fusarium oxysporum, Penicillium corylophilum, P. cyclopium* and *Rhizopus stolonifera*. All these fungal species have the ability to produce gibberellins (Hasan, 2002). Dobbelaere *et al*., (2003), reported that plant growth promoting micro-organisms including Rhizobium and Azospirillum can reduce the use of urea-N fertiliser by growth promotion through the production of cytokinins, gibberellins and ethylene. Even ubiquitous soil micro-organisms such as *Bacillus cereus* (MJ-1), *Bacillus macroides* (CJ-29) and *Bacillus pumilus* (CJ-69) have been shown to produce gibberellins (Joo *et al.,* 2004 & 2005).

The individual components of the active substance GA4/7, gibberellins GA4 and gibberellins GA7 are rapidly and extensively degraded in soil under aerobic conditions, which is likely to be indicative of their common occurrence in the terrestrial environment. In the modern guideline compliant aerobic soil degradation studies covering eight EU soils, which are submitted in support of this application, GA4 degraded with DT_{50} values of <2 days in all soils (DT_{90} 0.3 to 12.6 days) and GA7 degraded with DT_{50} values of <1 day in all soils (DT_{90} <2 days). Given that both components are rapidly and extensively degraded in soil, the temporal natural occurrence of metabolites of GA4 and GA7 in soil can also be expected. Biological metabolism by soil organisms in the natural environment is expected to result in similar metabolites to those formed following exogenous application of the active substance gibberellins GA4/7. Since gibberellins are expected to undergo relatively constant metabolism in the natural environment, naturally occurring levels of metabolites of GA4 and GA7 are expected to be continuous and therefore more persistent than those resulting from the limited and short-term use of the active substance gibberellins GA4/7 in plant protection products.

Gibberellins GA4 and GA7 can enter water systems through the plants and micro-organisms that inhabit the aquatic environment. For example, Jennings and McComb (1967) reported gibberellin-like activity in samples of red algae (*Hynea musciformis*) and Radley (1969) conducted bioassays to confirm the presence of gibberellins in kelp (*Macrocystis integrifolia*). Sciuto *et al*., (1980), used the stimulation or inhibition of amaranthin synthesis in Amaranthus sp. seedlings as biological tests to assay levels of cytokinin- or gibberellin-like activity in 21 species of marine algae from the central Mediterranean and Jennings (1968) reported gibberellin-like activity in extracts of green and brown algae (*Enteromorpha prolifera* and *Ecklonia radiata*).

Although stable to hydrolysis at environmentally relevant temperature and pH, the individual components of the active substance GA4/7, gibberellins GA4 and gibberellins GA7 are expected to be rapidly and extensively biodegraded in the aquatic environment. Both components can be classified as being inherently biodegradable, which is likely to be indicative of their common occurrence in the natural aquatic environment. Degradation in aquatic systems (pelagic water and water/sediments systems) was assessed by read across to studies conducted using the structurally similar active substance gibberellic acid GA3. GA3 degraded rapidly in these studies with DT_{50} values of ≤17.2 days in the aerobic mineralisation study and DT_{50} values of 10.9 to 11.2 days (DT_{90} 36.3 to 37.3 days) in the water-sediment study. Given that both gibberellins GA4 and gibberellins GA7 are expected to be rapidly degraded in water, the temporal natural occurrence of metabolites of GA4 and GA7 in the aquatic environment can also be expected. Biological metabolism in the natural aquatic environment is expected to result

in similar metabolites to those formed following exogenous application of the active substance gibberellins GA4/7. Since gibberellins are expected to undergo relatively constant metabolism in the natural environment, naturally occurring levels of metabolites of GA4 and GA7 are expected to be continuous and therefore more persistent than those resulting from the limited and short-term use of gibberellins GA4/7 in plant protection products.

The information in the published literature indicates that levels of naturally occurring gibberellins in untreated pome fruits can be found at levels up to 0.06 mg/kg at various stages of fruit development. This is considerably lower than the expected levels of gibberellins GA4 and GA7 in the environment following application of the active substance GA4/7 as a plant protection product. However, given the rapid and extensive degradation of gibberellins GA4 and gibberellins GA7 in both soil and water, increased levels of GA4 and GA7 in the environment resulting from the exogenous application of the active substance GA4/7 will be extremely short-lived and will rapidly decline to levels equivalent to or below naturally occurring background concentrations.

B.8.5. REFERENCES RELIED ON

Data Point	Author(s)	Year	Title Compagny Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA 7.1.1.1/01	Hurst, L.	2014	[^{14}C]-GA4 and [^{14}C]-GA7: Aerobic Soil Metabolism and Transformation. Smithers Viscient (ESG) Ltd., Study No. 3200135. GLP Unpublished	N	Y	New study submitted for the purpose of renewal	Fine Agrochemicals and VBC	In product RR
KCA 7.1.2.1.1/01	Traub, M.	2014	Gibberellins GA4, GA7: Aerobic degradation in four European soils. Eurofins Agroscience Services, Study code S14-01454. GLP Unpublished	N	Y	New study submitted for the purpose of renewal	Globachem NV	-
KCA 7.1.3.1.1/01	Hurst, L.	2013	[^{14}C]-GA4 and [^{14}C]-GA7: Adsorption/Desorption in soil. Smithers Viscient (ESG) Ltd., UK, study no. 3200134. GLP Unpublished	N	Y	New study submitted for the purpose of renewal	Fine Agrochemicals and VBC	-

KCA 7.2.1.1/01	Van der Kolk, J.	2000	Gibberellin A4/A7: Determination of the Hydrolysis as a Function of pH. Springborn Laboratories (Europe) AG, Report no. 1042.003.715. GLP Unpublished	N	N	-	VBC	In DAR B.8.4.1
KCA 7.2.1.2/01	McLaughlin, S.P.	2003	Gibberellins A4 and A7 Combined - Photodegradation in Water an Experimental Screening Test Based on the OECD Direct Photolysis Draft Guideline, Tier II. Springborn Smithers Report No. 12709.6214. GLP Unpublished	N	N	-	VBC	In DAR B.8.4.2
KCA 7.2.2.1/01	Barnes, S.P.	2005	Gibberellins A4 and A7 (Technical Grade) Assessment of Ready Biodegradability - Modified Sturm Test. Huntingdon Life Sciences Laboratory report no. ZAB 049/043181. GLP Unpublished	N	N	-	VBC	In DAR B.8.4.3.1

KCA 7.2.2.1/02	Drake, R.M.	2009	An evaluation of the ready biodegradability of GA4/7 Technical using the OECD 301B CO_2 evolution test (Modified Sturm Test). Chemex Environmental International Ltd., ref ENV8775/070905. GLP Unpublished	N	Y	New study submitted for the purpose of renewal	Globachem NV	-
KCA 7.2.2.2/01	Lamond, P.	2017	[^{14}C]Gibberellic acid: Aerobic mineralisation in surface water. Smithers Viscient (ESG) Ltd., Study No. 3201604. GLP Unpublished	N	Y	New study submitted for the purpose of renewal	EGAT F II	-
KCA 7.2.2.3/01	Cooper, T.	2017	[^{14}C]Gibberellic acid: Degradation in water-sediment systems under aerobic conditions. Smithers Viscient (ESG) Ltd., Study No. 3201605. GLP Unpublished	N	Y	New study submitted for the purpose of renewal	EGAT F II	-

B.8.6. DEFINITION OF THE RESIDUE

Based on the information provided in the dossier under previous points, the following residue definitions for risk assessment are proposed for soil, groundwater, surface water, sediment and air:

The residue definition for risk assessment in soil is based on the following studies:

- Studies investigating aerobic soil degradation found the following major components:
    - 7.1.1.1/01 - gibberellins GA4 and gibberellins GA7 only (although several major (>10% AR) metabolites were observed these are considered to be of no environmental concern due to the natural occurrence of the active substance)
    - 7.1.2.1.1/01 – gibberellins GA4 and gibberellins GA7 only
- Residue definition for risk assessment in soil: gibberellins GA4 and gibberellins GA7 only

The residue definition for risk assessment in groundwater is based on the residue definition for soil:

- Residue definition for risk assessment in groundwater: gibberellins GA4 and gibberellins GA7 only (although several major (>10% AR) metabolites were observed these are considered to be of no environmental concern due to the natural occurrence of the active substance)

The residue definition for risk assessment in surface water and sediment is based on the following studies:

- Studies investigating hydrolysis found the following major components:
    - 7.2.1.1/01 - gibberellins GA4 and gibberellins GA7 only
- Studies investigating direct aqueous photolysis found the following major components:
    - 7.2.1.2/01 - gibberellins GA4 and gibberellins GA7 only
- Studies investigating degradation in pelagic water systems found the following major components:
    - 7.2.2.2/01 - gibberellins GA4 and gibberellins GA7 only, based on a study conducted using gibberellins GA3 (although significant metabolites were observed these were considered to be of no environmental concern due to the natural occurrence of the active substance)
- Studies investigating degradation in water/sediment systems found the following major components:
    - 7.2.2.3/01 – For surface water gibberellins GA4 and gibberellins GA7 only, based on a study conducted using gibberellins GA3 (although significant metabolites were observed these were considered to be of no environmental concern due to the natural occurrence of the active substance). For sediment gibberellins GA4 and gibberellins GA7 only
- Residue definition for risk assessment in surface water and sediment: For surface water gibberellins GA4 and gibberellins GA7 only. For sediment gibberellins GA4 and gibberellins GA7 only (although several major (>10% AR) metabolites were observed these are considered to be of no environmental concern due to the natural occurrence of the active substance).

The residue definition in air for risk assessment is based on vapour pressure values of 0.160 and 0.067 Pa (22°C) for the components gibberellins GA4 and gibberellins GA7, respectively. Although potentially volatile from plant and soil surfaces, the components were not observed to be volatile in any of the environmental fate studies. Therefore volatility under the conditions of use is not expected. Furthermore, the estimated photochemical oxidative degradation half-lives in air of the components gibberellins GA4 and gibberellins GA7 (calculated using the Atkinson equation), are 1.67 and 0.99 hours, respectively (EFSA LoEP, page 39/50). Therefore, these components will not persist in the atmosphere, if present.

- Residue definition in air for risk assessment: gibberellins GA4 and gibberellins GA7 only

Definition of the residue for monitoring

Based on the information considered for the definition of the residue for risk assessment, the persistence and relative toxicity of the components involved and the general natural occurrence of the components and their degradation products, the following residue definitions for monitoring purposes are proposed for soil, groundwater, surface water, sediment and air.

The residue definition for monitoring purposes in soil is gibberellins GA4 and gibberellins GA7 only (as above).

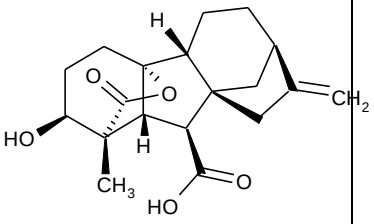
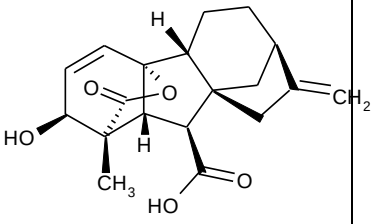
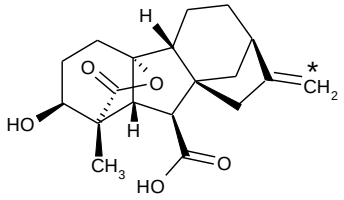
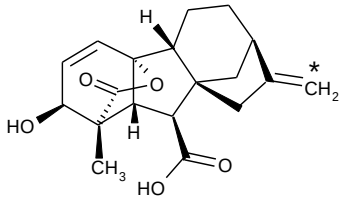
The residue definition for monitoring purposes in groundwater is gibberellins GA4 and gibberellins GA7 only (as above).

The residue definition for monitoring purposes in surface water is gibberellins GA4 and gibberellins GA7 only (as above).

The residue definition for monitoring purposes in sediment is gibberellins GA4 and gibberellins GA7 only (as above).

The residue definition for monitoring purposes in air is gibberellins GA4 and gibberellins GA7 only (as above).

Appendix1: Chemical names and structures of components

Code Number (Synonyms)	Description	Compound found:	Structure
GA4	(3*S*,3a*R*,4*S*,4a*R*,7*R*,9a*R*,9b*R*,12*S*)-12-hydroxy-3-methyl-6-methylene-2-oxoperhydro-4a,7-methano-3,9b-propanoazuleno[1,2-*b*]furan-4-carboxylic acid (IUPAC) Synonym(s): (1α,2β,4aα,4bβ,10β)-2,4a-dihydroxy-1-methyl-8-methylenegibbane,-1,10-dicarboxylic acid 1,4a-lactone (CAS), Gibberellins GA_4 CAS no. 468-44-0	active substance	 Mol wt – 332.40 g/mol
GA7	(3*S*,3a*R*,4*S*,4a*R*,7*R*,9a*R*,9b*R*,12*S*)-12-hydroxy-3-methyl-6-methylene-2-oxoperhydro-4a,7-methano-9b,3-propenoazuleno[1,2-*b*]furan-4-carboxylic acid (IUPAC) Synonym(s): (1α,2β,4aα,4bβ,10β)-2,4a-dihydroxy-1-methyl-8-methylenegibb-3-ene,-1,10-dicarboxylic acid 1,4a-lactone (CAS), Gibberellins GA_7 CAS no. 510-75-8	active substance	 Mol wt – 330.40 g/mol
Radiolabelled GA4 (* position of ^{14}C)	As above	active substance	
Radiolabelled GA7 (* position of ^{14}C)	As above	active substance	

Appendix 2: Kinetic fit Analysis of study CA 7.1.1.1/01; Hurst, L. (2014); (Aerobic soil metabolism and Transformation)

Rates of degradation of [^{14}C]-GA4 (SFO kinetics)

Speyer 5M soil treated with [^{14}C]-GA4 (primary extracts only)

Parent only (SFO)

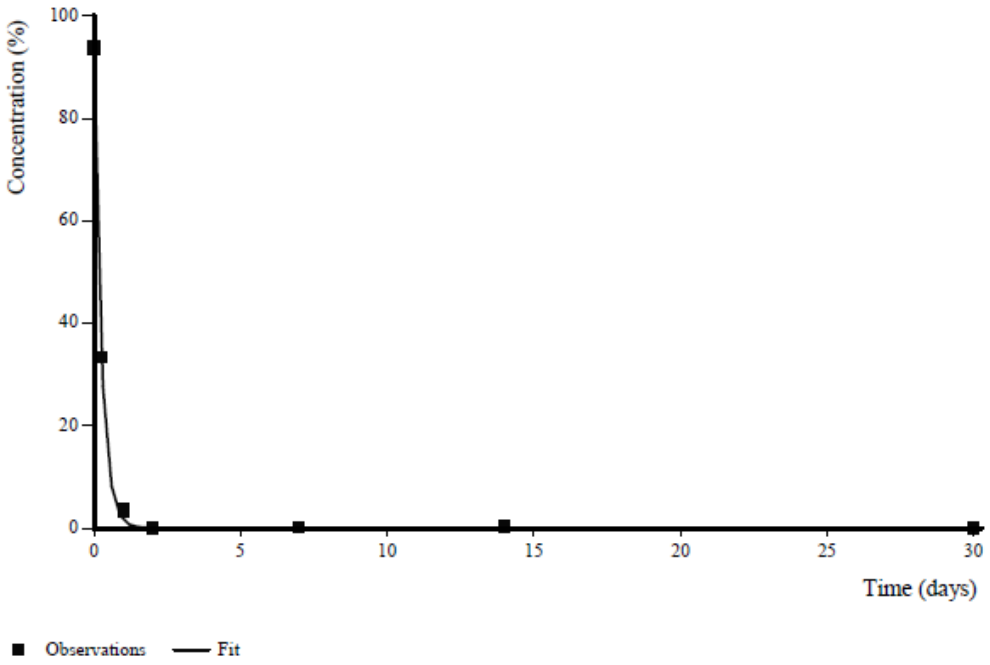
Initial Values of Sequence Parameters:

Parameter	Initial Value	Bounds	Fixed
Parent_0	100	0 to (unbounded)	No
k_Parent	0.1	0 to (unbounded)	No

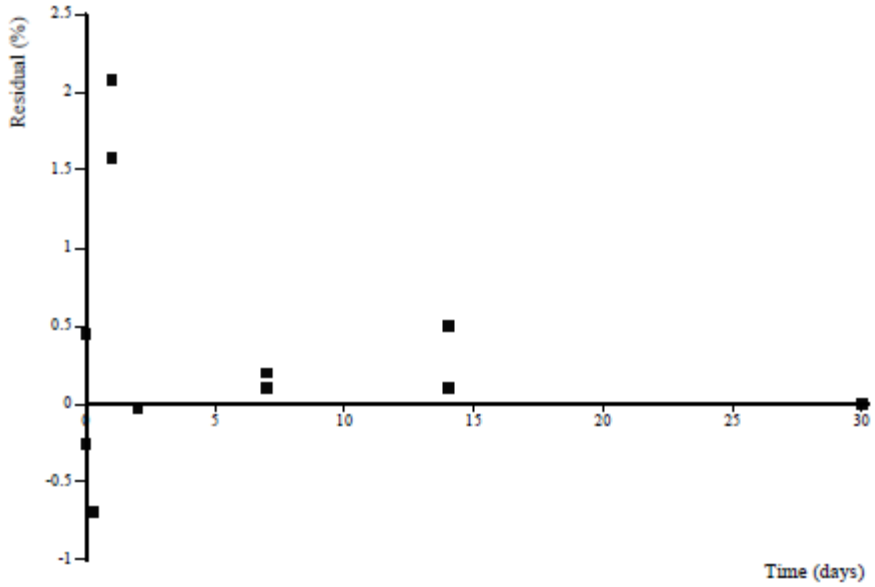
Fit step: Final

Graphical Summary:

Observations and Fitted Model:



Residuals:



Initial Values for This Step:

Parameter	Initial Value	Bounds	Fixed
Parent_0	100	0 to (unbounded)	No
k_Parent	0.1	0 to (unbounded)	No

Estimated Values:

Parameter	Value	σ	Prob. > t	Lower CI	Upper CI
Parent_0	93.67	0.7465	5.157E-19	92.02	95.31
k_Parent	4.453	0.1382	1.526E-12	4.149	4.757

χ^2

Parameter	Error %	Degrees of Freedom
All data	4.079	5
Parent	4.079	5

Decay Times:

Compartment	DT-50 (days)	DT-90 (days)
Parent	0.16	0.52

Additional Statistics:

Parameter	r^2 (Obs v Pred)	Efficiency
All data	0.9993	0.9992
Parent	0.9993	0.9992

Parameter Correlation:

	Parent_0	k_Parent
Parent_0	1	0.2241
k_Parent	0.2241	1

Observed v. Predicted:

Compartment Parent

Time (days)	Value (%)	Predicted Value	Residual
0	94.1	93.66	0.4449
0	93.4	93.66	-0.2551
0.25	33.3	34	-0.6976
1	3.7	1.626	2.074
1	3.2	1.626	1.574
2	0	0.02824	-0.02824
2	0	0.02824	-0.02824
8	0.1	-6.623E-08	0.1
8	0.2	-6.623E-08	0.2
14	0.5	-5.947E-09	0.5
14	0.1	-5.947E-09	0.1
30	0	-1.359E-11	1.359E-11
30	0	-1.359E-11	1.359E-11

Sequence Creation Information:
Fit generated by CAKE version 1.4 (Release)
running on R version 2.12.2 (2011-02-25)

Report Information:
Report generated by CAKE version 1.4 (Release)
CAKE developed by Tessella Plc, Abingdon, Oxfordshire, UK for Syngenta
Running on .Net version 2.0.50727.5472

Speyer 2.2 soil treated with [^{14}C]-GA4 (primary extracts only)

Parent only (SFO)

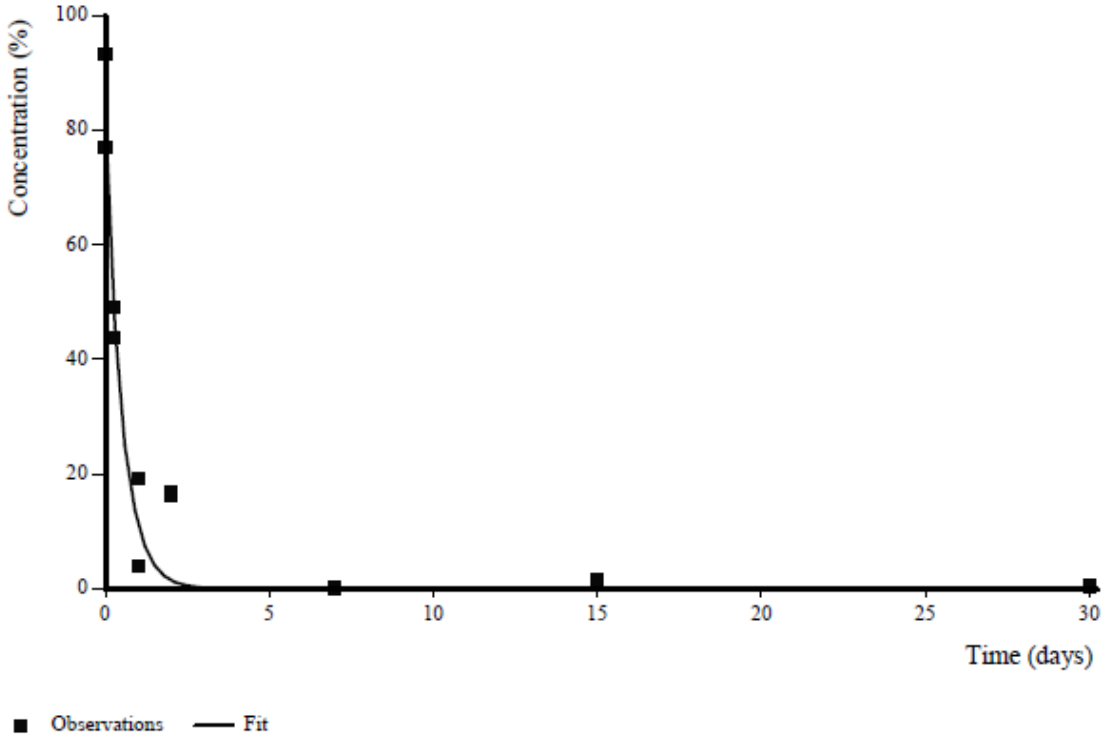
Initial Values of Sequence Parameters:

Parameter	Initial Value	Bounds	Fixed
Parent_0	100	0 to (unbounded)	No
k_Parent	0.1	0 to (unbounded)	No

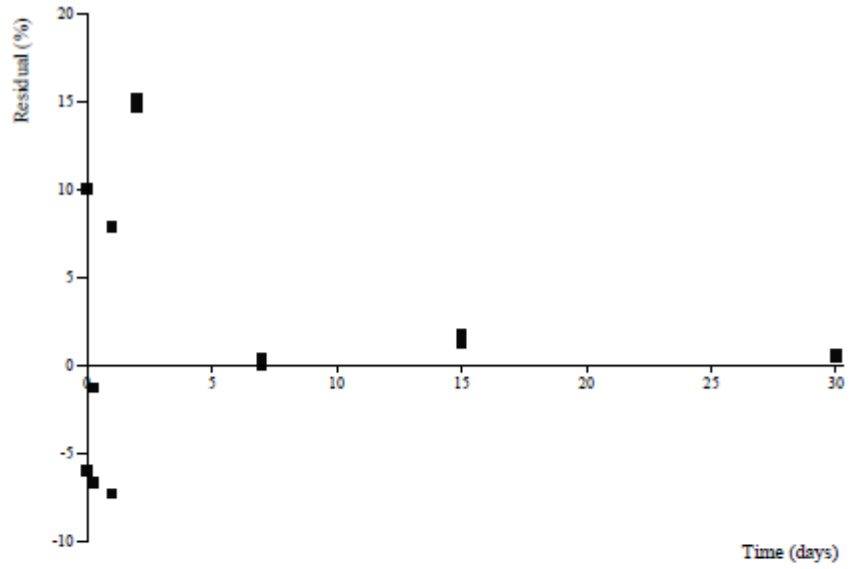
Fit step: Final

Graphical Summary:

Observations and Fitted Model:



Residuals:



Initial Values for This Step:

Parameter	Initial Value	Bounds	Fixed
Parent_0	100	0 to (unbounded)	No
k_Parent	0.1	0 to (unbounded)	No

Estimated Values:

Parameter	Value	σ	Prob. > t	Lower CI	Upper CI
Parent_0	83.08	5.357	1.326E-09	71.41	94.75
k_Parent	1.997	0.367	7.491E-05	1.197	2.796

χ^2

Parameter	Error %	Degrees of Freedom
All data	20.43	5
Parent	20.43	5

Decay Times:

Compartment	DT-50 (days)	DT-90 (days)
Parent	0.3472	1.153

Additional Statistics:

Parameter	r^2 (Obs v Pred)	Efficiency
All data	0.9475	0.939
Parent	0.9475	0.939

Parameter Correlation:

	Parent_0	k_Parent
Parent_0	1	0.4564
k_Parent	0.4564	1

Observed v. Predicted:

Compartment Parent

Time (days)	Value (%)	Predicted Value	Residual
0	93.2	83.08	10.12
0	77.1	83.08	-5.982
0.25	43.8	50.43	-6.634
0.25	49.2	50.43	-1.234
1	4	11.28	-7.282
1	19.2	11.28	7.918
2	16.3	1.532	14.77
2	16.8	1.532	15.27
7	3.6	7.137E-05	3.6
7	5.8	7.137E-05	5.8
15	1.3	-6.803E-08	1.3
15	1.8	-6.803E-08	1.8
30	0.5	-2.065E-09	0.5
30	0.7	-2.065E-09	0.7

Sequence Creation Information:
Fit generated by CAKE version 1.4 (Release)
running on R version 2.12.2 (2011-02-25)

Report Information:
Report generated by CAKE version 1.4 (Release)
CAKE developed by Tessella Plc, Abingdon, Oxfordshire, UK for Syngenta
Running on .Net version 2.0.50727.5472

Brierlow soil treated with [^{14}C]-GA4 (primary extracts only)

Parent only (SFO)

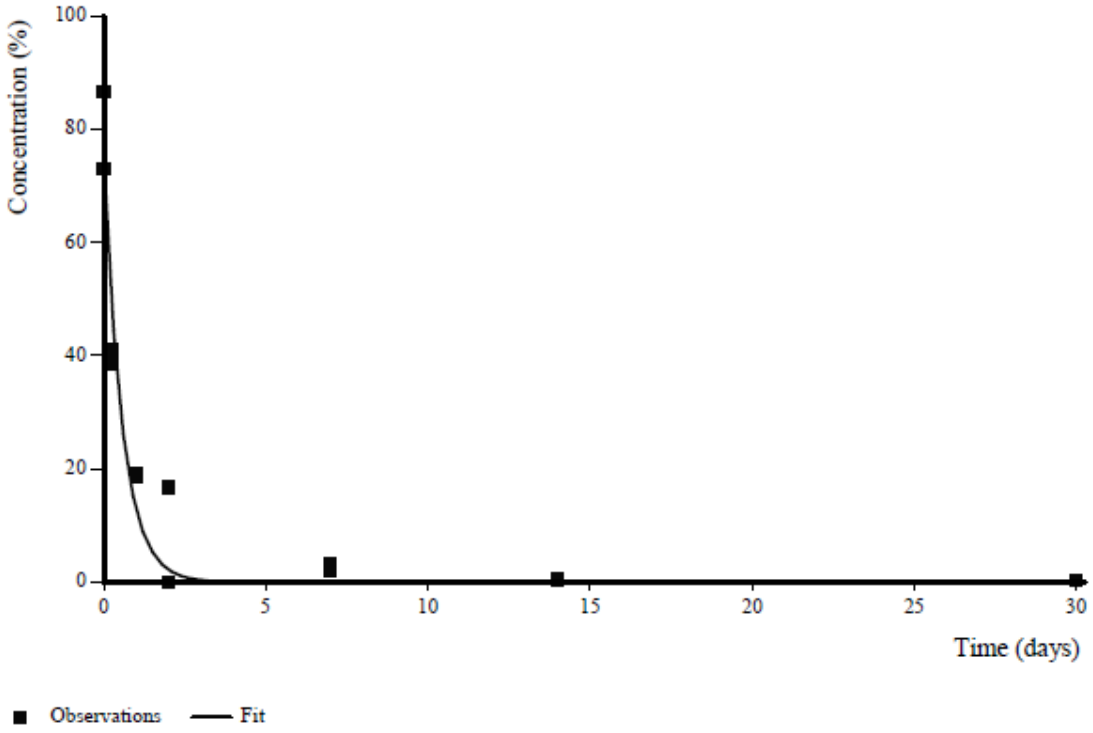
Initial Values of Sequence Parameters:

Parameter	Initial Value	Bounds	Fixed
Parent_0	100	0 to (unbounded)	No
k_Parent	0.1	0 to (unbounded)	No

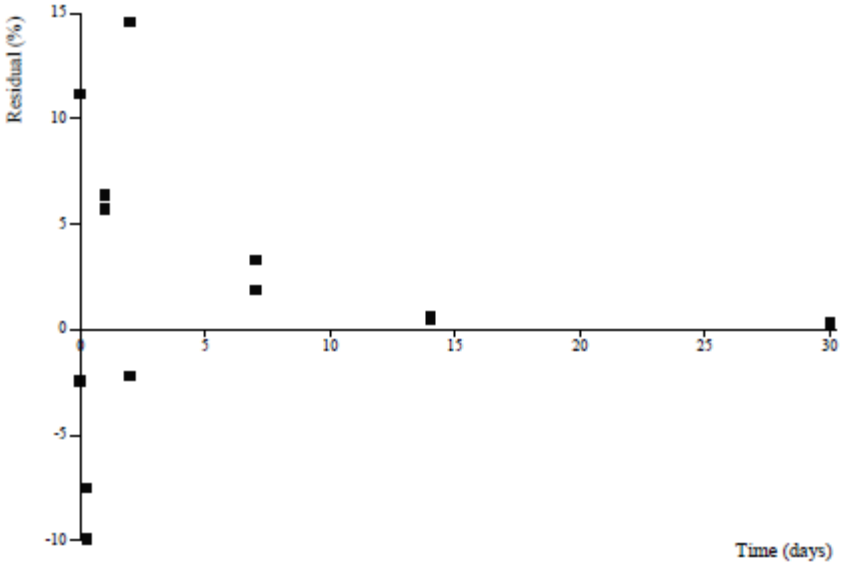
Fit step: Final

Graphical Summary:

Observations and Fitted Model:



Residuals:



Initial Values for This Step:

Parameter	Initial Value	Bounds	Fixed
Parent_0	100	0 to (unbounded)	No
k_Parent	0.1	0 to (unbounded)	No

Estimated Values:

Parameter	Value	σ	Prob. > t	Lower CI	Upper CI
Parent_0	75.43	4.661	8.128E-10	65.27	85.58
k_Parent	1.767	0.3071	4.555E-05	1.098	2.436

χ^2

Parameter	Error %	Degrees of Freedom
All data	18.61	5
Parent	18.61	5

Decay Times:

Compartment	DT-50 (days)	DT-90 (days)
Parent	0.3923	1.303

Additional Statistics:

Parameter	r^2 (Obs v Pred)	Efficiency
All data	0.9492	0.944
Parent	0.9492	0.944

Parameter Correlation:

	Parent_0	k_Parent
Parent_0	1	0.4622
k_Parent	0.4622	1

Observed v. Predicted:

Compartment Parent

Time (days)	Value (%)	Predicted Value	Residual
0	86.6	75.43	11.17
0	73	75.43	-2.426
0.25	38.6	48.5	-9.896
0.25	41	48.5	-7.496
1	19.3	12.89	6.41
1	18.6	12.89	5.71
2	0	2.203	-2.203
2	16.8	2.203	14.6
8	1.9	0.0003212	1.9
8	3.3	0.0003212	3.3
14	0.6	-2.155E-07	0.6
14	0.5	-2.155E-07	0.5
30	0.3	-4.723E-09	0.3
30	0.2	-4.723E-09	0.2

Sequence Creation Information:
Fit generated by CAKE version 1.4 (Release)
running on R version 2.12.2 (2011-02-25)

Report Information:
Report generated by CAKE version 1.4 (Release)
CAKE developed by Tessella Plc, Abingdon, Oxfordshire, UK for Syngenta
Running on .Net version 2.0.50727.5472

South Witham soil treated with [^{14}C]-GA4 (primary extracts only)

Parent only (SFO)

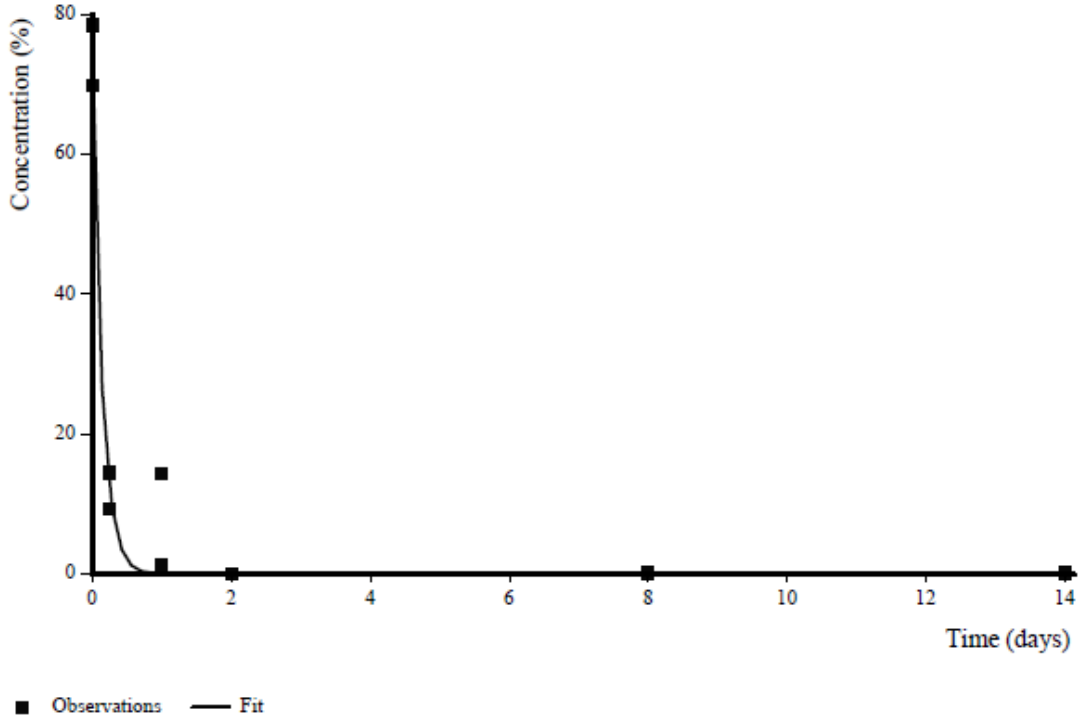
Initial Values of Sequence Parameters:

Parameter	Initial Value	Bounds	Fixed
Parent_0	100	0 to (unbounded)	No
k_Parent	0.1	0 to (unbounded)	No

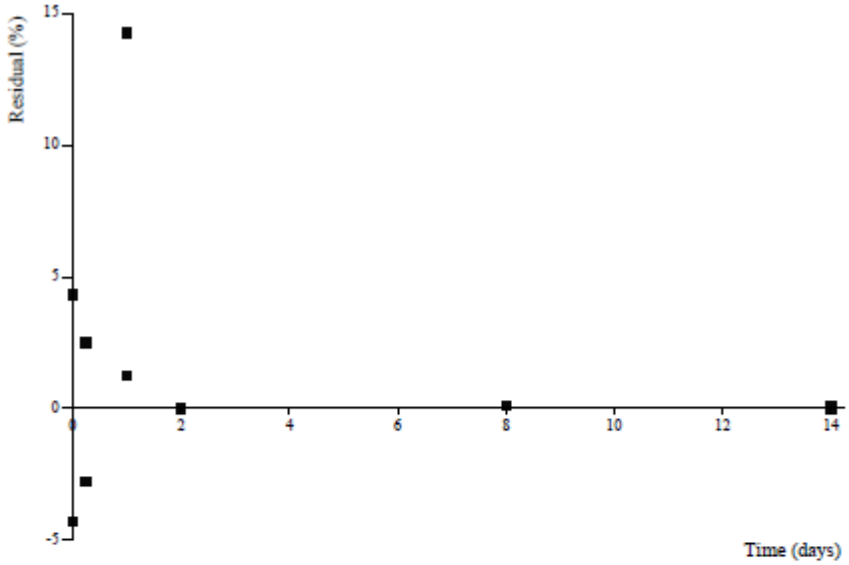
Fit step: Final

Graphical Summary:

Observations and Fitted Model:



Residuals:



Initial Values for This Step:

Parameter	Initial Value	Bounds	Fixed
Parent_0	100	0 to (unbounded)	No
k_Parent	0.1	0 to (unbounded)	No

Estimated Values:

Parameter	Value	σ	Prob. > t	Lower CI	Upper CI
Parent_0	74.18	3.576	7.494E-10	66.22	82.15
k_Parent	7.293	1.209	6.331E-05	4.599	9.987

χ^2

Parameter	Error %	Degrees of Freedom
All data	16.06	4
Parent	16.06	4

Decay Times:

Compartment	DT-50 (days)	DT-90 (days)
Parent	0.09504	0.3157

Additional Statistics:

Parameter	r^2 (Obs v Pred)	Efficiency
All data	0.9733	0.9703
Parent	0.9733	0.9703

Parameter Correlation:

	Parent_0	k_Parent
Parent_0	1	0.1857
k_Parent	0.1857	1

Observed v. Predicted:

Compartment Parent

Time (days)	Value (%)	Predicted Value	Residual
0	69.9	74.14	-4.242
0	78.5	74.14	4.358
0.25	15.4	14.01	1.388
0.25	11.8	14.01	-2.212
1	14.3	0.09458	14.21
1	16.4	0.09458	16.31
2	0	0.0001211	-0.0001211
2	0	0.0001211	-0.0001211
8	0.1	-1.626E-08	0.1
8	0.1	-1.626E-08	0.1
14	0.1	-4.125E-11	0.1
14	0	-4.125E-11	4.125E-11

Sequence Creation Information:
Fit generated by CAKE version 1.4 (Release)
running on R version 2.12.2 (2011-02-25)

Report Information:
Report generated by CAKE version 1.4 (Release)
CAKE developed by Tessella Plc, Abingdon, Oxfordshire, UK for Syngenta
Running on .Net version 2.0.50727.5472

Rates of degradation of [^{14}C]-GA7 (SFO kinetics)

Speyer 5M soil treated with [^{14}C]-GA7 (primary extracts only)

Parent only (SFO)

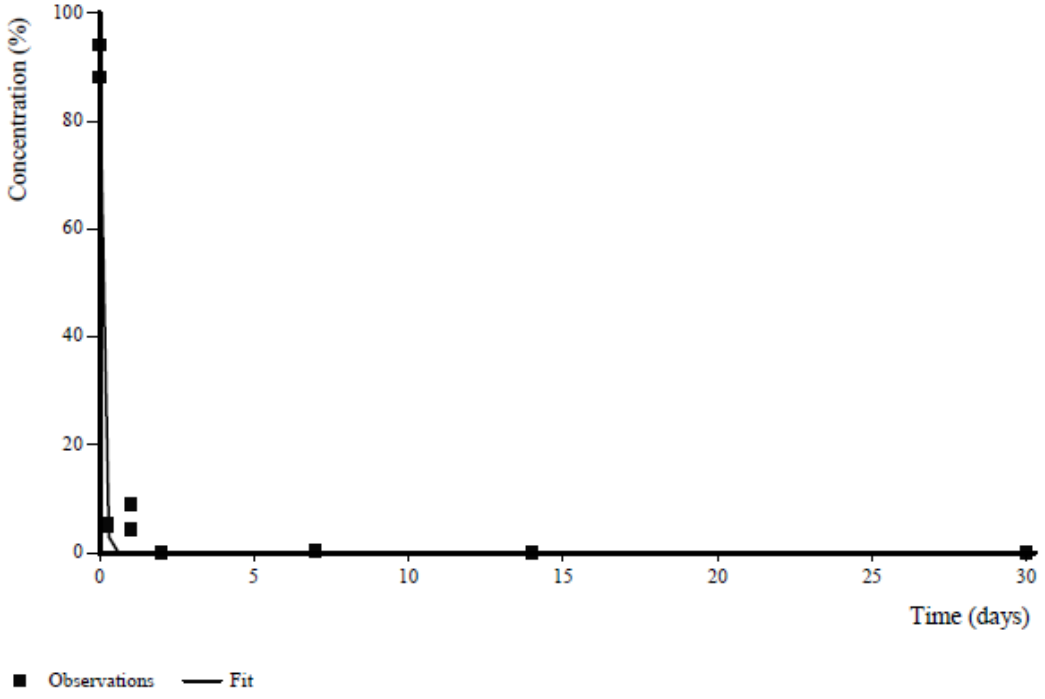
Initial Values of Sequence Parameters:

Parameter	Initial Value	Bounds	Fixed
Parent_0	100	0 to (unbounded)	No
k_Parent	0.1	0 to (unbounded)	No

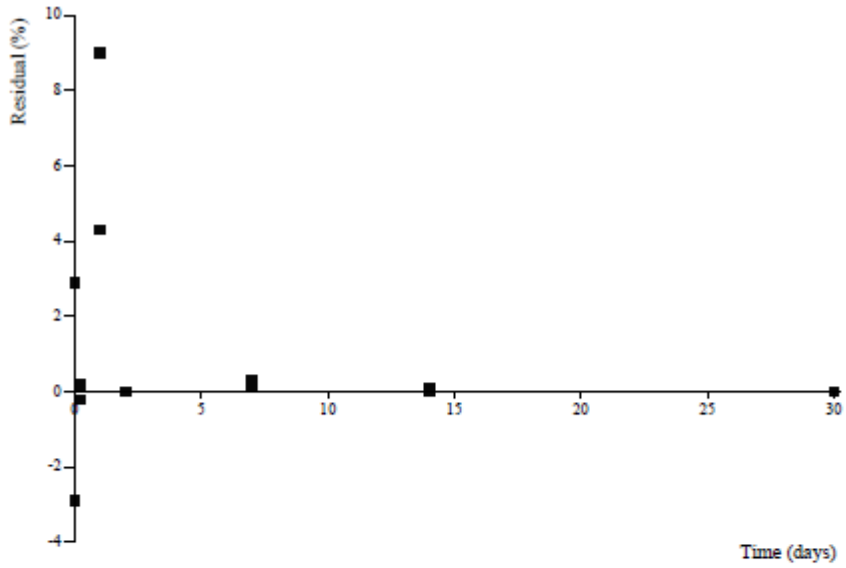
Fit step: Final

Graphical Summary:

Observations and Fitted Model:



Residuals:



Initial Values for This Step:

Parameter	Initial Value	Bounds	Fixed
Parent_0	100	0 to (unbounded)	No
k_Parent	0.1	0 to (unbounded)	No

Estimated Values:

Parameter	Value	σ	Prob. > t	Lower CI	Upper CI
Parent_0	91.1	2.301	1.62E-13	86.03	96.17
k_Parent	11.53	1.806	2.603E-05	7.552	15.5

χ^2

Parameter	Error %	Degrees of Freedom
All data	13.57	5
Parent	13.57	5

Decay Times:

Compartment	DT-50 (days)	DT-90 (days)
Parent	0.06013	0.1998

Additional Statistics:

Parameter	r^2 (Obs v Pred)	Efficiency
All data	0.9927	0.9914
Parent	0.9927	0.9914

Speyer 2.2 soil treated with [^{14}C]-GA7 (primary extracts only)

Parent only (SFO)

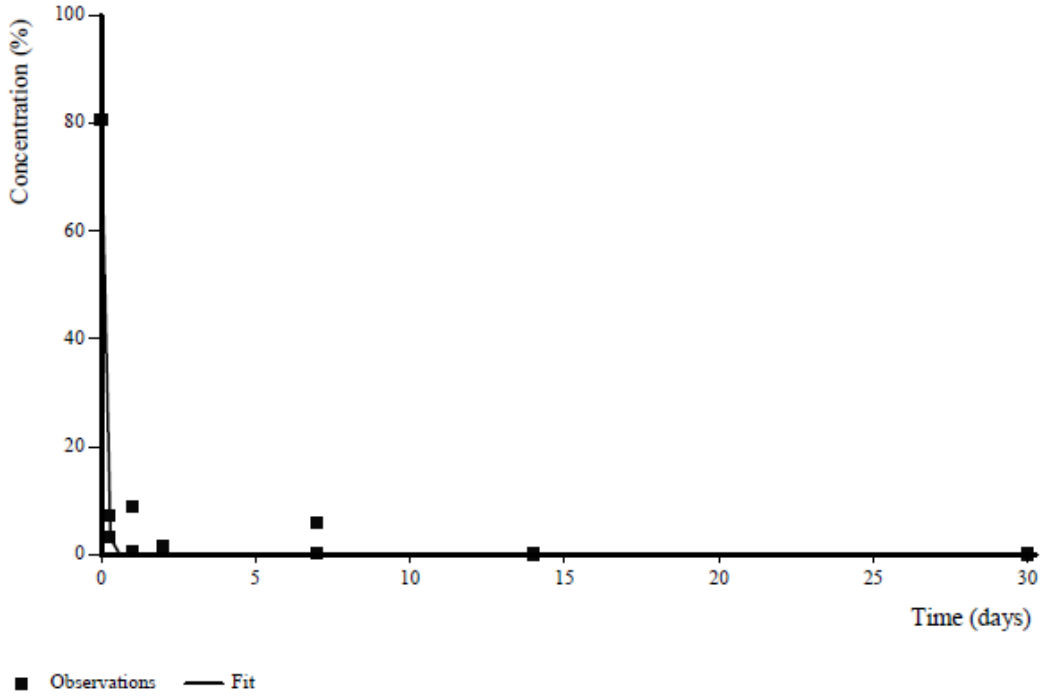
Initial Values of Sequence Parameters:

Parameter	Initial Value	Bounds	Fixed
Parent_0	100	0 to (unbounded)	No
k_Parent	0.1	0 to (unbounded)	No

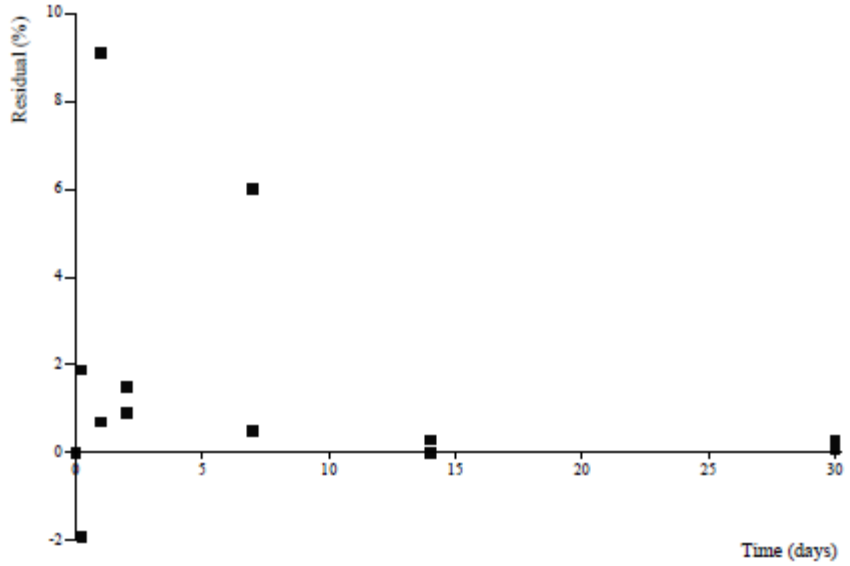
Fit step: Final

Graphical Summary:

Observations and Fitted Model:



Residuals:



Initial Values for This Step:

Parameter	Initial Value	Bounds	Fixed
Parent_0	100	0 to (unbounded)	No
k_Parent	0.1	0 to (unbounded)	No

Estimated Values:

Parameter	Value	σ	Prob. > t	Lower CI	Upper CI
Parent_0	80.7	3.438	4.761E-11	73.13	88.27
k_Parent	10.89	1.841	5.04E-05	6.837	14.94

χ^2

Parameter	Error %	Degrees of Freedom
All data	13.2	5
Parent	13.2	5

Decay Times:

Compartment	DT-50 (days)	DT-90 (days)
Parent	0.06366	0.2115

Additional Statistics:

Parameter	r^2 (Obs v Pred)	Efficiency
All data	0.983	0.9774
Parent	0.983	0.9774

Parameter Correlation:

	Parent_0	k_Parent
Parent_0	1	0.09258
k_Parent	0.09258	1

Observed v. Predicted:

Compartment Parent

Time (days)	Value (%)	Predicted Value	Residual
0	80.7	80.7	0.0005489
0.25	7.2	5.306	1.894
0.25	3.4	5.306	-1.906
1	0.7	0.001508	0.6985
1	9.1	0.001508	9.098
2	1.5	-2.937E-07	1.5
2	0.9	-2.937E-07	0.9
7	0.5	-4.569E-10	0.5
7	6	-4.569E-10	6
15	0	-1.447E-11	1.447E-11
15	0.3	-1.447E-11	0.3
30	0.3	-8.26E-14	0.3
30	0.1	-8.26E-14	0.1

Sequence Creation Information:
Fit generated by CAKE version 1.4 (Release)
running on R version 2.12.2 (2011-02-25)

Report Information:
Report generated by CAKE version 1.4 (Release)
CAKE developed by Tessella Plc, Abingdon, Oxfordshire, UK for Syngenta
Running on .Net version 2.0.50727.5472

Brierlow soil treated with [^{14}C]-GA7 (primary extracts only)

Parent only (SFO)

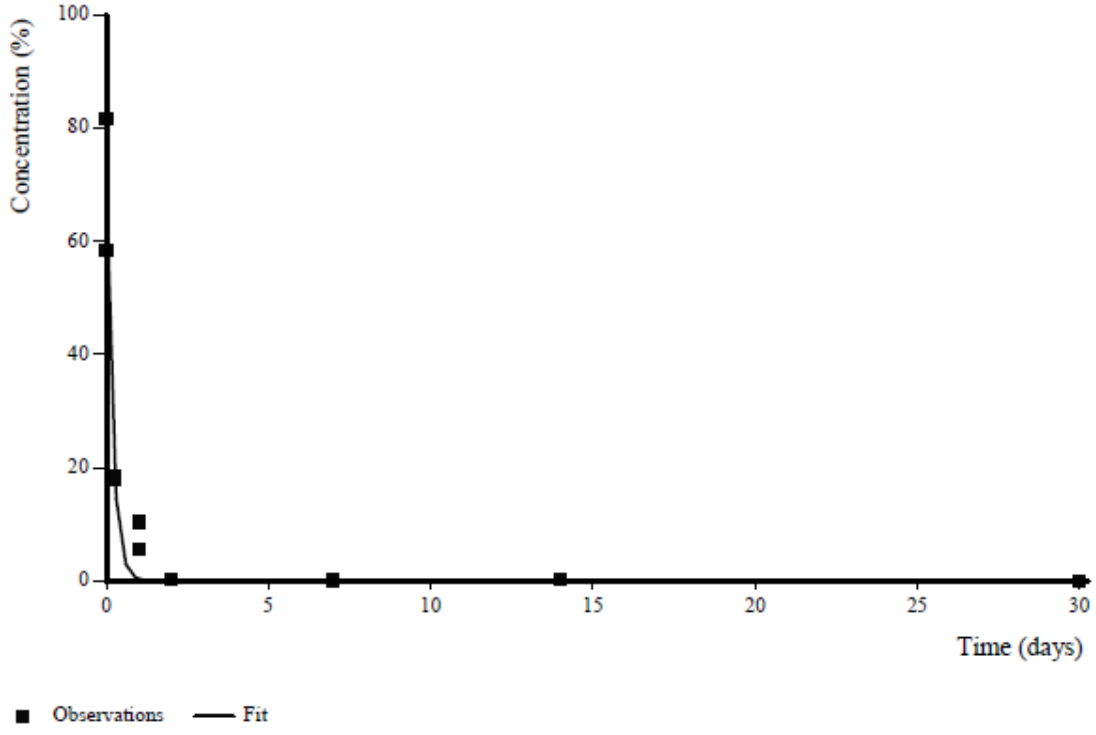
Initial Values of Sequence Parameters:

Parameter	Initial Value	Bounds	Fixed
Parent_0	100	0 to (unbounded)	No
k_Parent	0.1	0 to (unbounded)	No

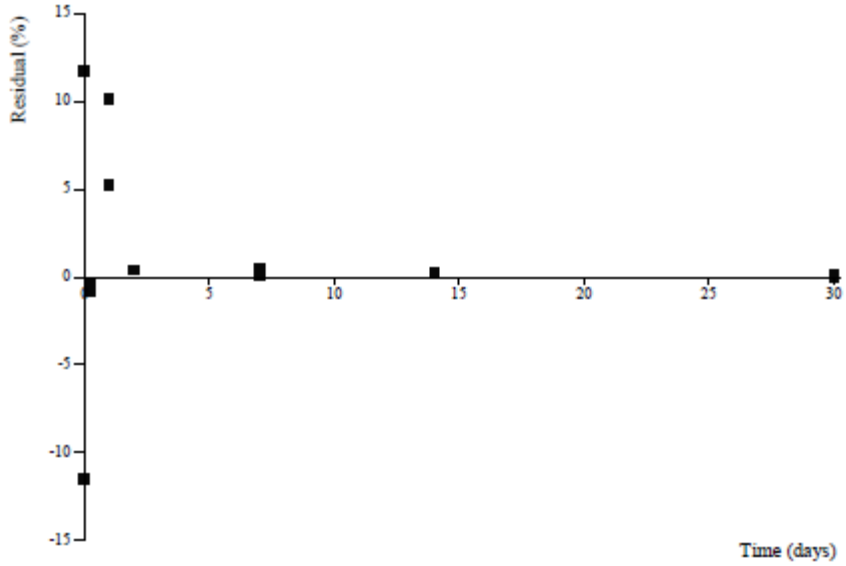
Fit step: Final

Graphical Summary:

Observations and Fitted Model:



Residuals:



Initial Values for This Step:

Parameter	Initial Value	Bounds	Fixed
Parent_0	100	0 to (unbounded)	No
k_Parent	0.1	0 to (unbounded)	No

Estimated Values:

Parameter	Value	σ	Prob. > t	Lower CI	Upper CI
Parent_0	69.98	4.085	4.217E-10	61.08	78.88
k_Parent	5.235	0.8925	3.826E-05	3.29	7.179

χ^2

Parameter	Error %	Degrees of Freedom
All data	16.67	5
Parent	16.67	5

Decay Times:

Compartment	DT-50 (days)	DT-90 (days)
Parent	0.1324	0.4399

Additional Statistics:

Parameter	r^2 (Obs v Pred)	Efficiency
All data	0.9541	0.9511
Parent	0.9541	0.9511

Parameter Correlation:

	Parent_0	k_Parent
Parent_0	1	0.2604
k_Parent	0.2604	1

Observed v. Predicted:

Compartment Parent

Time (days)	Value (%)	Predicted Value	Residual
0	81.7	69.98	11.72
0	58.5	69.98	-11.48
0.25	18.5	18.91	-0.4059
0.25	18.1	18.91	-0.8059
1	5.6	0.3728	5.227
1	10.5	0.3728	10.13
2	0.4	0.001986	0.398
2	0.4	0.001986	0.398
8	0.1	-2.823E-08	0.1
8	0.5	-2.823E-08	0.5
14	0.2	-6.375E-10	0.2
14	0.3	-6.375E-10	0.3
30	0.1	-7.521E-12	0.1
30	0	-7.521E-12	7.521E-12

Sequence Creation Information:
Fit generated by CAKE version 1.4 (Release)
running on R version 2.12.2 (2011-02-25)

Report Information:
Report generated by CAKE version 1.4 (Release)
CAKE developed by Tessella Plc, Abingdon, Oxfordshire, UK for Syngenta
Running on .Net version 2.0.50727.5472

South Witham soil treated with [^{14}C]-GA7 (primary extracts only)

Parent only (SFO)

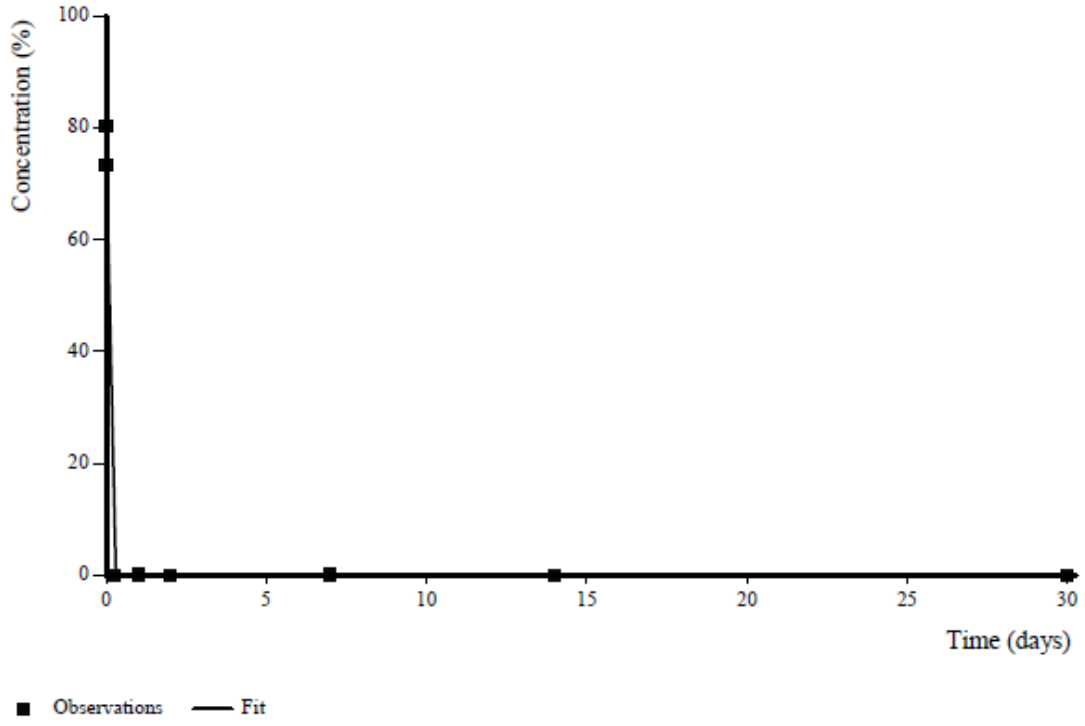
Initial Values of Sequence Parameters:

Parameter	Initial Value	Bounds	Fixed
Parent_0	100	0 to (unbounded)	No
k_Parent	0.1	0 to (unbounded)	No

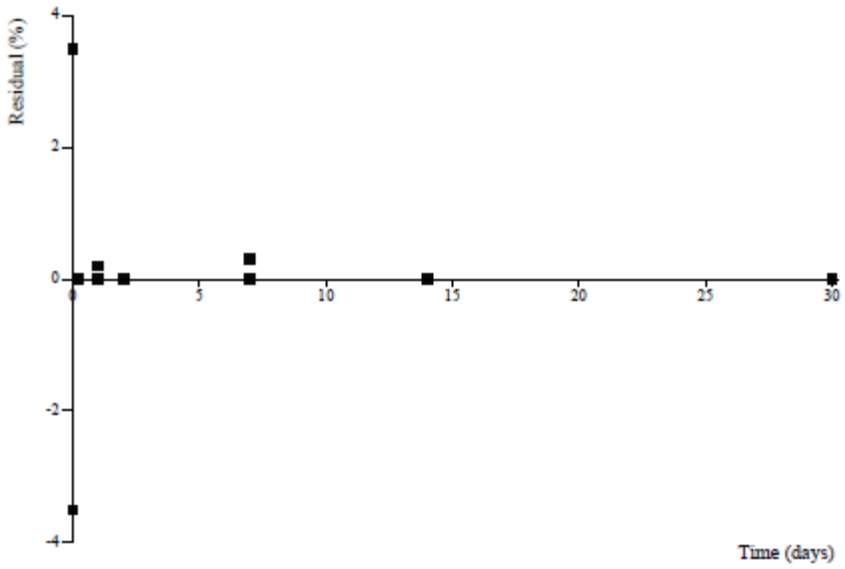
Fit step: Final

Graphical Summary:

Observations and Fitted Model:



Residuals:



Initial Values for This Step:

Parameter	Initial Value	Bounds	Fixed
Parent_0	100	0 to (unbounded)	No
k_Parent	0.1	0 to (unbounded)	No

Estimated Values:

Parameter	Value	σ	Prob. > t	Lower CI	Upper CI
Parent_0	76.9	1.013	9.094E-18	74.69	79.1
k_Parent	63.41	4.178E+05	0.4999	-9.102E+05	9.103E+05

χ^2

Parameter	Error %	Degrees of Freedom
All data	1.005	5
Parent	1.005	5

Decay Times:

Compartment	DT-50 (days)	DT-90 (days)
Parent	0.01093	0.03631

Additional Statistics:

Parameter	r^2 (Obs v Pred)	Efficiency
All data	0.9976	0.9976
Parent	0.9976	0.9976

Parameter Correlation:

	Parent_0	k_Parent
Parent_0	1	1.258E-07
k_Parent	1.258E-07	1

Observed v. Predicted:

Compartment Parent

Time (days)	Value (%)	Predicted Value	Residual
0	73.4	76.9	-3.5
0	80.4	76.9	3.5
0.25	0	8.677E-06	-8.677E-06
0.25	0	8.677E-06	-8.677E-06
1	0	-9.935E-10	9.935E-10
1	0.2	-9.935E-10	0.2
2	0	-1.658E-11	1.658E-11
2	0	-1.658E-11	1.658E-11
8	0.3	-5.183E-14	0.3
8	0	-5.183E-14	5.183E-14
14	0	-1.359E-16	1.359E-16
14	0	-1.359E-16	1.359E-16
30	0	-1.334E-19	1.334E-19
30	0	-1.334E-19	1.334E-19

Sequence Creation Information:
Fit generated by CAKE version 1.4 (Release)
running on R version 2.12.2 (2011-02-25)

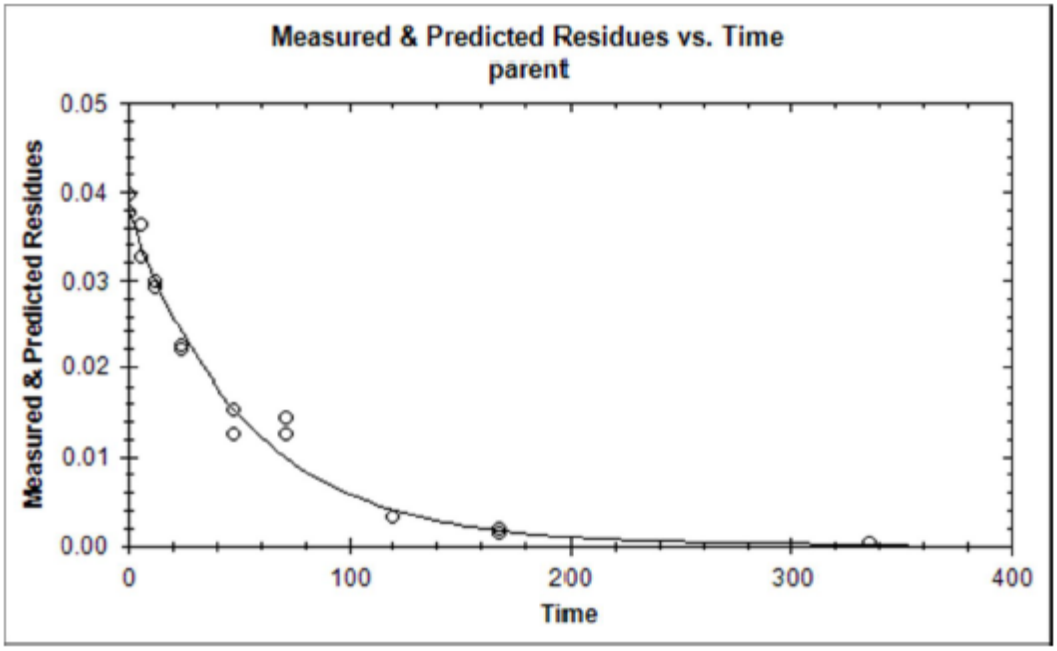
Report Information:
Report generated by CAKE version 1.4 (Release)
CAKE developed by Tessella Plc, Abingdon, Oxfordshire, UK for Syngenta
Running on .Net version 2.0.50727.5472

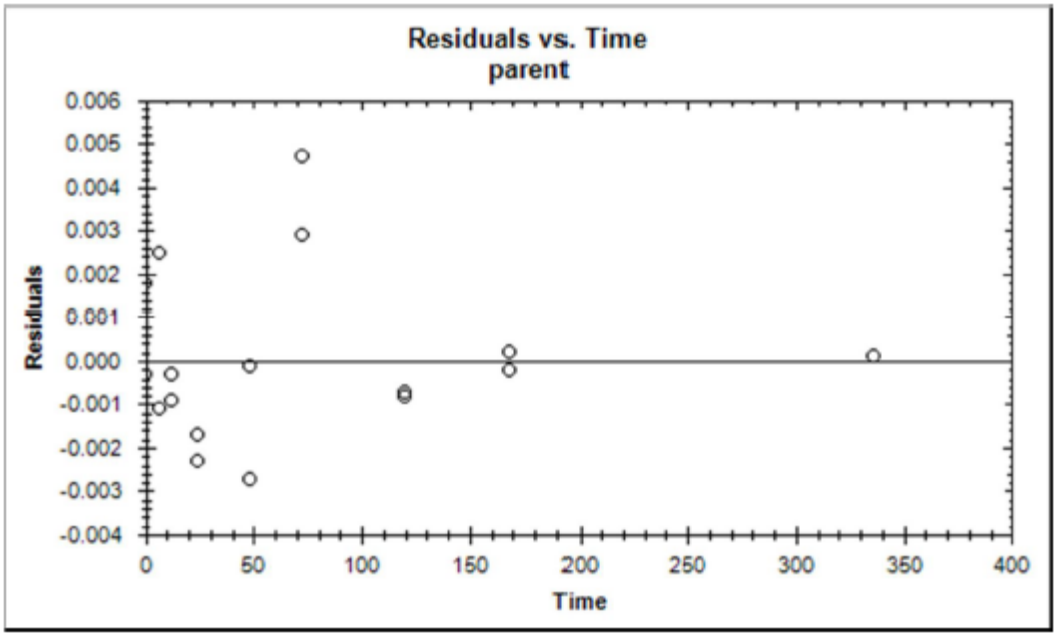
Appendix 3: Kinetic fit Analysis of study; CA 7.1.2.1.1/01; Traub, M. (2014) (Aerobic degradation in four European soils)

1.) Kinetic for Degradation of the Test Items in Soil LUFA 2.1

Single First Order (SFO) for GA4

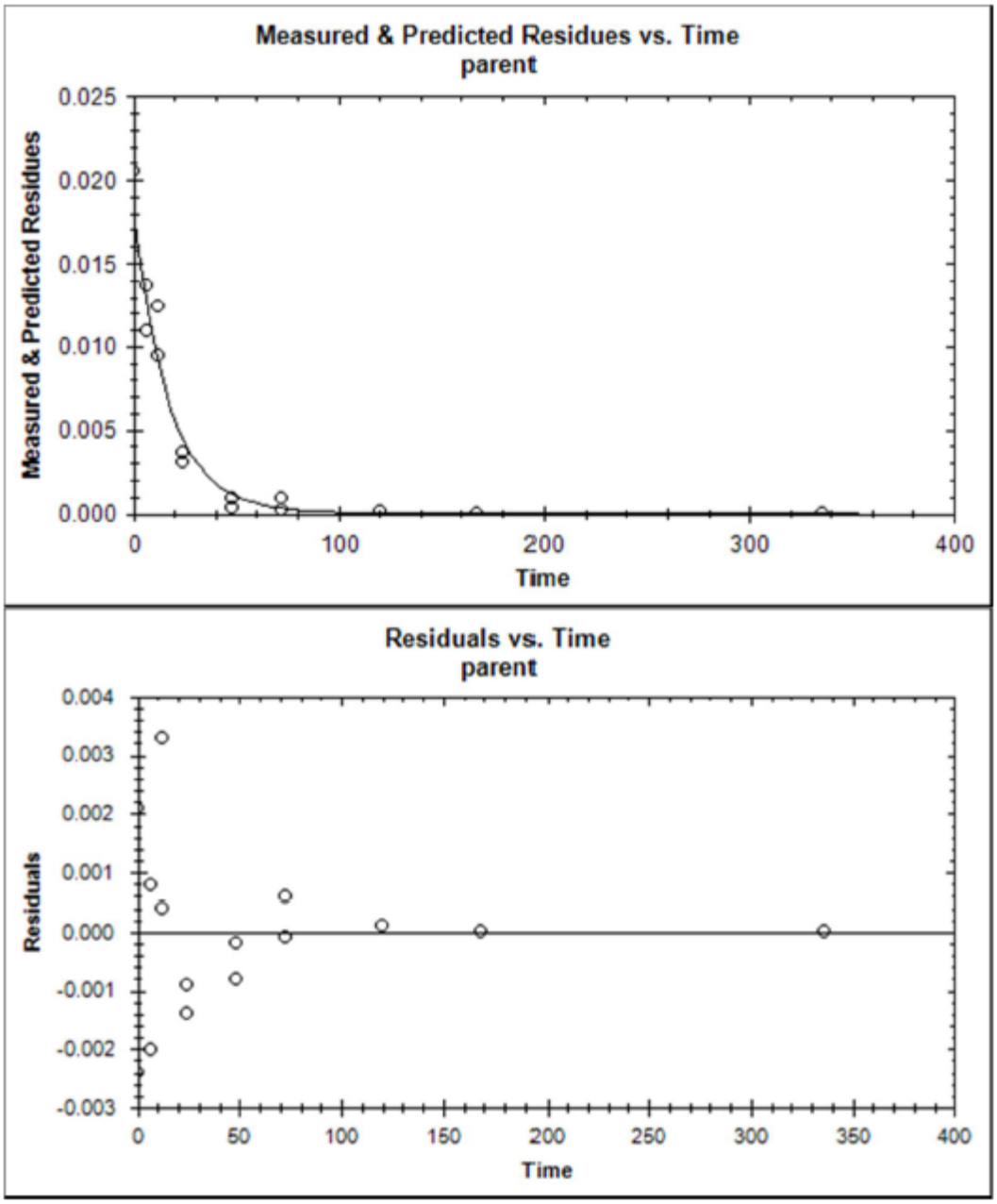
```
SFO
# KinGUII version : 2.2012.320.1629
# Project:    S14-01454
# Testsystem:no weights
# Comment:    GA4 2.1
# ================================
# Results of the kinetic evaluation
# ================================
# --------------------------------
# Initial values
# --------------------------------
                          Initial Value    Lower Bound      Upper Bound      Fixed
 M(0)  parent         :             0.1              0              Inf      False
 k     parent         :             0.1              0              Inf      False
# --------------------------------
# Chi2 error estimation
# --------------------------------
                                 parent          All
  Number of data sets :               9            9
 Number of parameters :               2            2
   Degrees of Freedom :               7            7

                                 parent          All
                RMSE :         0.001802     0.001802
            Chi2Sigma :         0.001255     0.001255
             Chi2Err% :            7.184        7.184
# --------------------------------
# Parameter estimation
# --------------------------------
  Degrees of Freedom : 16
  Parameter          Estimate     Lower CI     Upper CI       St.Dev         Prob > t
 M(0)  parent:       0.037852     0.035589        0.040     0.001154          < 2e-16
 k     parent:       0.018852     0.014426        0.023     0.002259         1.59e-07
# --------------------------------
# DT50 and DT90 values
# --------------------------------
                                 parent
                DT50 :           36.767
                DT90 :           122.14
        Kinetic model :              SFO
# --------------------------------
# Additional Statistics
# --------------------------------
# Correlation and R-square of predicted vs. measured data
                                 parent          All
                  EF :           0.9828       0.9828
           R-square :            0.9829       0.9829
# Correlation matrix of parameters
                          M(0) parent    k parent
 M(0)  parent         :           1.0000       0.7437
 k     parent         :           0.7437       1.0000
# --------------------------------
# Measured vs. predicted values
```


Measured & Predicted Residues vs. Time
parent
Measured & Predicted Residues
0.05
0.04
0.03
0.02
0.01
0.00
0
100
200
300
400
Time


Residuals vs. Time
parent
Residuals
0.006
0.005
0.004
0.003
0.002
0.001
0.000
-0.001
-0.002
-0.003
-0.004
0
50
100
150
200
250
300
350
400
Time

Single First Order (SFO) for GA7

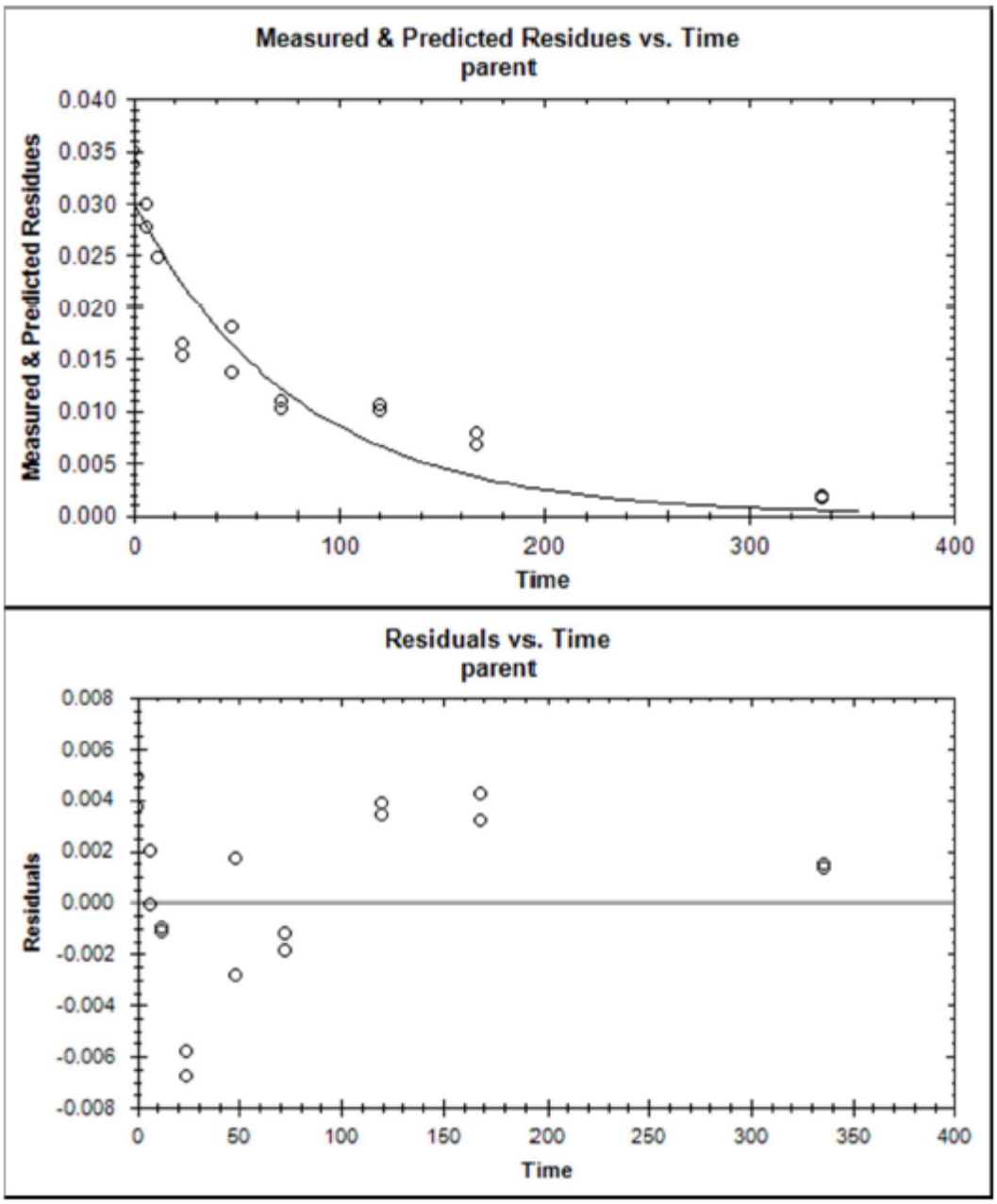
```
SFO
# KinGUII version : 2.2012.320.1629
# Project:    S14-01454
# Testsystem:no weights
# Comment:    GA7 2.1
# ================================
# Results of the kinetic evaluation
# ================================
# --------------------------------
# Initial values
# --------------------------------
                        Initial Value     Lower Bound       Upper Bound       Fixed
 M(0)  parent         :            0.1               0               Inf       False
 k     parent         :            0.1               0               Inf       False
# --------------------------------
# Chi2 error estimation
# --------------------------------
                                 parent          All
  Number of data sets :               9            9
 Number of parameters :               2            2
   Degrees of Freedom :               7            7
                                 parent          All
                 RMSE :       0.001287     0.001287
            Chi2Sigma :       0.000626     0.000626
             Chi2Err% :          12.22        12.22
# --------------------------------
# Parameter estimation
# --------------------------------
  Degrees of Freedom : 16
  Parameter         Estimate      Lower CI      Upper CI        St.Dev         Prob > t
 M(0)  parent:      0.0183813     0.0166906          0.02     0.0008626         1.80e-13
 k     parent:      0.0583991     0.0463391          0.07     0.0061532         2.83e-08
# --------------------------------
# DT50 and DT90 values
# --------------------------------
                                 parent
                 DT50 :          11.869
                 DT90 :          39.428
        Kinetic model :             SFO
# --------------------------------
# Additional Statistics
# --------------------------------
# Correlation and R-square of predicted vs. measured data
                                 parent          All
                    EF :         0.9617       0.9617
              R-square :         0.9617       0.9617
# Correlation matrix of parameters
                          M(0) parent    k parent
 M(0)  parent         :          1.0000       0.5028
 k     parent         :          0.5028       1.0000
# --------------------------------
# Measured vs. predicted values
# --------------------------------
```


Measured & Predicted Residues vs. Time
parent
Measured & Predicted Residues
0.025
0.020
0.015
0.010
0.005
0.000
0
100
200
300
400
Time
Residuals vs. Time
parent
Residuals
0.004
0.003
0.002
0.001
0.000
-0.001
-0.002
-0.003
0
50
100
150
200
250
300
350
400
Time

2.) Kinetic for Degradation of the Test Items in Soil LUFA 2.2

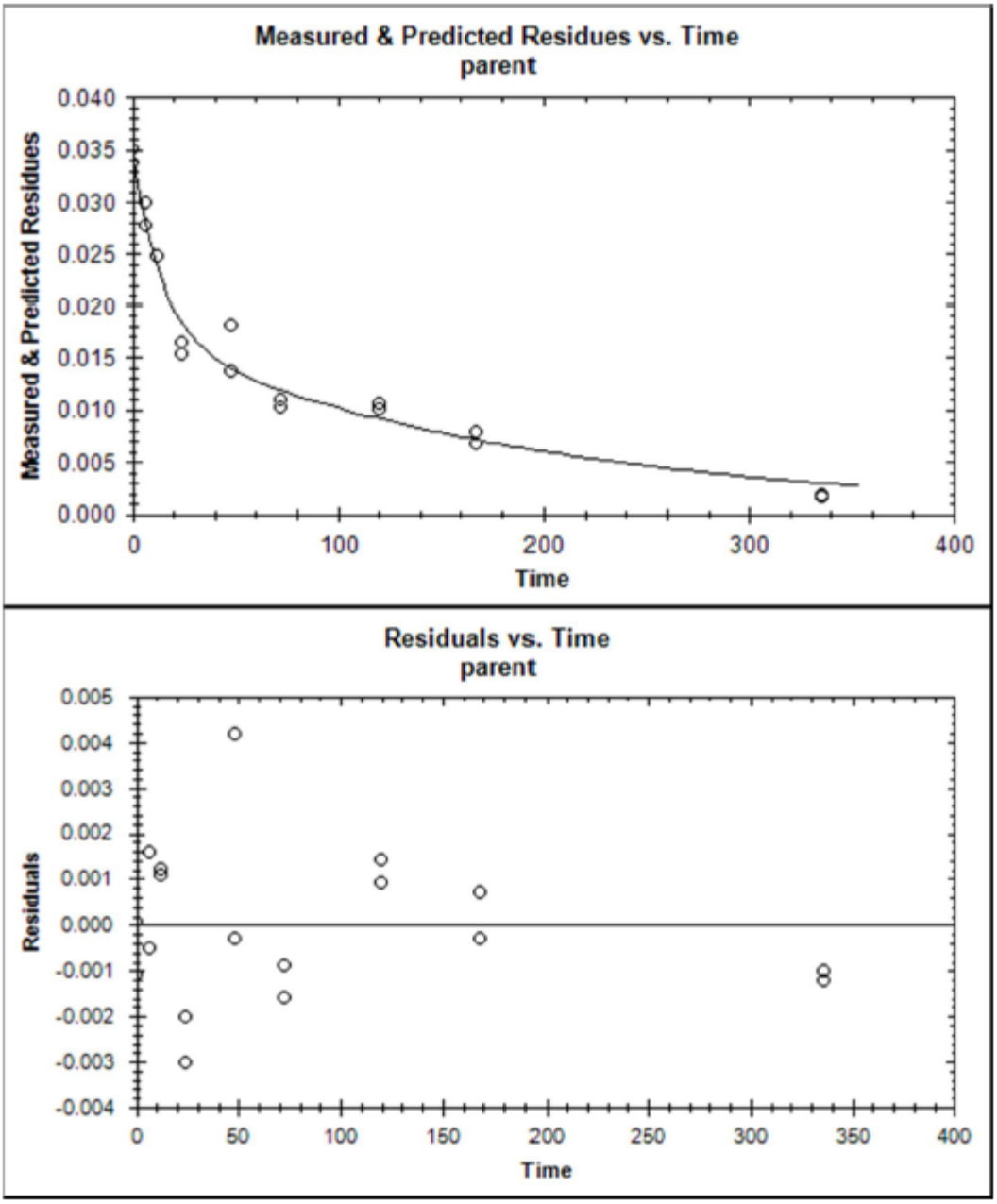
Single First Order (SFO) for GA4

```
SFO
# KinGUI version : 2.2012.320.1629
# Project:   S14-01454
# Testsystem:no weights
# Comment:   GA4 2.2
# ================================
# Results of the kinetic evaluation
# ================================
# --------------------------------
# Initial values
# --------------------------------
                        Initial Value    Lower Bound      Upper Bound      Fixed
 M(0)  parent         :           0.1              0              Inf      False
 k     parent         :           0.1              0              Inf      False
# --------------------------------
# Chi2 error estimation
# --------------------------------
                                parent          All
  Number of data sets :              9            9
 Number of parameters :              2            2
   Degrees of Freedom :              7            7
                                parent          All
                 RMSE :      0.003321     0.003321
            Chi2Sigma :      0.002558     0.002558
             Chi2Err% :         15.37        15.37
# --------------------------------
# Parameter estimation
# --------------------------------
  Degrees of Freedom : 16
  Parameter          Estimate     Lower CI     Upper CI       St.Dev        Prob > t
 M(0)  parent:       0.030087     0.026675        0.033     0.001741        4.48e-12
 k     parent:       0.012634     0.007544        0.018     0.002597        8.60e-05
# --------------------------------
# DT50 and DT90 values
# --------------------------------
                                parent
                 DT50 :         54.864
                 DT90 :         182.25
        Kinetic model :            SFO
# --------------------------------
# Additional Statistics
# --------------------------------
# Correlation and R-square of predicted vs. measured data
                                parent          All
                     EF :       0.8928       0.8928
              R-square :       0.9012       0.9012
# Correlation matrix of parameters
                          M(0) parent    k parent
 M(0)  parent         :       1.0000       0.6324
 k     parent         :       0.6324       1.0000
# --------------------------------
# Measured vs. predicted values
# --------------------------------
```


Measured & Predicted Residues vs. Time
parent
Measured & Predicted Residues
0.040
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
0
100
200
300
400
Time
Residuals vs. Time
parent
Residuals
0.008
0.006
0.004
0.002
0.000
-0.002
-0.004
-0.006
-0.008
0
50
100
150
200
250
300
350
400
Time

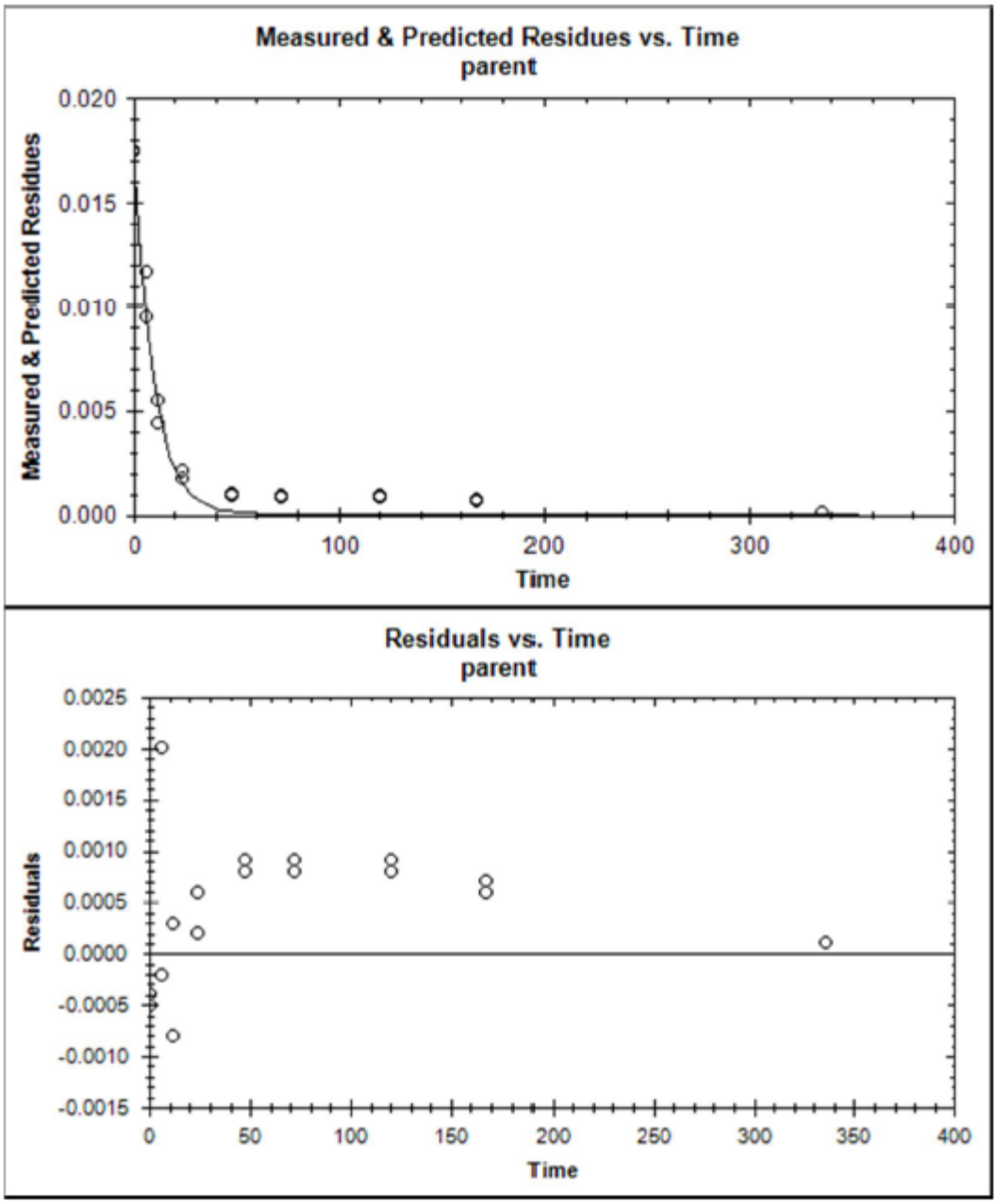
Double First Order (DFOP) for GA4

```
DFOP
# KinGUII version : 2.2012.320.1629
# Project:    S14-01454
# Testsystem:no weights
# Comment:    GA4 2.2
# ================================
# Results of the kinetic evaluation
# ================================
# --------------------------------
# Initial values
# --------------------------------
                          Initial Value    Lower Bound      Upper Bound      Fixed
 M(0)   parent         :           0.10              0              Inf      False
 k1     parent         :           0.10              0              Inf      False
 k2     parent         :           0.01              0              Inf      False
 g      parent         :           0.50              0                1      False
# --------------------------------
# Chi2 error estimation
# --------------------------------
                                 parent          All
  Number of data sets :               9            9
 Number of parameters :               4            4
   Degrees of Freedom :               5            5
                                 parent          All
                 RMSE :       0.001609     0.001609
            Chi2Sigma :       0.001205     0.001205
             Chi2Err% :          7.243        7.243
# --------------------------------
# Parameter estimation
# --------------------------------
  Degrees of Freedom : 14
  Parameter          Estimate     Lower CI      Upper CI        St.Dev         Prob > t
 M(0)   parent:      0.0349353    0.0325931         0.037    0.0011950         2.98e-14
 k1     parent:      0.0702271    0.0471983         0.093    0.0117496         1.69e-05
 k2     parent:      0.0052527    0.0040996         0.006    0.0005884         1.87e-07
 g      parent:      0.5109899    0.4920404         0.530    0.0096683          < 2e-16
# --------------------------------
# DT50 and DT90 values
# --------------------------------
                                 parent
                 DT50 :          27.142
                 DT90 :          302.17
        Kinetic model :            DFOP
# --------------------------------
# Additional Statistics
# --------------------------------
# Correlation and R-square of predicted vs. measured data
                                 parent          All
                   EF :          0.9748       0.9748
             R-square :          0.9749       0.9749
# Correlation matrix of parameters
                          M(0) parent    k1 parent    k2 parent     g parent
 M(0)   parent         :          1.0000      0.65634      0.21782       0.6553
 k1     parent         :          0.6563      1.00000     -0.09483       0.9138
 k2     parent         :          0.2178     -0.09483      1.00000      -0.1300
 g      parent         :          0.6553      0.91381     -0.12997       1.0000
# --------------------------------
# Measured vs. predicted values
# --------------------------------
```


Measured & Predicted Residues vs. Time
parent
Measured & Predicted Residues
0.040
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
0
100
200
300
400
Time
Residuals vs. Time
parent
Residuals
0.005
0.004
0.003
0.002
0.001
0.000
-0.001
-0.002
-0.003
-0.004
0
50
100
150
200
250
300
350
400
Time

Single First Order (SFO) for GA7

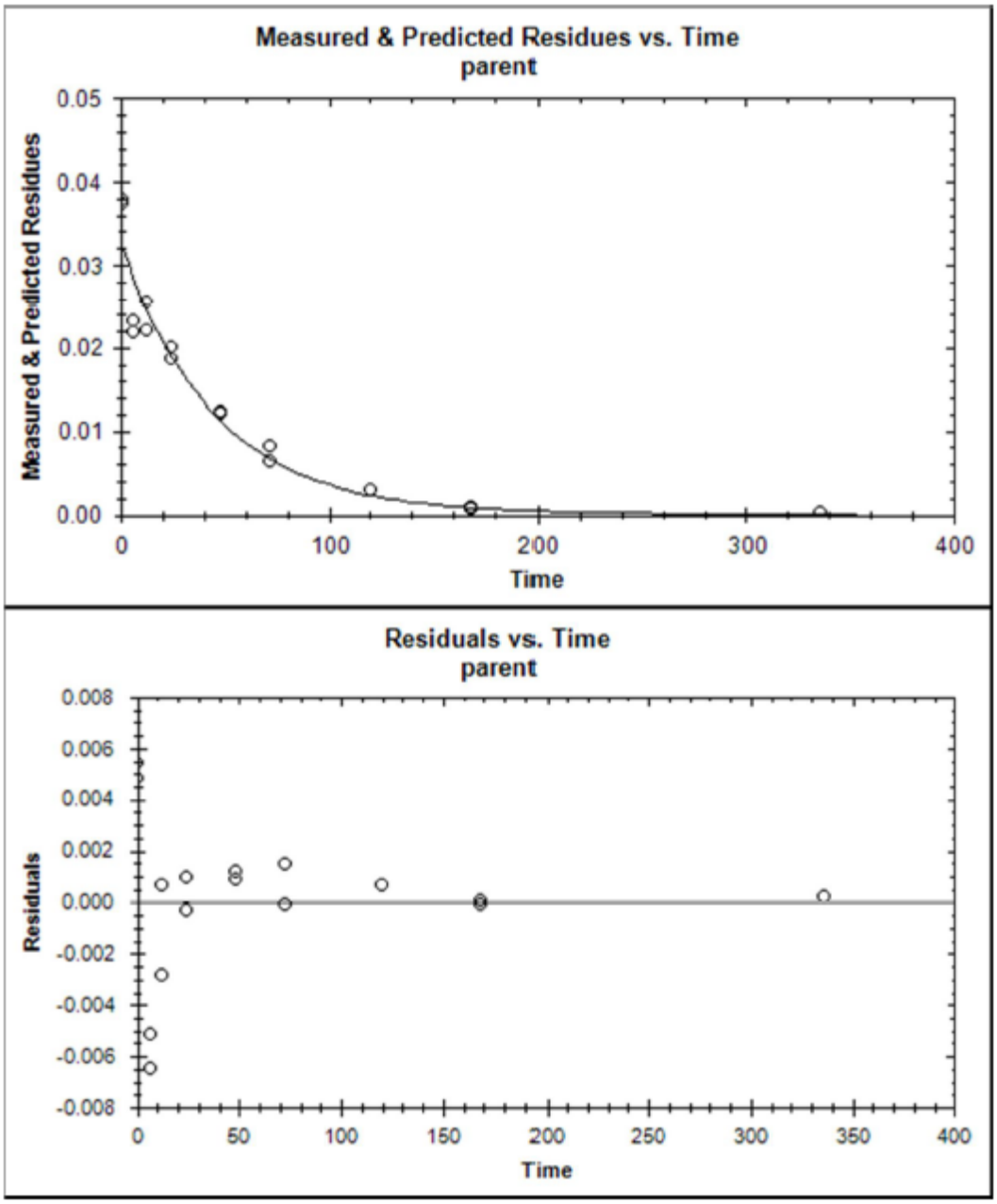
```
SFO
# KinGUII version : 2.2012.320.1629
# Project:    S14-01454
# Testsystem:no weights
# Comment:    GA7 2.2
# ================================
# Results of the kinetic evaluation
# ================================
# --------------------------------
# Initial values
# --------------------------------
                         Initial Value    Lower Bound      Upper Bound      Fixed
 M(0)  parent          :           0.1              0              Inf      False
 k     parent          :           0.1              0              Inf      False
# --------------------------------
# Chi2 error estimation
# --------------------------------
                                 parent          All
  Number of data sets :               9            9
 Number of parameters :               2            2
   Degrees of Freedom :               7            7
                                 parent          All
                RMSE :     0.0007739   0.0007739
             Chi2Sigma :     0.0005215   0.0005215
              Chi2Err% :         12.25       12.25
# --------------------------------
# Parameter estimation
# --------------------------------
  Degrees of Freedom : 16
  Parameter        Estimate     Lower CI     Upper CI       St.Dev        Prob > t
 M(0)  parent:     0.0178968    0.0169114        0.019    0.0005028          < 2e-16
 k     parent:     0.1027186    0.1005493        0.105    0.0011068          < 2e-16
# --------------------------------
# DT50 and DT90 values
# --------------------------------
                                 parent
                  DT50 :         6.7480
                  DT90 :         22.416
         Kinetic model :            SFO
# --------------------------------
# Additional Statistics
# --------------------------------
# Correlation and R-square of predicted vs. measured data
                                 parent          All
                    EF :         0.9812       0.9812
               R-square :         0.9893       0.9893
# Correlation matrix of parameters
                           M(0) parent    k parent
 M(0)  parent          :         1.0000       0.2323
 k     parent          :         0.2323       1.0000
# --------------------------------
# Measured vs. predicted values
# --------------------------------
```


Measured & Predicted Residues vs. Time
parent
Measured & Predicted Residues
0.020
0.015
0.010
0.005
0.000
0
100
200
300
400
Time
Residuals vs. Time
parent
Residuals
0.0025
0.0020
0.0015
0.0010
0.0005
0.0000
-0.0005
-0.0010
-0.0015
0
50
100
150
200
250
300
350
400
Time

3.) Kinetic for Degradation of the Test Items in Soil LUFA 2.3

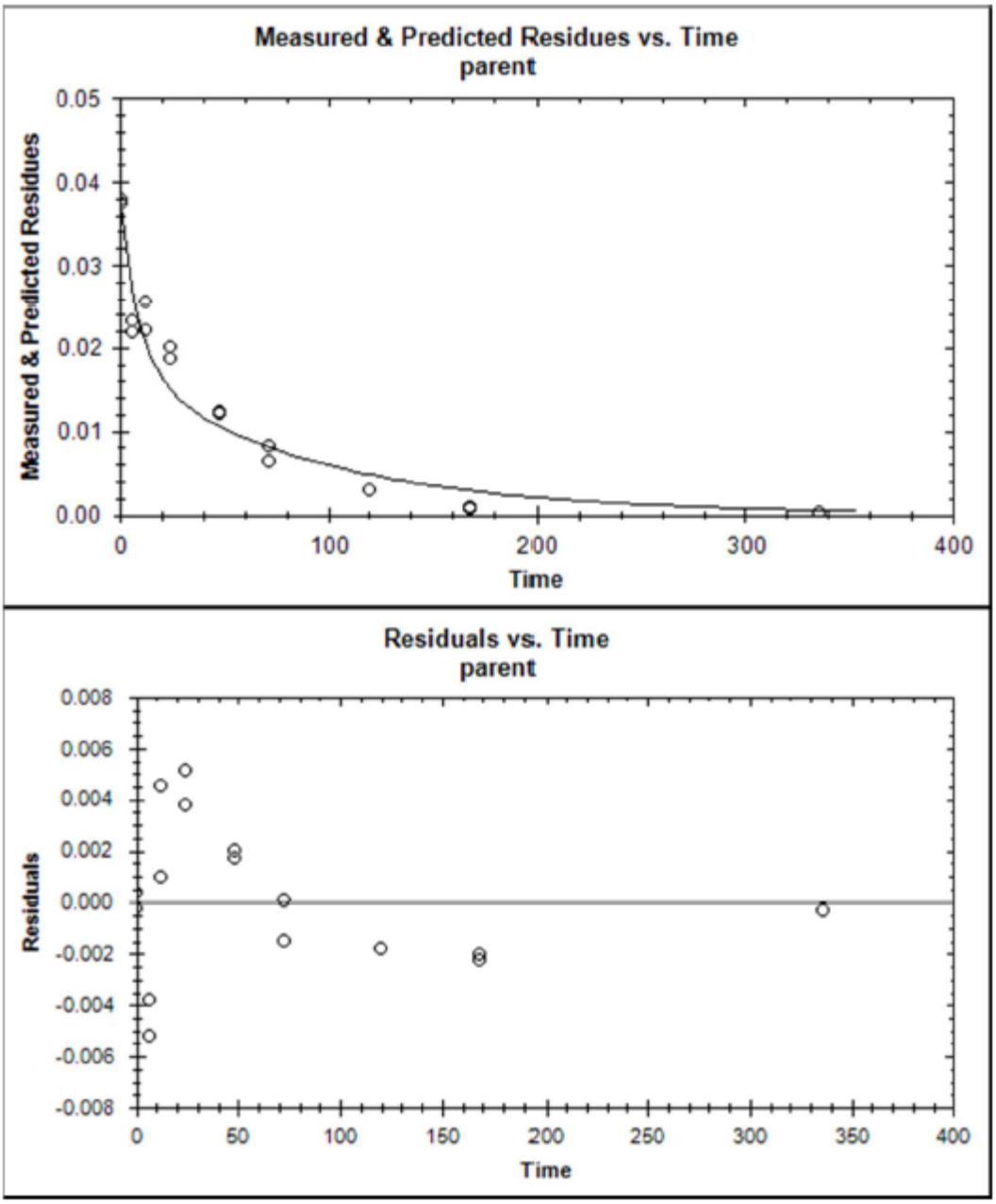
Single First Order (SFO) for GA4

```
SFO
# KinGUI version : 2.2012.320.1629
# Project:   S14-01454
# Testsystem:no weights
# Comment:   GA4 2.3
# ================================
# Results of the kinetic evaluation
# ================================
# --------------------------------
# Initial values
# --------------------------------
                       Initial Value    Lower Bound      Upper Bound      Fixed
 M(0)  parent         :           0.1              0              Inf      False
 k     parent         :           0.1              0              Inf      False
# --------------------------------
# Chi2 error estimation
# --------------------------------
                                parent          All
  Number of data sets :              9            9
 Number of parameters :              2            2
   Degrees of Freedom :              7            7
                                parent          All
                 RMSE :       0.002757     0.002757
            Chi2Sigma :       0.002127     0.002127
             Chi2Err% :          15.07        15.07
# --------------------------------
# Parameter estimation
# --------------------------------
  Degrees of Freedom : 16
  Parameter        Estimate     Lower CI     Upper CI       St.Dev        Prob > t
 M(0)  parent:      0.032494     0.029580        0.035     0.001487        1.21e-13
 k     parent:      0.022223     0.016870        0.028     0.002731        2.22e-07
# --------------------------------
# DT50 and DT90 values
# --------------------------------
                                parent
                 DT50 :         31.190
                 DT90 :         103.61
       Kinetic model :            SFO
# --------------------------------
# Additional Statistics
# --------------------------------
# Correlation and R-square of predicted vs. measured data
                                parent          All
                   EF :         0.9472       0.9472
            R-square :         0.9476       0.9476
# Correlation matrix of parameters
                         M(0) parent     k parent
 M(0)  parent         :         1.0000       0.5712
 k     parent         :         0.5712       1.0000
# --------------------------------
# Measured vs. predicted values
# --------------------------------
```


Measured & Predicted Residues vs. Time
parent
Measured & Predicted Residues
0.05
0.04
0.03
0.02
0.01
0.00
0
100
200
300
400
Time
Residuals vs. Time
parent
Residuals
0.008
0.006
0.004
0.002
0.000
-0.002
-0.004
-0.006
-0.008
0
50
100
150
200
250
300
350
400
Time

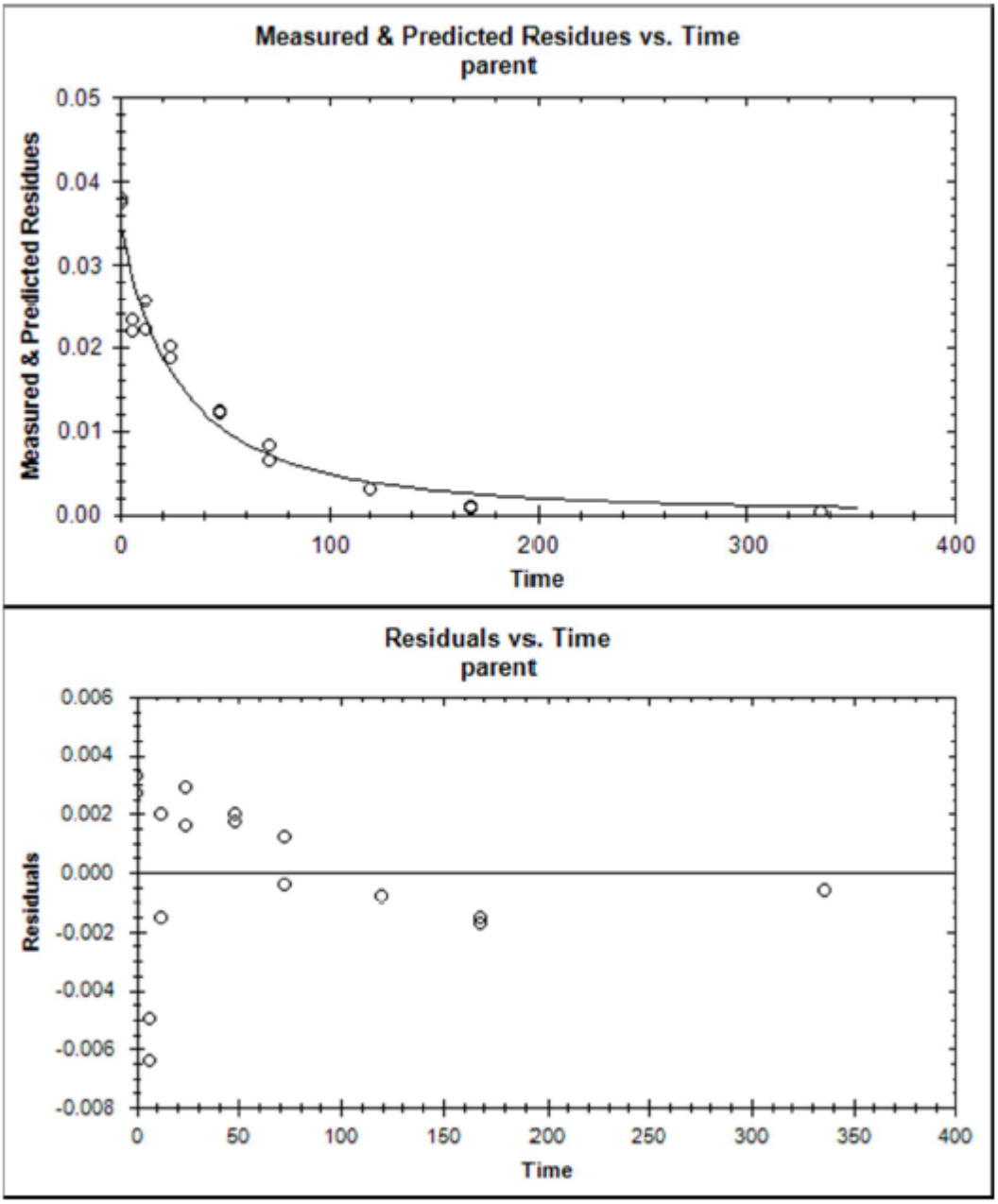
Double First Order (DFOP) for GA4

```
DFOP
# KinGUII version : 2.2012.320.1629
# Project:    S14-01454
# Testsystem:no weights
# Comment:    GA4 2.3
# ================================
# Results of the kinetic evaluation
# ================================
# --------------------------------
# Initial values
# --------------------------------
                          Initial Value    Lower Bound      Upper Bound      Fixed
 M(0)   parent          :          0.10              0              Inf      False
 k1     parent          :          0.10              0              Inf      False
 k2     parent          :          0.01              0              Inf      False
 g      parent          :          0.50              0                1      False
# --------------------------------
# Chi2 error estimation
# --------------------------------
                                 parent          All
  Number of data sets :               9            9
 Number of parameters :               4            4
   Degrees of Freedom :               5            5
                                 parent          All
                 RMSE :       0.002660     0.002660
            Chi2Sigma :       0.002307     0.002307
             Chi2Err% :         16.34        16.34
# --------------------------------
# Parameter estimation
# --------------------------------
  Degrees of Freedom : 14
  Parameter          Estimate     Lower CI     Upper CI        St.Dev        Prob > t
 M(0)   parent:       0.037468     0.032532        0.042      0.002518        2.84e-10
 k1     parent:       0.101701     0.093158        0.110      0.004359        6.59e-13
 k2     parent:       0.010656     0.002685        0.019      0.004067          0.0101
 g      parent:       0.543239     0.531180        0.555      0.006153          < 2e-16
# --------------------------------
# DT50 and DT90 values
# --------------------------------
                                 parent
                 DT50 :          15.469
                 DT90 :          142.55
         Kinetic model :            DFOP
# --------------------------------
# Additional Statistics
# --------------------------------
# Correlation and R-square of predicted vs. measured data
                                 parent          All
                   EF :          0.9509       0.9509
              R-square :           0.951        0.951
# Correlation matrix of parameters
                          M(0) parent    k1 parent    k2 parent     g parent
 M(0)   parent          :        1.0000       0.1270       0.7801      -0.1524
 k1     parent          :        0.1270       1.0000      -0.1015       0.1013
 k2     parent          :        0.7801      -0.1015       1.0000      -0.3654
 g      parent          :       -0.1524       0.1013      -0.3654       1.0000
# --------------------------------
# Measured vs. predicted values
# --------------------------------
```


Measured & Predicted Residues vs. Time
parent
Measured & Predicted Residues
0.05
0.04
0.03
0.02
0.01
0.00
0
100
200
300
400
Time
Residuals vs. Time
parent
Residuals
0.008
0.006
0.004
0.002
0.000
-0.002
-0.004
-0.006
-0.008
0
50
100
150
200
250
300
350
400
Time

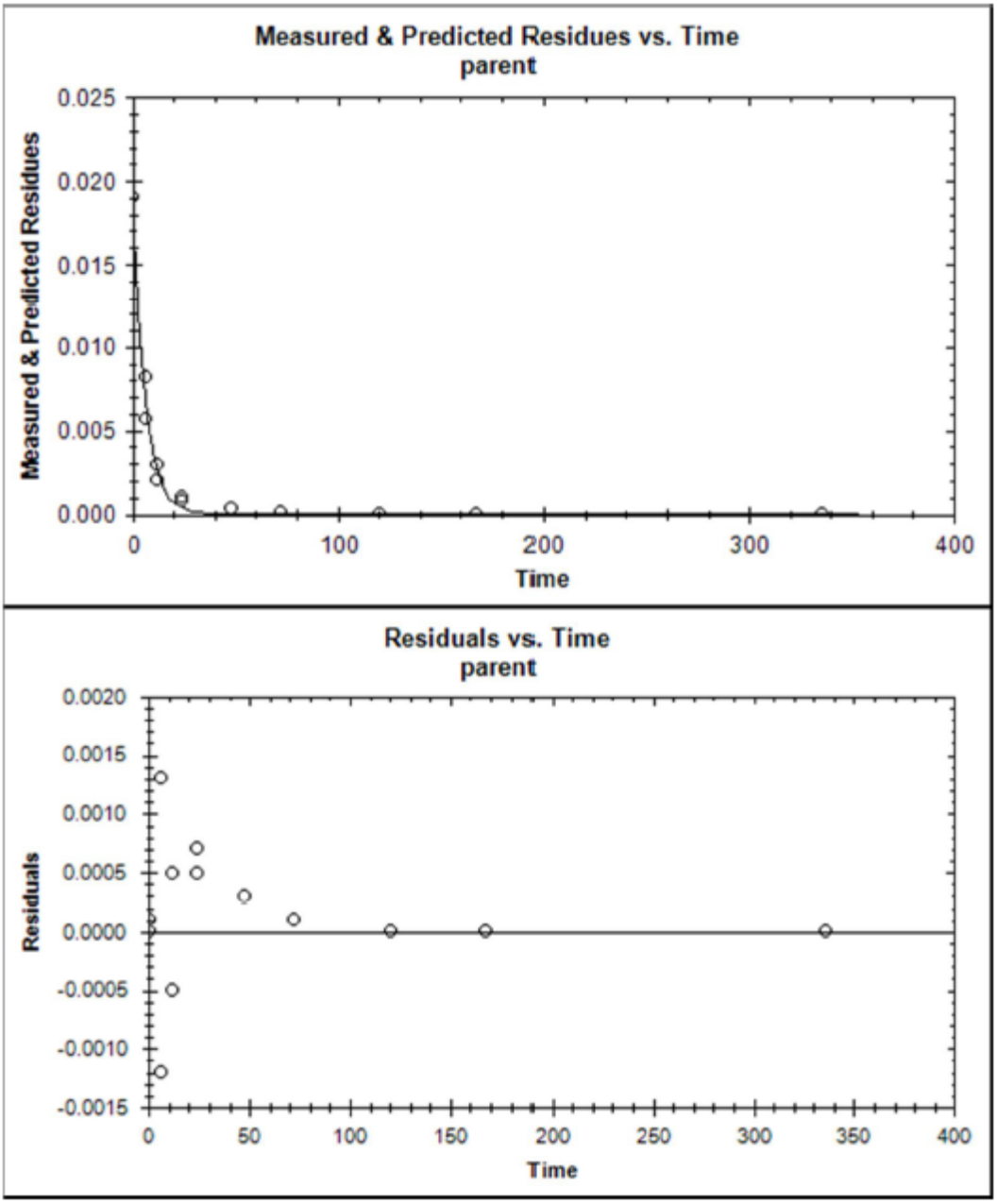
First Order Multiple Compartment (FOMC) for GA4

```
FMOC
# KinGUII version : 2.2012.320.1629
# Project:    S14-01454
# Testsystem:no weights
# Comment:    GA4 2.3
# ================================
# Results of the kinetic evaluation
# ================================
# --------------------------------
# Initial values
# --------------------------------
                        Initial Value    Lower Bound      Upper Bound      Fixed
 M(0)  parent         :           0.1              0              Inf      False
 alpha parent         :           0.1              0              Inf      False
 beta  parent         :           0.1              0              Inf      False
# --------------------------------
# Chi2 error estimation
# --------------------------------
                                parent          All
  Number of data sets :              9            9
 Number of parameters :              3            3
   Degrees of Freedom :              6            6
                                parent          All
                 RMSE :       0.002554     0.002554
            Chi2Sigma :       0.002070     0.002070
             Chi2Err% :          14.67        14.67
# --------------------------------
# Parameter estimation
# --------------------------------
  Degrees of Freedom : 15
  Parameter          Estimate     Lower CI     Upper CI       St.Dev         Prob > t
 M(0)  parent:       0.034630     0.030192        0.039     0.002264         7.37e-11
 alpha parent:       1.866065     0.858024        2.874     0.514316          0.00124
 beta  parent:      52.751618     7.504272       97.999    23.085804          0.01864
# --------------------------------
# DT50 and DT90 values
# --------------------------------
                                parent
                 DT50 :         23.729
                 DT90 :         128.43
        Kinetic model :           FOMC
# --------------------------------
# Additional Statistics
# --------------------------------
# Correlation and R-square of predicted vs. measured data
                                parent          All
                    EF :         0.9547       0.9547
             R-square :          0.955        0.955
# Correlation matrix of parameters
                          M(0) parent alpha parent  beta parent
 M(0)  parent         :          1.0000       -0.6942      -0.6952
 alpha parent         :         -0.6942        1.0000       0.9849
 beta  parent         :         -0.6952        0.9849       1.0000
# --------------------------------
# Measured vs. predicted values
# --------------------------------
```


Measured & Predicted Residues vs. Time
parent
Measured & Predicted Residues
0.05
0.04
0.03
0.02
0.01
0.00
0
100
200
300
400
Time
Residuals vs. Time
parent
Residuals
0.006
0.004
0.002
0.000
-0.002
-0.004
-0.006
-0.008
0
50
100
150
200
250
300
350
400
Time

Single First Order (SFO) for GA7

```
SFO
# KinGUII version : 2.2012.320.1629
# Project:   S14-01454
# Testsystem:no weights
# Comment:   GA7 2.3
# ================================
# Results of the kinetic evaluation
# ================================
# --------------------------------
# Initial values
# --------------------------------
                        Initial Value    Lower Bound      Upper Bound      Fixed
 M(0)  parent         :           0.1              0              Inf      False
 k     parent         :           0.1              0              Inf      False
# --------------------------------
# Chi2 error estimation
# --------------------------------
                                parent          All
  Number of data sets :              9            9
 Number of parameters :              2            2
   Degrees of Freedom :              7            7
                                parent          All
                 RMSE :    0.0004992    0.0004992
             Chi2Sigma :    0.0001723    0.0001723
              Chi2Err% :        5.203        5.203
# --------------------------------
# Parameter estimation
# --------------------------------
  Degrees of Freedom : 16
  Parameter          Estimate     Lower CI     Upper CI       St.Dev         Prob > t
 M(0)  parent:     0.0189922    0.0183038         0.02    0.0003512          < 2e-16
 k     parent:     0.1679265    0.1554456         0.18    0.0063679         6.49e-15
# --------------------------------
# DT50 and DT90 values
# --------------------------------
                                parent
                  DT50 :        4.1277
                  DT90 :        13.712
         Kinetic model :           SFO
# --------------------------------
# Additional Statistics
# --------------------------------
# Correlation and R-square of predicted vs. measured data
                                parent          All
                    EF :         0.993        0.993
             R-square :        0.9934       0.9934

# Correlation matrix of parameters
                          M(0) parent    k parent
 M(0)  parent         :        1.0000       0.6895
 k     parent         :        0.6895       1.0000
# --------------------------------
# Measured vs. predicted values
# --------------------------------
```


Measured & Predicted Residues vs. Time
parent
Measured & Predicted Residues
0.025
0.020
0.015
0.010
0.005
0.000
0
100
200
300
400
Time
Residuals vs. Time
parent
Residuals
0.0020
0.0015
0.0010
0.0005
0.0000
-0.0005
-0.0010
-0.0015
0
50
100
150
200
250
300
350
400
Time

4.) Kinetic for Degradation of the Test Items in Soil LUFA 6S

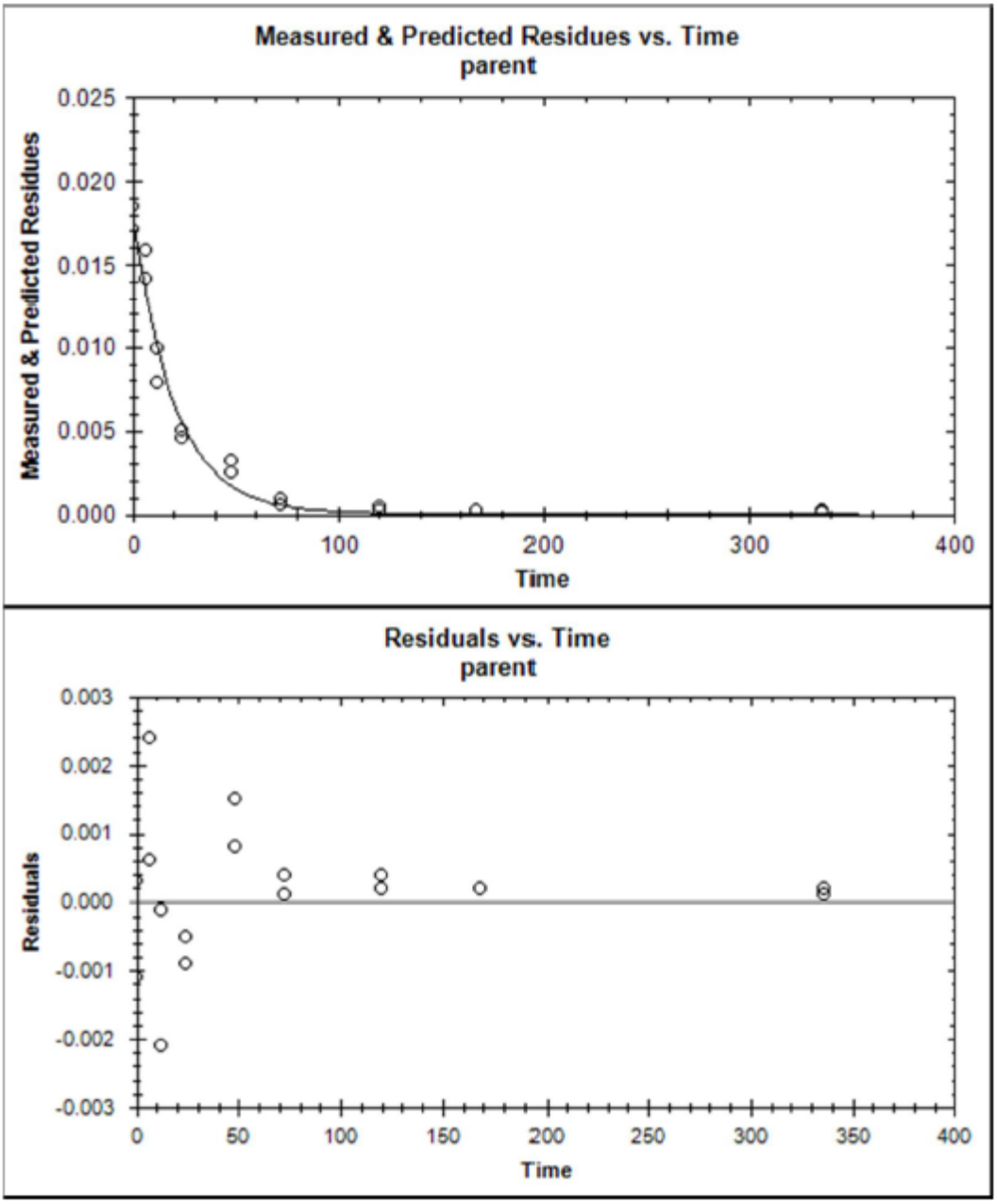
Single First Order (SFO) for GA4

```
SFO
# KinGUII version : 2.2012.320.1629
# Project:    S14-01454
# Testsystem:no weights
# Comment:    GA4 6S
# ================================
# Results of the kinetic evaluation
# ================================
# --------------------------------
# Initial values
# --------------------------------
                        Initial Value    Lower Bound      Upper Bound      Fixed
 M(0)  parent         :           0.1              0              Inf      False
 k     parent         :           0.1              0              Inf      False
# --------------------------------
# Chi2 error estimation
# --------------------------------
                                parent          All
  Number of data sets :              9            9
 Number of parameters :              2            2
   Degrees of Freedom :              7            7
                                parent          All
                 RMSE :       0.002049     0.002049
            Chi2Sigma :       0.000747     0.000747
             Chi2Err% :          5.582        5.582
# --------------------------------
# Parameter estimation
# --------------------------------
  Degrees of Freedom : 16
  Parameter          Estimate      Lower CI      Upper CI        St.Dev         Prob > t
 M(0)  parent:       0.033193      0.030931         0.035      0.001154         1.67e-15
 k     parent:       0.026518      0.021772         0.031      0.002422         3.83e-09
# --------------------------------
# DT50 and DT90 values
# --------------------------------
                                parent
                 DT50 :         26.138
                 DT90 :         86.830
        Kinetic model :            SFO
# --------------------------------
# Additional Statistics
# --------------------------------
# Correlation and R-square of predicted vs. measured data
                                parent          All
                     EF :        0.9717       0.9717
              R-square :         0.972        0.972
# Correlation matrix of parameters
                         M(0) parent    k parent
 M(0)  parent         :        1.0000       0.5506
 k     parent         :        0.5506       1.0000
# --------------------------------
# Measured vs. predicted values
# --------------------------------
```

Single First Order (SFO) for GA7

SFO

```
# KinGUI version : 2.2012.320.1629
# Project:   S14-01454
# Testsystem:no weights
# Comment:   GA7 6S
# ================================
# Results of the kinetic evaluation
# ================================
# --------------------------------
# Initial values
# --------------------------------
                        Initial Value    Lower Bound      Upper Bound      Fixed
 M(0)  parent         :           0.1              0              Inf      False
 k     parent         :           0.1              0              Inf      False
# --------------------------------
# Chi2 error estimation
# --------------------------------
                               parent          All
  Number of data sets :             9            9
 Number of parameters :             2            2
   Degrees of Freedom :             7            7
                               parent          All
                 RMSE :    0.0009565    0.0009565
            Chi2Sigma :    0.0006386    0.0006386
             Chi2Err% :        11.32        11.32
# --------------------------------
# Parameter estimation
# --------------------------------
  Degrees of Freedom : 16
  Parameter                     Estimate     Lower CI     Upper CI       St.Dev
Prob > t
 M(0)  parent         :     0.0182047    0.0169625        0.019    0.0006338
1.70e-15
 k     parent         :     0.0498298    0.0415713        0.058    0.0042136
1.27e-09
# --------------------------------
# DT50 and DT90 values
# --------------------------------
                               parent
                 DT50 :        13.910
                 DT90 :        46.209
        Kinetic model :           SFO
# --------------------------------
# Additional Statistics
# --------------------------------
# Correlation and R-square of predicted vs. measured data
                               parent          All
                   EF :        0.9777       0.9777
            R-square :        0.9786       0.9786
# Correlation matrix of parameters
                         M(0) parent    k parent
 M(0)  parent         :        1.0000       0.5647
 k     parent         :        0.5647       1.0000
# --------------------------------
# Measured vs. predicted values
# --------------------------------
```


Measured & Predicted Residues vs. Time
parent
Measured & Predicted Residues
0.025
0.020
0.015
0.010
0.005
0.000
0
100
200
300
400
Time
Residuals vs. Time
parent
Residuals
0.003
0.002
0.001
0.000
-0.001
-0.002
-0.003
0
50
100
150
200
250
300
350
400
Time

Appendix 4: Kinetic fit Analysis of study; CA 7.2.2.3/01; Cooper, T. (2017) (Degradation in water-sediment systems under aerobic conditions

CAKE Kinetic Evaluation Report

Study: 3201605

Data set: Experiment 1 (SFO)

Group A Water

Study date: 04/07/2017
Report generated: 10/10/2017

Model Setup:

Topology: Parent only
Optimiser: IRLS (IRLS Its. 10, IRLS Tol. 1E-05, Max. Its. 100, Tol. 1E-05)
SANN Max Iterations: 10000
Use Extra Solver: True

Initial Values of Sequence Parameters:

Parameter	Initial Value	Bounds	Fixed
Parent_0	100	0 to (unbounded)	No
k_Parent	0.1	0 to (unbounded)	No

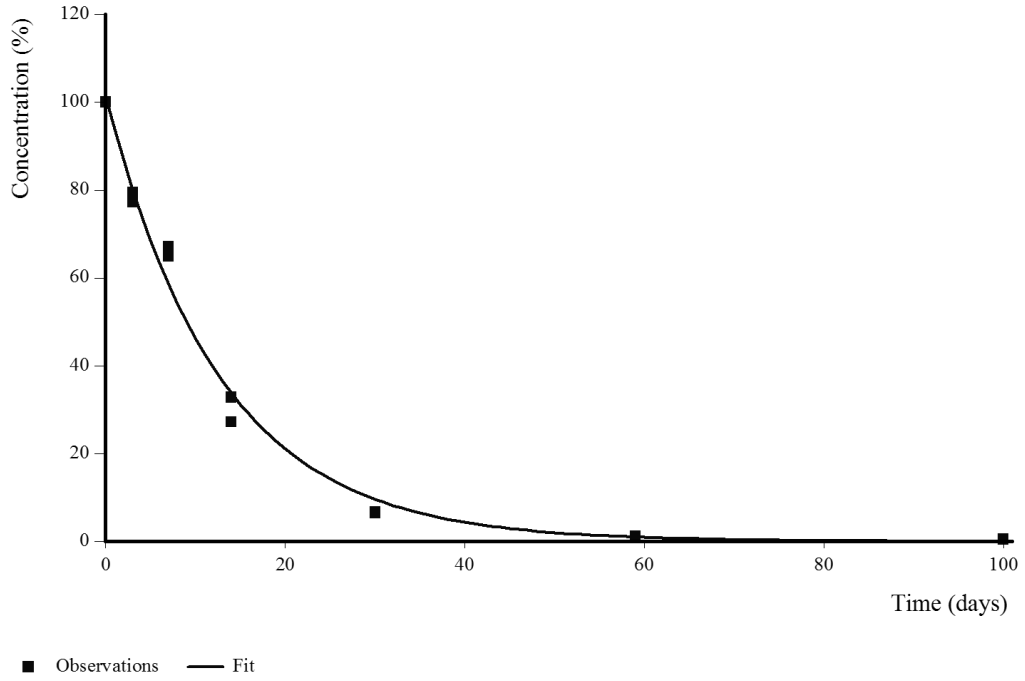
Fit step: Final

Reference Table:

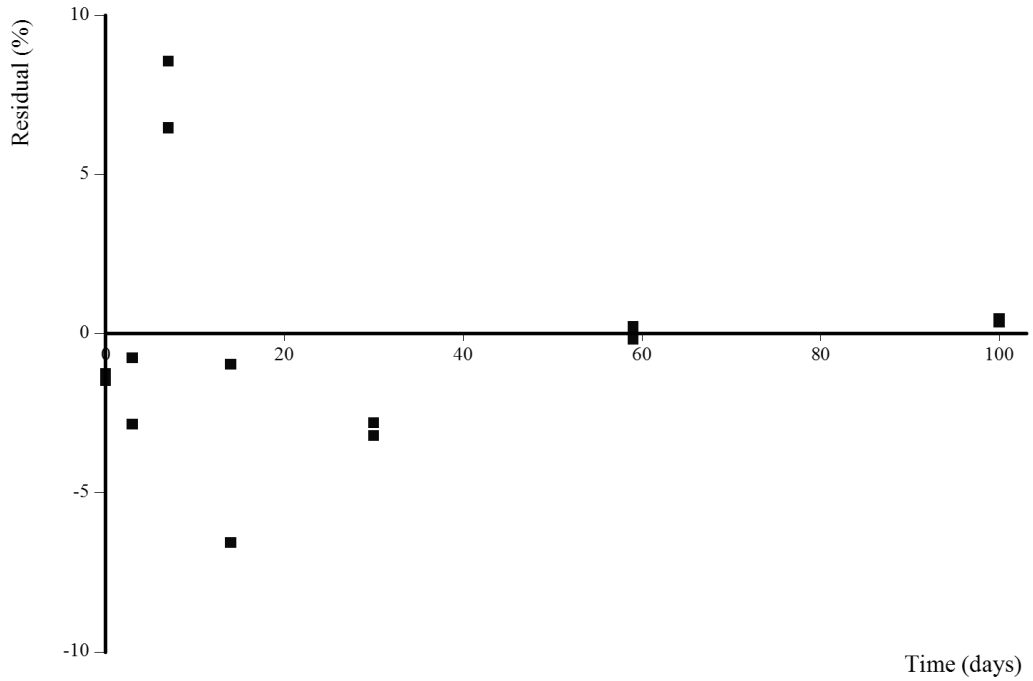
Compartment	Name
Parent	Parent

Graphical Summary:

Observations and Fitted Model:



Residuals:



Initial Values for This Step:

Parameter	Initial Value	Bounds	Fixed
Parent_0	100	0 to (unbounded)	No
k_Parent	0.1	0 to (unbounded)	No

Estimated Values:

Parameter	Value	σ	Prob. > t	Lower (90%) CI	Upper (90%) CI	Lower (95%) CI	Upper (95%) CI
Parent_0	101.5	2.375		97.24	105.7	96.29	106.6
k_Parent	0.07859	0.004538	3.72E-010				

χ^2

Parameter	Error %	Degrees of Freedom
All data	6.87	5
Parent	6.87	5

Decay Times:

Compartment	DT50 (days)	DT90 (days)
Parent	8.82	29.3

Additional Statistics:

Parameter	r² (Obs v Pred)	Efficiency
All data	0.9907	0.9906
Parent	0.9907	0.9906

Parameter Correlation:

	Parent_0	k_Parent
Parent_0	1	0.5585
k_Parent	0.5585	1

Observed v. Predicted:

Compartment Parent

Time (days)	Value (%)	Predicted Value	Residual
0	100	101.5	-1.469
0	100.2	101.5	-1.269
3	77.3	80.16	-2.857
3	79.4	80.16	-0.7573
7	67.1	58.54	8.564
7	65	58.54	6.464
14	27.2	33.77	-6.569
14	32.8	33.77	-0.9685
30	6.8	9.604	-2.804
30	6.4	9.604	-3.204
59	1.2	0.9832	0.2168
59	0.8	0.9832	-0.1832
100	0.4	0.0392	0.3608
100	0.5	0.0392	0.4608

Sequence Creation Information:
Fit generated by CAKE version 2.0 (Release)
running on R version 3.0.0 (2013-04-03)

Report Information:
Report generated by CAKE version 2.0 (Release)
CAKE developed by Tessella Ltd, Abingdon, Oxfordshire, UK for Syngenta
Running on .Net version 4.0.30319.18444

CAKE Kinetic Evaluation Report

Study: 3201605

Data set: Experiment 1 (SFO)

Group B Water

Study date: 04/07/2017
Report generated: 10/10/2017

Model Setup:

Topology: Parent only
Optimiser: IRLS (IRLS Its. 10, IRLS Tol. 1E-05, Max. Its. 100, Tol. 1E-05)
SANN Max Iterations: 10000
Use Extra Solver: True

Initial Values of Sequence Parameters:

Parameter	Initial Value	Bounds	Fixed
Parent_0	100	0 to (unbounded)	No
k_Parent	0.1	0 to (unbounded)	No

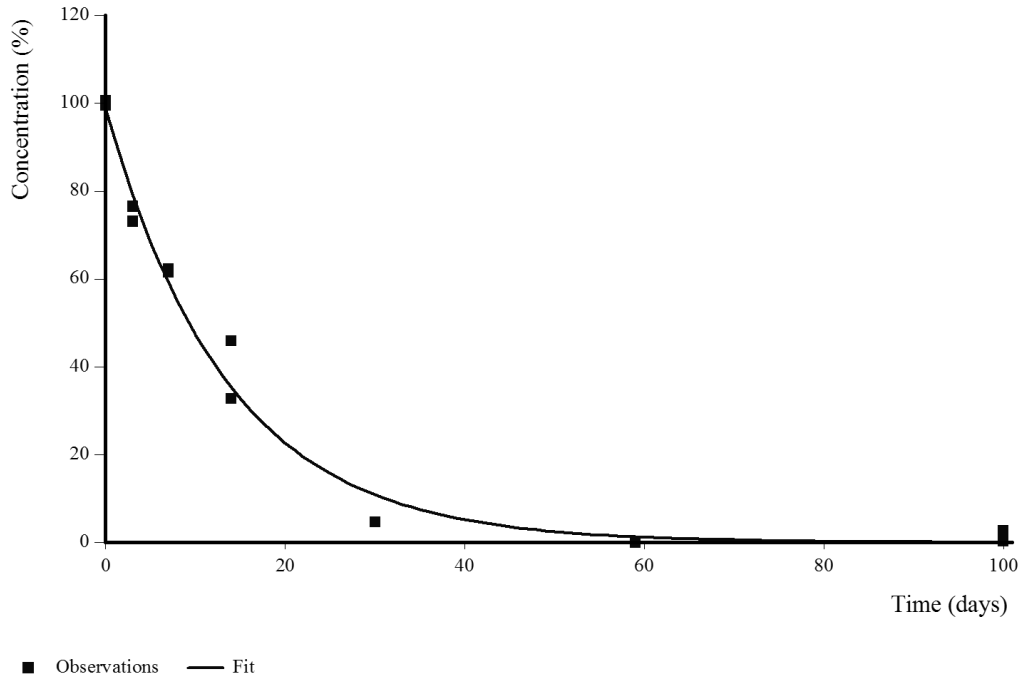
Fit step: Final

Reference Table:

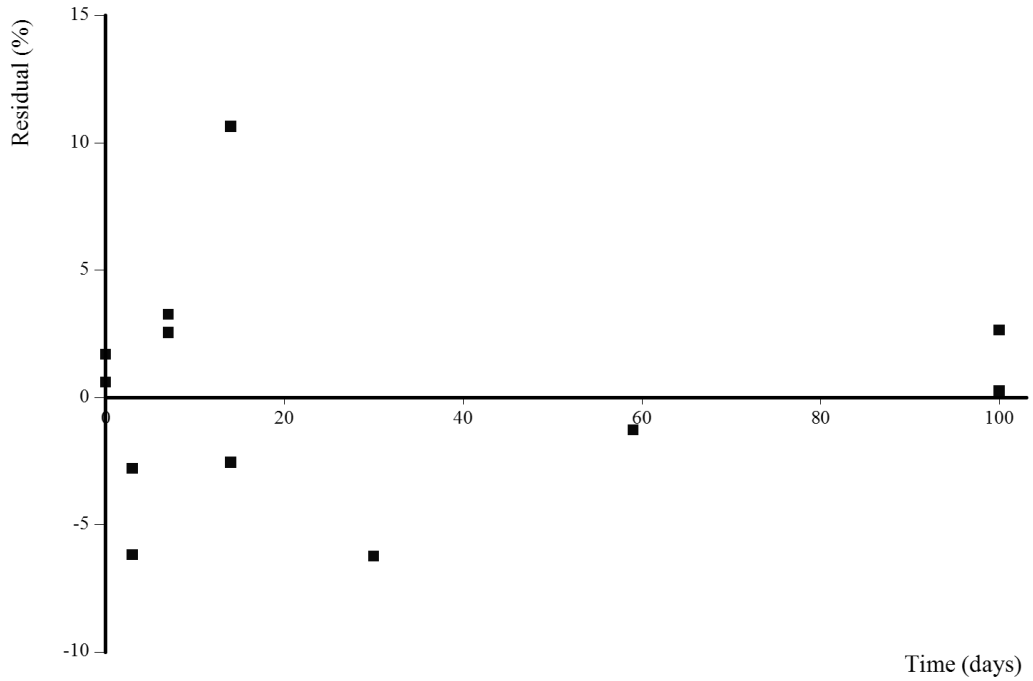
Compartment	Name
Parent	Parent

Graphical Summary:

Observations and Fitted Model:



Residuals:



Initial Values for This Step:

Parameter	Initial Value	Bounds	Fixed
Parent_0	100	0 to (unbounded)	No
k_Parent	0.1	0 to (unbounded)	No

Estimated Values:

Parameter	Value	σ	Prob. > t	Lower (90%) CI	Upper (90%) CI	Lower (95%) CI	Upper (95%) CI
Parent_0	98.91	2.778		93.96	103.9	92.86	105
k_Parent	0.07368	0.005237	4.04E-009				

χ^2

Parameter	Error %	Degrees of Freedom
All data	7.03	5
Parent	7.03	5

Decay Times:

Compartment	DT50 (days)	DT90 (days)
Parent	9.41	31.3

Additional Statistics:

Parameter	r² (Obs v Pred)	Efficiency
All data	0.986	0.9858
Parent	0.986	0.9858

Parameter Correlation:

	Parent_0	k_Parent
Parent_0	1	0.5722
k_Parent	0.5722	1

Observed v. Predicted:

Compartment Parent

Time (days)	Value (%)	Predicted Value	Residual
0	99.5	98.91	0.588
0	100.6	98.91	1.688
3	73.1	79.3	-6.197
3	76.5	79.3	-2.797
7	61.6	59.06	2.544
7	62.3	59.06	3.244
14	45.9	35.26	10.64
14	32.7	35.26	-2.56
30	4.6	10.85	-6.247
30	4.6	10.85	-6.247
59	0	1.281	-1.281
59	0	1.281	-1.281
100	2.7	0.06244	2.638
100	0.3	0.06244	0.2376

Sequence Creation Information:
Fit generated by CAKE version 2.0 (Release)
running on R version 3.0.0 (2013-04-03)

Report Information:
Report generated by CAKE version 2.0 (Release)
CAKE developed by Tessella Ltd, Abingdon, Oxfordshire, UK for Syngenta
Running on .Net version 4.0.30319.18444

CAKE Kinetic Evaluation Report

Study: 3201605

Group A Sediment

Study date: 13/07/2017
Report generated: 13/07/2017

Experiment 1 (SFO)

Model Setup:

Topology: Parent only
Optimiser: IRLS (IRLS Its. 10, IRLS Tol. 1E-05, Max. Its. 100, Tol. 1E-05)
SANN Max Iterations: 10000
Use Extra Solver: True

Initial Values of Sequence Parameters:

Parameter	Initial Value	Bounds	Fixed
Parent_0	100	0 to (unbounded)	No
k_Parent	0.1	0 to (unbounded)	No

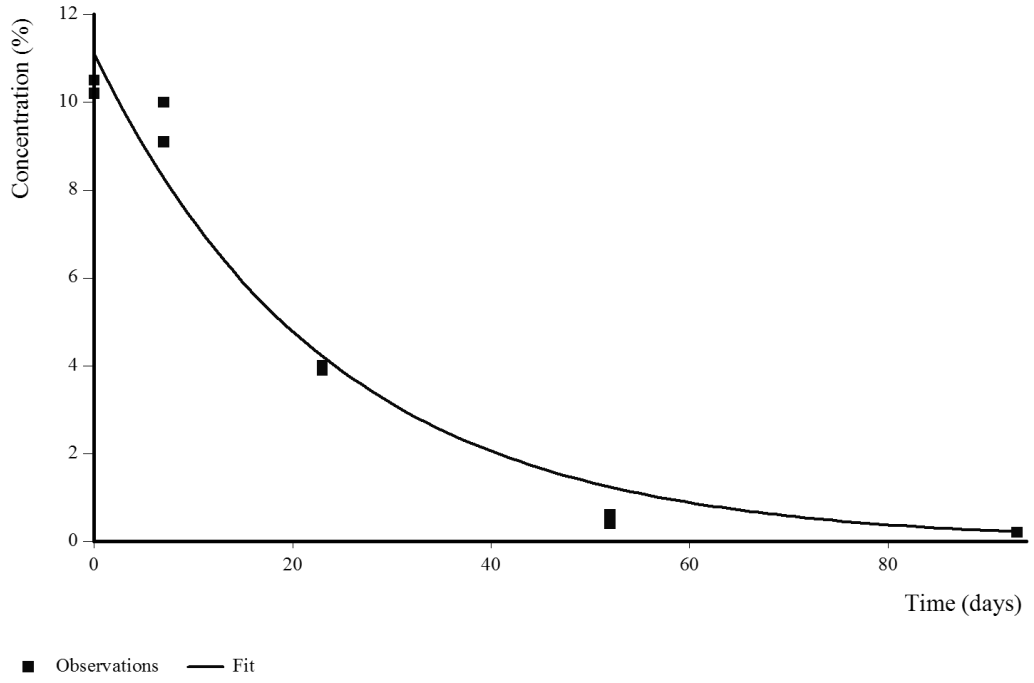
Fit step: Final

Reference Table:

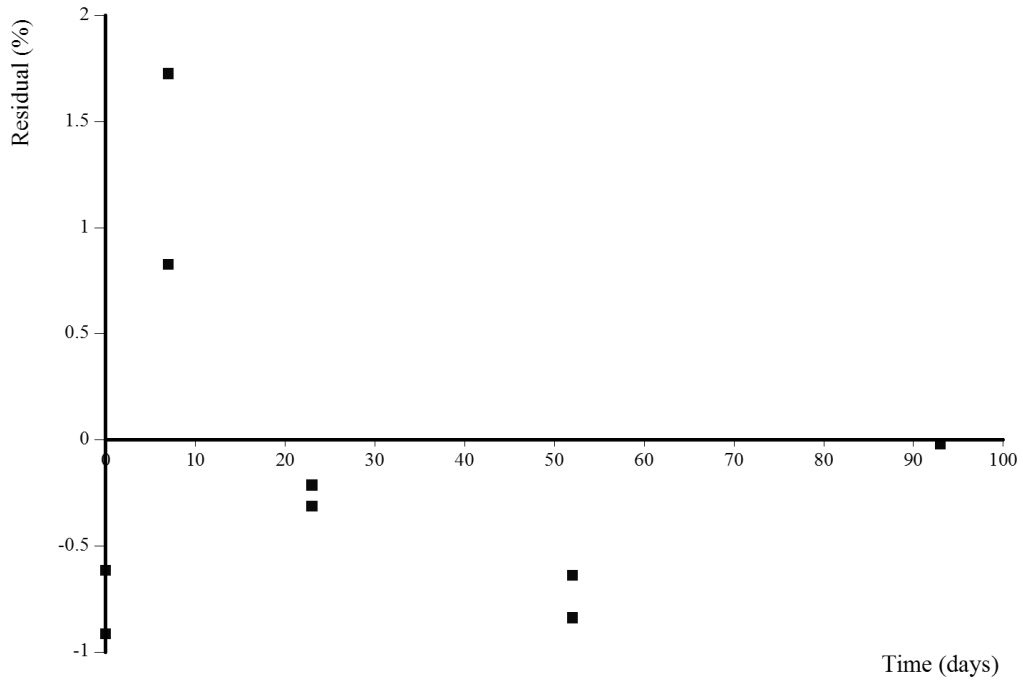
Compartment	Name
Parent	Parent

Graphical Summary:

Observations and Fitted Model:



Residuals:



Initial Values for This Step:

Parameter	Initial Value	Bounds	Fixed
Parent_0	100	0 to (unbounded)	No
k_Parent	0.1	0 to (unbounded)	No

Estimated Values:

Parameter	Value	σ	Prob. > t	Lower (90%) CI	Upper (90%) CI	Lower (95%) CI	Upper (95%) CI
Parent_0	11.12	0.5337		10.12	12.11	9.885	12.35
k_Parent	0.04217	0.004979	1.45E-005				

χ^2

Parameter	Error %	Degrees of Freedom
All data	12.3	3
Parent	12.3	3

Decay Times:

Compartment	DT50 (days)	DT90 (days)
Parent	16.4	54.6

Additional Statistics:

Parameter	r² (Obs v Pred)	Efficiency
All data	0.9687	0.9673
Parent	0.9687	0.9673

Parameter Correlation:

	Parent_0	k_Parent
Parent_0	1	0.4741
k_Parent	0.4741	1

Observed v. Predicted:

Compartment Parent

Time (days)	Value (%)	Predicted Value	Residual
0	10.5	11.12	-0.6159
0	10.2	11.12	-0.9159
7	9.1	8.275	0.8252
7	10	8.275	1.725
23	4	4.215	-0.2146
23	3.9	4.215	-0.3146
52	0.6	1.241	-0.6408
52	0.4	1.241	-0.8408
93	0.2	0.2202	-0.02025
93	0.2	0.2202	-0.02025

Sequence Creation Information:
Fit generated by CAKE version 2.0 (Release)
running on R version 3.0.0 (2013-04-03)

CAKE Kinetic Evaluation Report

Study: 3201605

Group B Sediment

Study date: 10/07/2017
Report generated: 13/07/2017

Experiment 1 (SFO)

Model Setup:

Topology: Parent only
Optimiser: IRLS (IRLS Its. 10, IRLS Tol. 1E-05, Max. Its. 100, Tol. 1E-05)
SANN Max Iterations: 10000
Use Extra Solver: True

Initial Values of Sequence Parameters:

Parameter	Initial Value	Bounds	Fixed
Parent_0	100	0 to (unbounded)	No
k_Parent	0.1	0 to (unbounded)	No

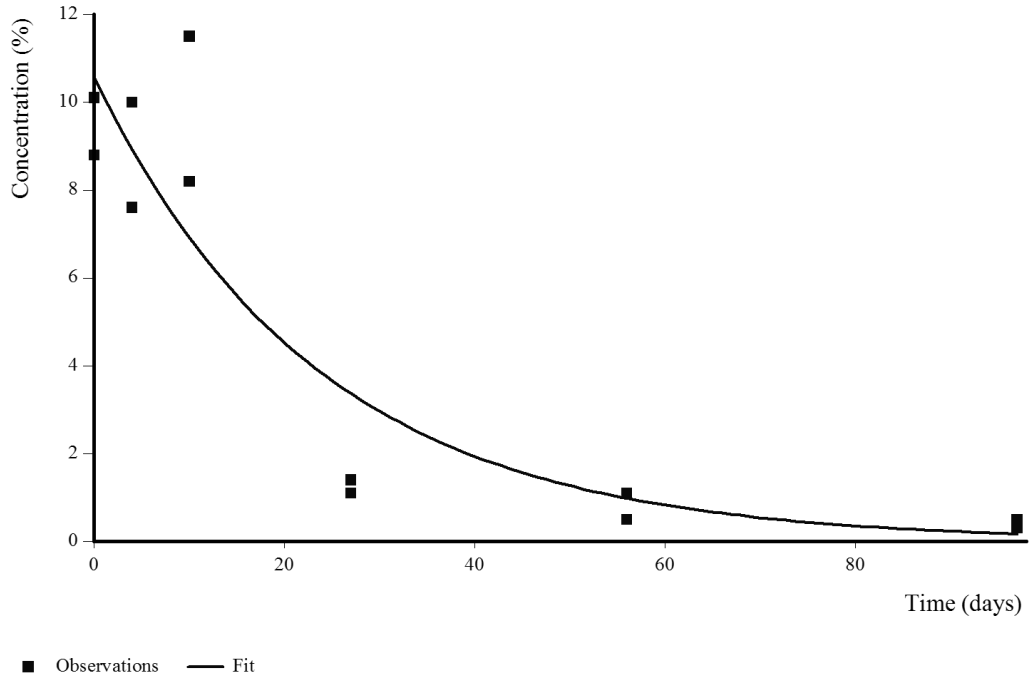
Fit step: Final

Reference Table:

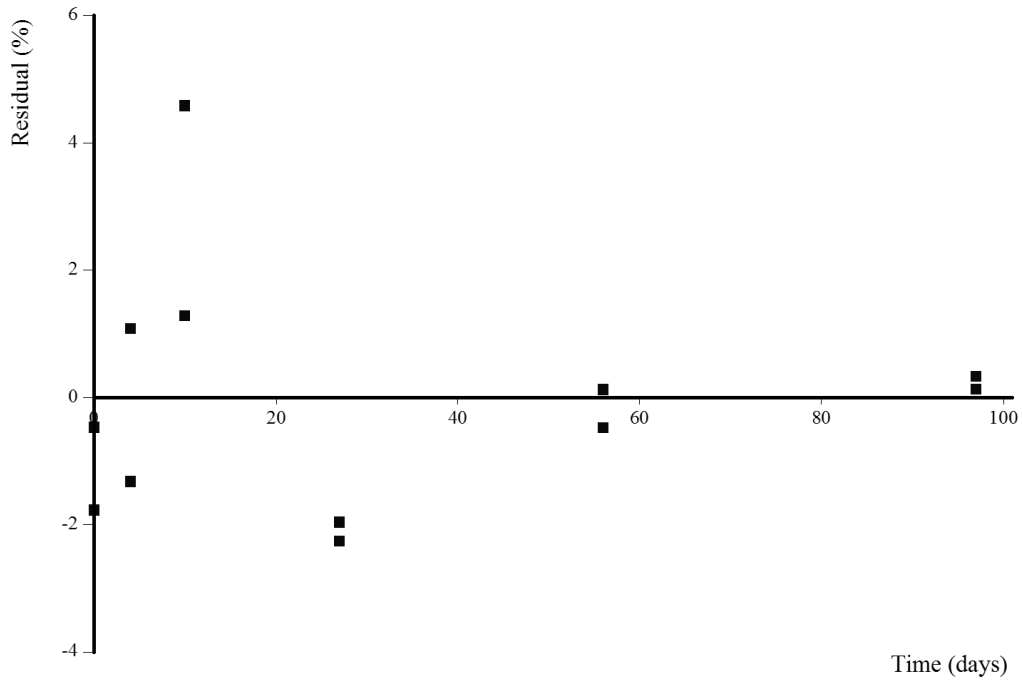
Compartment	Name
Parent	Parent

Graphical Summary:

Observations and Fitted Model:



Residuals:



Initial Values for This Step:

Parameter	Initial Value	Bounds	Fixed
Parent_0	100	0 to (unbounded)	No
k_Parent	0.1	0 to (unbounded)	No

Estimated Values:

Parameter	Value	σ	Prob. > t	Lower (90%) CI	Upper (90%) CI	Lower (95%) CI	Upper (95%) CI
Parent_0	10.58	1.081		8.616	12.54	8.167	12.98
k_Parent	0.04242	0.01134	0.001921				

χ^2

Parameter	Error %	Degrees of Freedom
All data	24.2	4
Parent	24.2	4

Decay Times:

Compartment	DT50 (days)	DT90 (days)
Parent	16.3	54.3

Additional Statistics:

Parameter	r^2 (Obs v Pred)	Efficiency
All data	0.8346	0.834
Parent	0.8346	0.834

Parameter Correlation:

	Parent_0	**k_Parent**
Parent_0	1	0.5206
k_Parent	0.5206	1

Observed v. Predicted:

Compartment Parent

Time (days)	**Value (%)**	**Predicted Value**	**Residual**
0	10.1	10.58	-0.4758
0	8.8	10.58	-1.776
4	7.6	8.925	-1.325
4	10	8.925	1.075
10	8.2	6.92	1.28
10	11.5	6.92	4.58
27	1.4	3.364	-1.964
27	1.1	3.364	-2.264
56	0.5	0.9831	-0.4831
56	1.1	0.9831	0.1169
97	0.3	0.1727	0.1273
97	0.5	0.1727	0.3273

Sequence Creation Information:
Fit generated by CAKE version 2.0 (Release)
running on R version 3.0.0 (2013-04-03)

CAKE Kinetic Evaluation Report

Study: 3201605

Group A including metabolites

A1 = Unknown WS1, B1 = Unknown WS2

Study date: 11/07/2017
Report generated: 10/10/2017

Experiment 1 (SFO)

Model Setup:

Topology: Parent, A1, B1
Optimiser: IRLS (IRLS Its. 10, IRLS Tol. 1E-05, Max. Its. 100, Tol. 1E-05)
SANN Max Iterations: 10000
Use Extra Solver: True

Initial Values of Sequence Parameters:

Parameter	Initial Value	Bounds	Fixed
Parent_0	104.6	0 to (unbounded)	Yes
k_Parent	0.06344	0 to (unbounded)	Yes
f_Parent_to_A1	0.3333	0 to 1	No
f_Parent_to_B1	0.3333	0 to 1	No
k_A1	0.1	0 to (unbounded)	No
k_B1	0.1	0 to (unbounded)	No

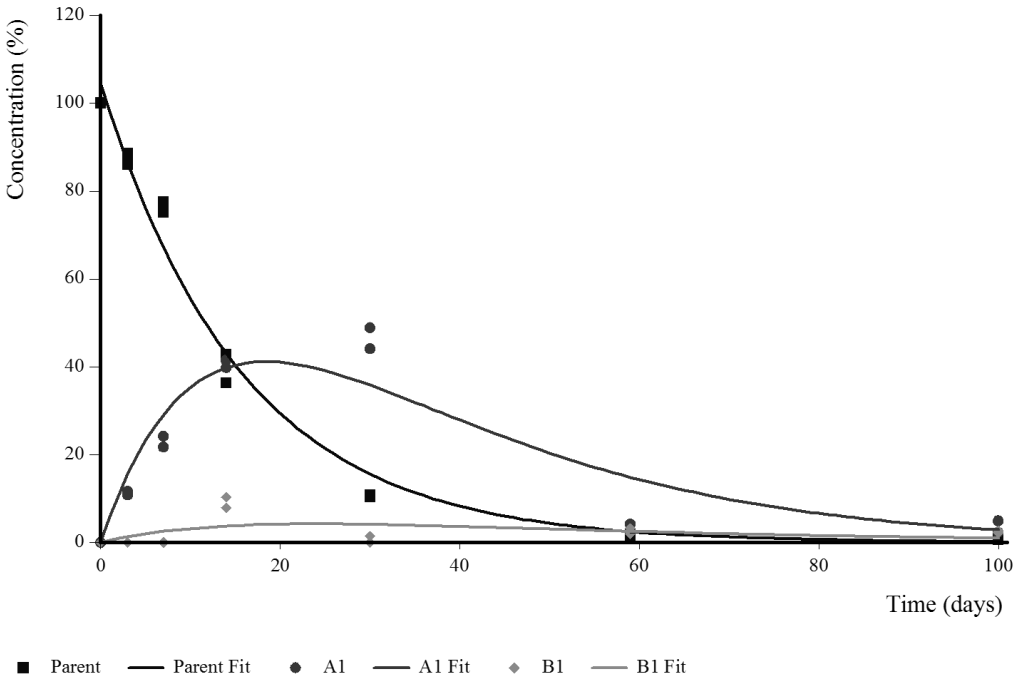
Fit step: Final

Reference Table:

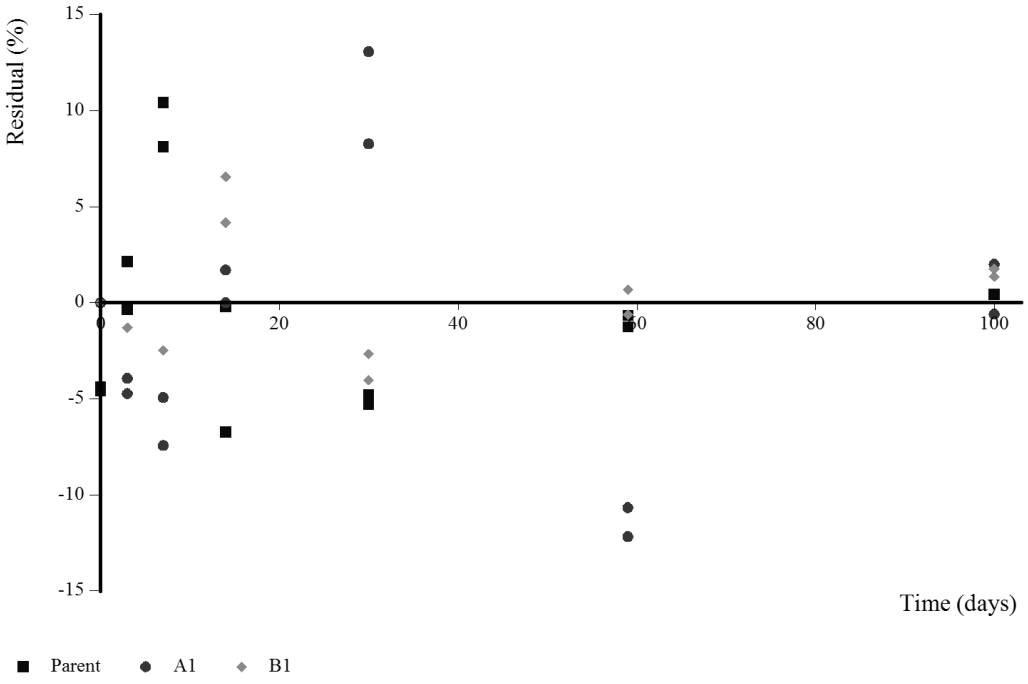
Compartment	Name
Parent	Parent
A1	A1
B1	B1

Graphical Summary:

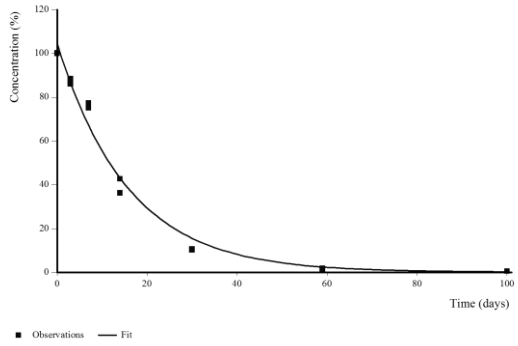
Observations and Fitted Model:

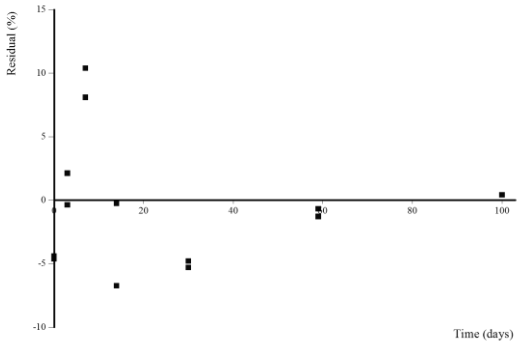


Residuals:

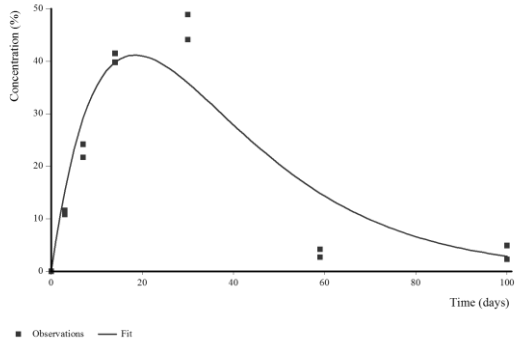


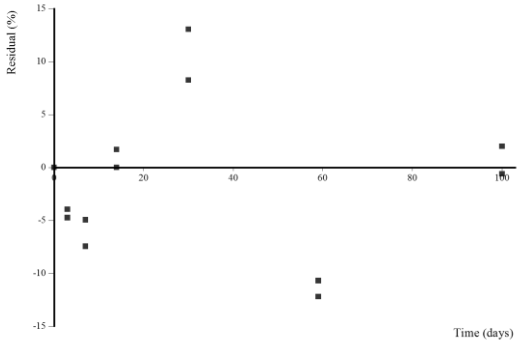
Compartment Parent:



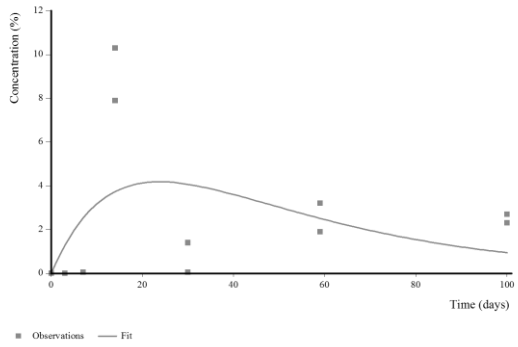


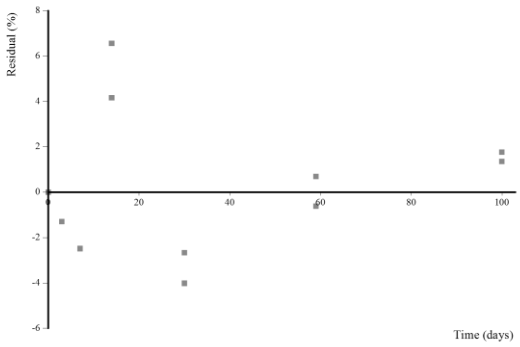
Compartment A1:





Compartment B1:





Initial Values for This Step:

Parameter	Initial Value	Bounds	Fixed
Parent_0	104.6	0 to (unbounded)	Yes
k_Parent	0.06344	0 to (unbounded)	Yes
f_Parent_to_A1	0.9212	0 to 1	No
f_Parent_to_B1	0.07878	0 to 1	No
k_A1	0.04605	0 to (unbounded)	No
k_B1	0.02901	0 to (unbounded)	No

Estimated Values:

Parameter	Value	σ	Prob. > t	Lower (90%) CI	Upper (90%) CI	Lower (95%) CI	Upper (95%) CI
f_Parent_to_A1	0.9198	0.07881		0.7869	1.053	0.7603	
k_A1	0.04605	0.006091	2.17E-009				
f_Parent_to_B1	0.07453	0.08929		-0.07601	0.2251	-0.1062	
k_B1	0.02622	0.06908	0.3532				

χ^2

Parameter	Error %	Degrees of Freedom
All data	19.4	17
Parent	7.12	7
A1	28.5	5
B1	90	5

Decay Times:

Compartment	DT50 (days)	DT90 (days)
Parent	10.9	36.3
A1	15.1	50
B1	26.4	87.8

Additional Statistics:

Parameter	r^2 (Obs v Pred)	Efficiency
All data	0.9741	0.9734
Parent	0.9858	0.9854
A1	0.8723	0.8543
B1	0.2109	0.2107

Parameter Correlation:

	f_Parent_to_A1	k_A1	f_Parent_to_B1	k_B1
f_Parent_to_A1	1	0.7412	0.02962	0.04355
k_A1	0.7412	1	0.03781	0.0575
f_Parent_to_B1	0.02962	0.03781	1	0.8834
k_B1	0.04355	0.0575	0.8834	1

Observed v. Predicted:

Compartment Parent

Time (days)	Value (%)	Predicted Value	Residual
0	100	104.6	-4.6

0	100.2	104.6	-4.4
3	86.1	86.47	-0.3723
3	88.6	86.47	2.128
7	77.5	67.09	10.41
7	75.2	67.09	8.108
14	36.3	43.03	-6.734
14	42.8	43.03	-0.2337
30	10.8	15.59	-4.795
30	10.3	15.59	-5.295
59	1.8	2.477	-0.6773
59	1.2	2.477	-1.277
100	0.6	0.1838	0.4162
100	0.6	0.1838	0.4162

Compartment A1

Time (days)	Value (%)	Predicted Value	Residual
0	0	0	0
0	0	0	0
3	11.6	15.54	-3.939
3	10.8	15.54	-4.739
7	21.7	29.14	-7.442
7	24.2	29.14	-4.942
14	41.5	39.8	1.697
14	39.8	39.8	-0.003334
30	48.9	35.84	13.06
30	44.1	35.84	8.263
59	2.7	14.88	-12.18
59	4.2	14.88	-10.68
100	4.9	2.893	2.007
100	2.3	2.893	-0.5929

Compartment B1

Time (days)	Value (%)	Predicted Value	Residual
0	0	0	0
0	0	0	0
3	0	1.298	-1.298
3	0	1.298	-1.298
7	0.05	2.537	-2.487

7	0.05	2.537	-2.487
14	10.3	3.738	6.562
14	7.9	3.738	4.162
30	0.05	4.07	-4.02
30	1.4	4.07	-2.67
59	1.9	2.514	-0.6137
59	3.2	2.514	0.6863
100	2.3	0.9419	1.358
100	2.7	0.9419	1.758

Sequence Creation Information:
Fit generated by CAKE version 2.0 (Release)
running on R version 3.0.0 (2013-04-03)

Report Information:
Report generated by CAKE version 2.0 (Release)
CAKE developed by Tessella Ltd, Abingdon, Oxfordshire, UK for Syngenta
Running on .Net version 4.0.30319.18444

CAKE Kinetic Evaluation Report

Study: 3201605

Group A including metabolites

A1 = Unknown WS3, B1 = Unknown WS4

Study date: 11/07/2017
Report generated: 10/10/2017

Experiment 1 (SFO)

Model Setup:

Topology: Parent, A1, B1
Optimiser: IRLS (IRLS Its. 10, IRLS Tol. 1E-05, Max. Its. 100, Tol. 1E-05)
SANN Max Iterations: 10000
Use Extra Solver: True

Initial Values of Sequence Parameters:

Parameter	Initial Value	Bounds	Fixed
Parent_0	104.5	0 to (unbounded)	Yes
k_Parent	0.06344	0 to (unbounded)	Yes
f_Parent_to_A1	0.3333	0 to 1	No
f_Parent_to_B1	0.3333	0 to 1	No
k_A1	0.1	0 to (unbounded)	No
k_B1	0.1	0 to (unbounded)	No

Fit step: Final

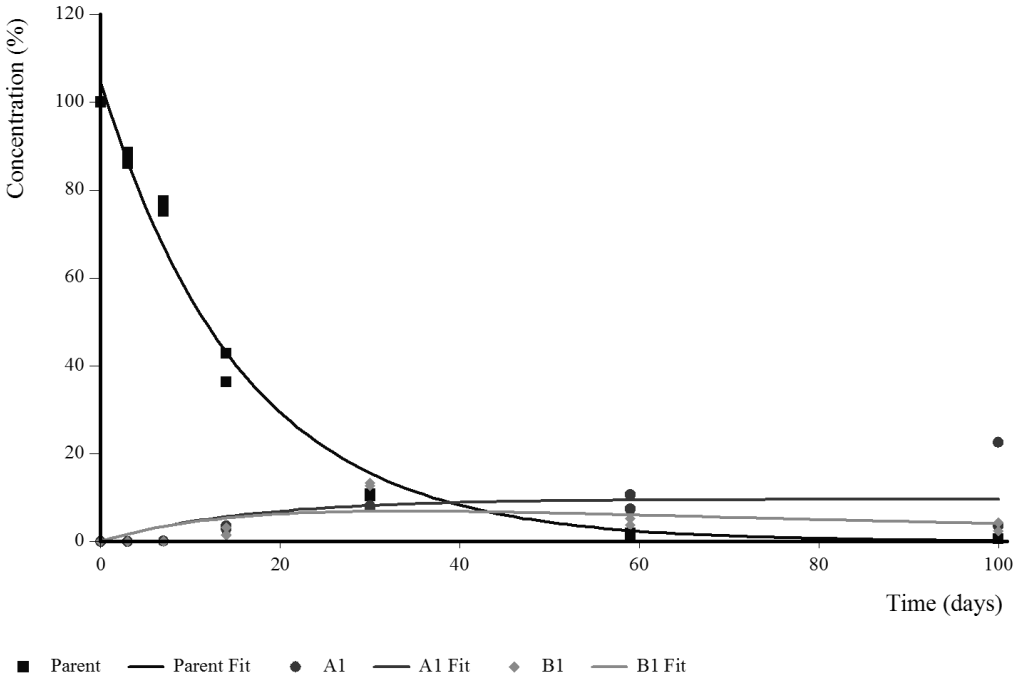
Reference Table:

Compartment	Name
Parent	Parent
A1	A1

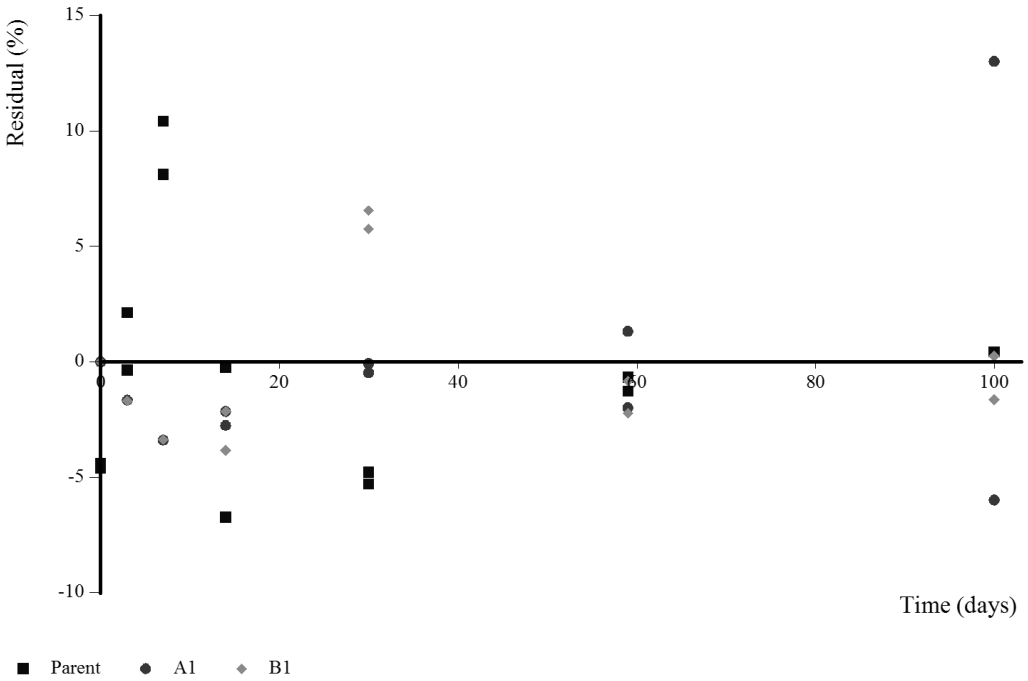
B1	B1

Graphical Summary:

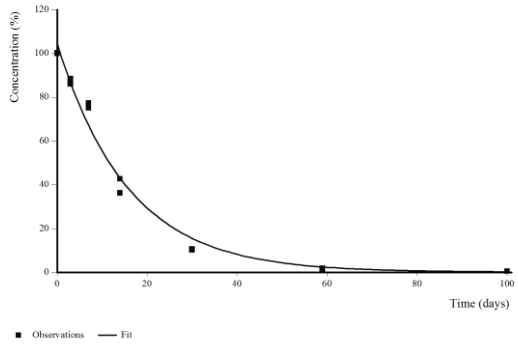
Observations and Fitted Model:

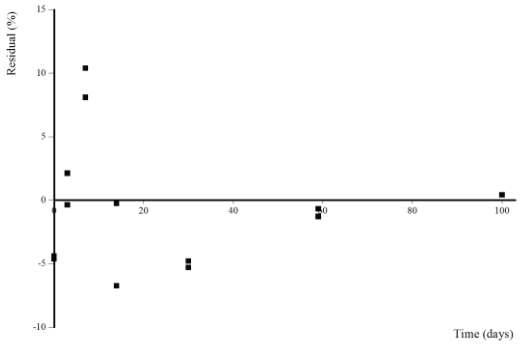


Residuals:

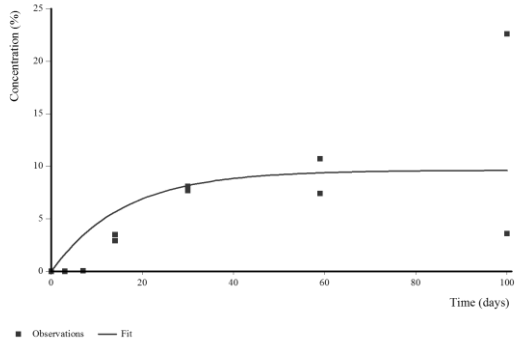


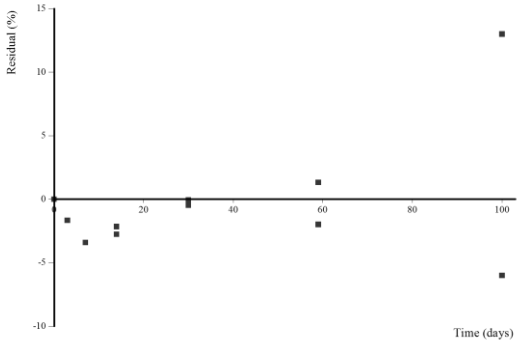
Compartment Parent:



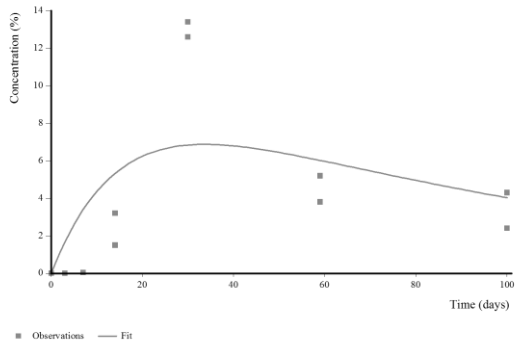


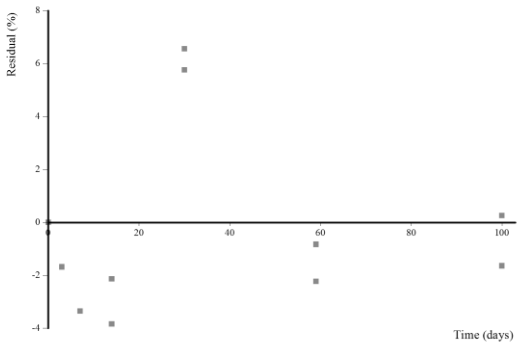
Compartment A1:





Compartment B1:





Initial Values for This Step:

Parameter	Initial Value	Bounds	Fixed
Parent_0	104.6	0 to (unbounded)	Yes
k_Parent	0.06344	0 to (unbounded)	Yes
f_Parent_to_A1	0.09184	0 to 1	No
f_Parent_to_B1	0.09438	0 to 1	No
k_A1	7.694E-11	0 to (unbounded)	No
k_B1	0.01074	0 to (unbounded)	No

Estimated Values:

Parameter	Value	σ	Prob. > t	Lower (90%) CI	Upper (90%) CI	Lower (95%) CI	Upper (95%) CI
f_Parent_to_A1	0.09184	0.02766		0.0452	0.1385	0.03584	
k_A1	5.49E-011	0.006426	0.5				
f_Parent_to_B1	0.09438	0.03618		0.03338	0.1554	0.02113	
k_B1	0.01074	0.01073	0.1617				

χ^2

Parameter	Error %	Degrees of Freedom
All data	16.7	17
Parent	7.12	7
A1	36.2	5
B1	72.2	5

Decay Times:

Compartment	DT50 (days)	DT90 (days)
Parent	10.9	36.3
A1	1.26E+10	4.19E+10
B1	64.5	214

Additional Statistics:

Parameter	r^2 (Obs v Pred)	Efficiency
All data	0.9822	0.9817
Parent	0.9858	0.9854
A1	0.5559	0.5172
B1	0.5686	0.4963

Parameter Correlation:

	f_Parent_to_A1	k_A1	f_Parent_to_B1	k_B1
f_Parent_to_A1	1	0.8013	0.109	0.1457
k_A1	0.8013	1	0.1134	0.1525
f_Parent_to_B1	0.109	0.1134	1	0.7545
k_B1	0.1457	0.1525	0.7545	1

Observed v. Predicted:

Compartment Parent

Time (days)	Value (%)	Predicted Value	Residual
0	100	104.6	-4.6

0	100.2	104.6	-4.4
3	86.1	86.47	-0.3723
3	88.6	86.47	2.128
7	77.5	67.09	10.41
7	75.2	67.09	8.108
14	36.3	43.03	-6.734
14	42.8	43.03	-0.2337
30	10.8	15.59	-4.795
30	10.3	15.59	-5.295
59	1.8	2.477	-0.6773
59	1.2	2.477	-1.277
100	0.6	0.1838	0.4162
100	0.6	0.1838	0.4162

Compartment A1

Time (days)	Value (%)	Predicted Value	Residual
0	0	0	0
0	0	0	0
3	0	1.665	-1.665
3	0	1.665	-1.665
7	0.05	3.445	-3.395
7	0.05	3.445	-3.395
14	3.5	5.654	-2.154
14	2.9	5.654	-2.754
30	8.1	8.174	-0.07405
30	7.7	8.174	-0.4741
59	7.4	9.379	-1.979
59	10.7	9.379	1.321
100	3.6	9.589	-5.989
100	22.6	9.589	13.01

Compartment B1

Time (days)	Value (%)	Predicted Value	Residual
0	0	0	0
0	0	0	0
3	0	1.683	-1.683
3	0	1.683	-1.683
7	0.05	3.401	-3.351

7	0.05	3.401	-3.351
14	3.2	5.336	-2.136
14	1.5	5.336	-3.836
30	13.4	6.839	6.561
30	12.6	6.839	5.761
59	3.8	6.025	-2.225
59	5.2	6.025	-0.8251
100	4.3	4.04	0.2605
100	2.4	4.04	-1.64

Sequence Creation Information:
Fit generated by CAKE version 2.0 (Release)
running on R version 3.0.0 (2013-04-03)

Report Information:
Report generated by CAKE version 2.0 (Release)
CAKE developed by Tessella Ltd, Abingdon, Oxfordshire, UK for Syngenta
Running on .Net version 4.0.30319.18444

CAKE Kinetic Evaluation Report

Study: 3201605

Group B including metabolites

A1 = Unknown WS1, B1 = Unknown WS2

Study date: 11/07/2017
Report generated: 10/10/2017

Experiment 1 (SFO)

Model Setup:

Topology: Parent, A1, B1
Optimiser: IRLS (IRLS Its. 10, IRLS Tol. 1E-05, Max. Its. 100, Tol. 1E-05)
SANN Max Iterations: 10000
Use Extra Solver: True

Initial Values of Sequence Parameters:

Parameter	Initial Value	Bounds	Fixed
Parent_0	102.7	0 to (unbounded)	Yes
k_Parent	0.06179	0 to (unbounded)	Yes
f_Parent_to_A1	0.3333	0 to 1	No
f_Parent_to_B1	0.3333	0 to 1	No
k_A1	0.1	0 to (unbounded)	No
k_B1	0.1	0 to (unbounded)	No

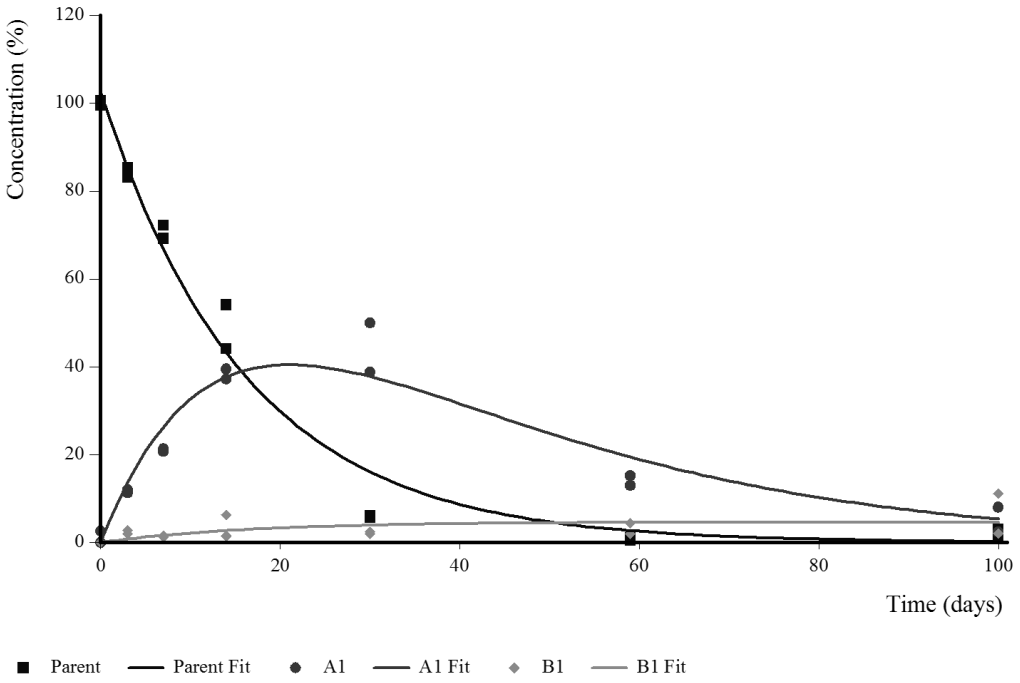
Fit step: Final

Reference Table:

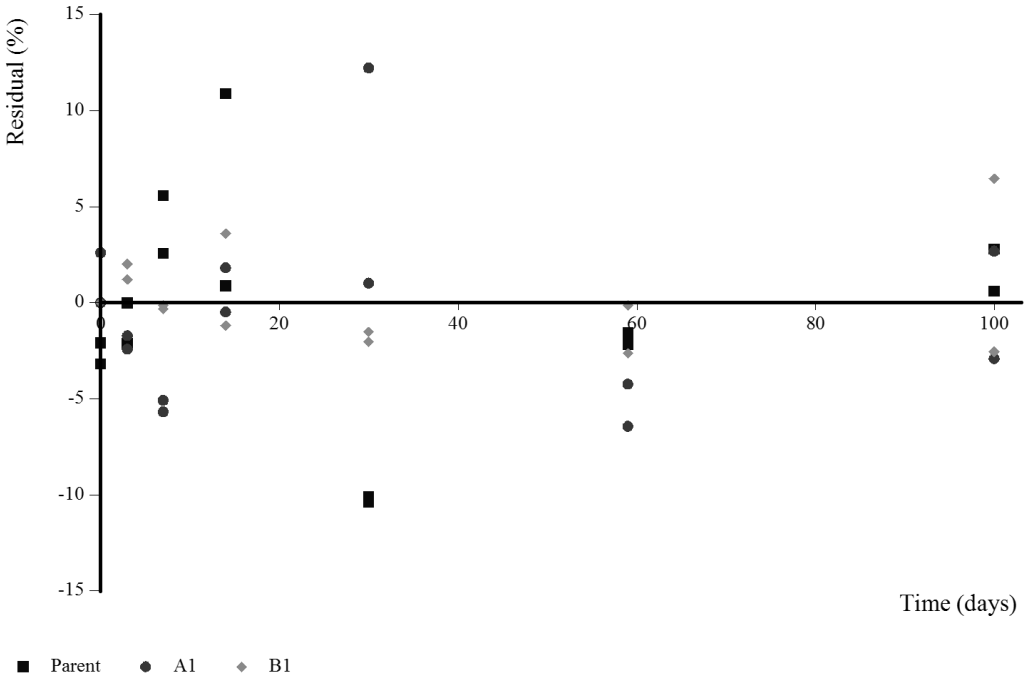
Compartment	Name
Parent	Parent
A1	A1
B1	B1

Graphical Summary:

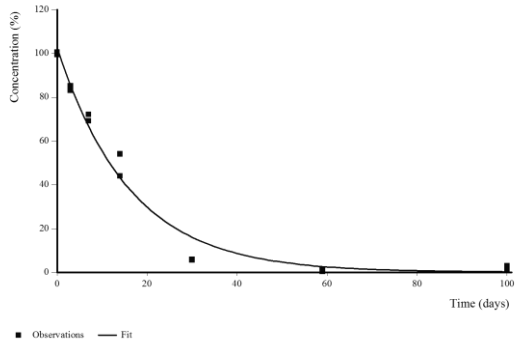
Observations and Fitted Model:

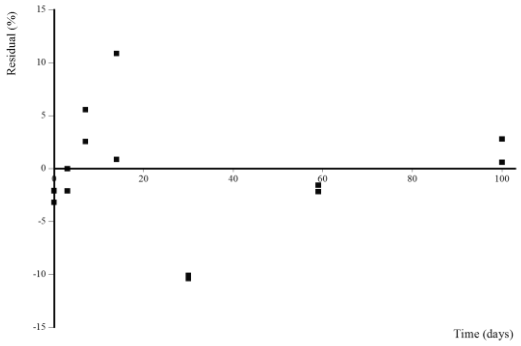


Residuals:

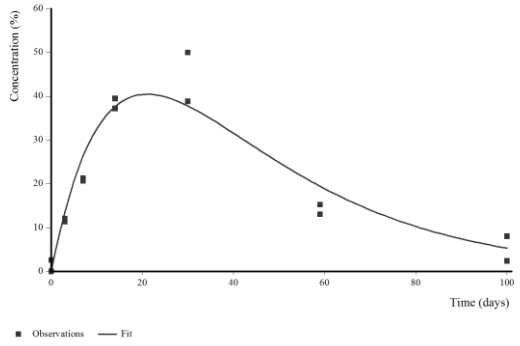


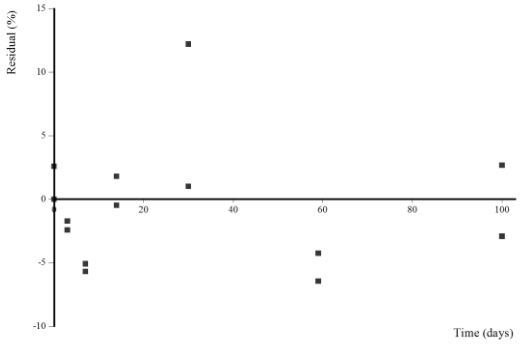
Compartment Parent:



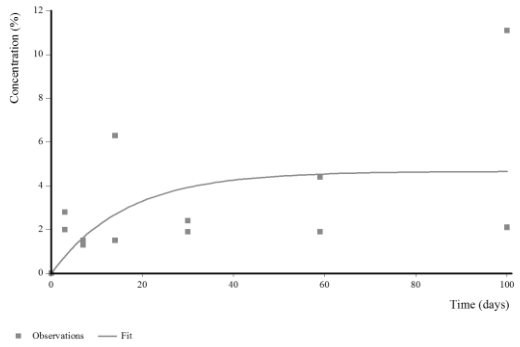


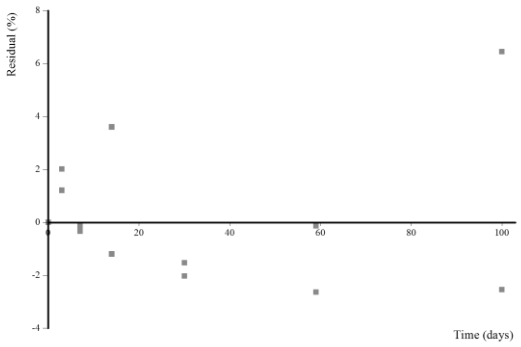
Compartment A1:





Compartment B1:





Initial Values for This Step:

Parameter	Initial Value	Bounds	Fixed
Parent_0	102.7	0 to (unbounded)	Yes
k_Parent	0.06179	0 to (unbounded)	Yes
f_Parent_to_A1	0.8342	0 to 1	No
f_Parent_to_B1	0.04531	0 to 1	No
k_A1	0.03563	0 to (unbounded)	No
k_B1	6.413E-10	0 to (unbounded)	No

Estimated Values:

Parameter	Value	σ	Prob. > t	Lower (90%) CI	Upper (90%) CI	Lower (95%) CI	Upper (95%) CI
f_Parent_to_A1	0.8342	0.04187		0.7636	0.9048	0.7494	
k_A1	0.03563	0.003704	4.94E-012				
f_Parent_to_B1	0.04531	0.1263		-0.1676	0.2583	-0.2104	
k_B1	9.57E-010	0.06404	0.5				

χ^2

Parameter	Error %	Degrees of Freedom
All data	14.6	17
Parent	7.79	7
A1	16.1	5
B1	38.7	5

Decay Times:

Compartment	DT50 (days)	DT90 (days)
Parent	11.2	37.3
A1	19.5	64.6
B1	7.24E+08	2.41E+09

Additional Statistics:

Parameter	r^2 (Obs v Pred)	Efficiency
All data	0.979	0.9788
Parent	0.9817	0.9812
A1	0.9132	0.9081
B1	0.2607	0.2439

Parameter Correlation:

	f_Parent_to_A1	k_A1	f_Parent_to_B1	k_B1
f_Parent_to_A1	1	0.6142	0.0641	0.0252
k_A1	0.6141	1	-0.0298	-0.05467
f_Parent_to_B1	0.0641	-0.0298	1	0.9858
k_B1	0.0252	-0.05467	0.9858	1

Observed v. Predicted:

Compartment Parent

Time (days)	Value (%)	Predicted Value	Residual
0	99.5	102.7	-3.2

0	100.6	102.7	-2.1
3	83.2	85.32	-2.123
3	85.3	85.32	-0.02284
7	69.2	66.64	2.562
7	72.2	66.64	5.562
14	54.1	43.24	10.86
14	44.1	43.24	0.8606
30	6	16.09	-10.09
30	5.7	16.09	-10.39
59	0.5	2.681	-2.181
59	1.1	2.681	-1.581
100	3	0.2128	2.787
100	0.8	0.2128	0.5872

Compartment A1

Time (days)	Value (%)	Predicted Value	Residual
0	0	0	0
0	2.6	0	2.6
3	12	13.73	-1.726
3	11.3	13.73	-2.426
7	20.7	26.39	-5.687
7	21.3	26.39	-5.087
14	39.5	37.68	1.817
14	37.2	37.68	-0.4828
30	38.8	37.78	1.015
30	50	37.78	12.22
59	15.2	19.44	-4.242
59	13	19.44	-6.442
100	8	5.317	2.683
100	2.4	5.317	-2.917

Compartment B1

Time (days)	Value (%)	Predicted Value	Residual
0	0	0	0
0	0	0	0
3	2.8	0.7873	2.013
3	2	0.7873	1.213
7	1.3	1.634	-0.3338

7	1.5	1.634	-0.1338
14	1.5	2.694	-1.194
14	6.3	2.694	3.606
30	1.9	3.924	-2.024
30	2.4	3.924	-1.524
59	1.9	4.532	-2.632
59	4.4	4.532	-0.1315
100	11.1	4.643	6.457
100	2.1	4.643	-2.543

Sequence Creation Information:
Fit generated by CAKE version 2.0 (Release)
running on R version 3.0.0 (2013-04-03)

Report Information:
Report generated by CAKE version 2.0 (Release)
CAKE developed by Tessella Ltd, Abingdon, Oxfordshire, UK for Syngenta
Running on .Net version 4.0.30319.18444

CAKE Kinetic Evaluation Report

Study: 3201605

Group B including metabolites

A1 = Unknown WS3, B1 = Unknown WS4

Study date: 11/07/2017
Report generated: 10/10/2017

Experiment 1 (SFO)

Model Setup:

Topology: Parent, A1, B1
Optimiser: IRLS (IRLS Its. 10, IRLS Tol. 1E-05, Max. Its. 100, Tol. 1E-05)
SANN Max Iterations: 10000
Use Extra Solver: True

Initial Values of Sequence Parameters:

Parameter	Initial Value	Bounds	Fixed
Parent_0	102.7	0 to (unbounded)	Yes
k_Parent	0.06179	0 to (unbounded)	Yes
f_Parent_to_A1	0.3333	0 to 1	No
f_Parent_to_B1	0.3333	0 to 1	No
k_A1	0.1	0 to (unbounded)	No
k_B1	0.1	0 to (unbounded)	No

Fit step: Final

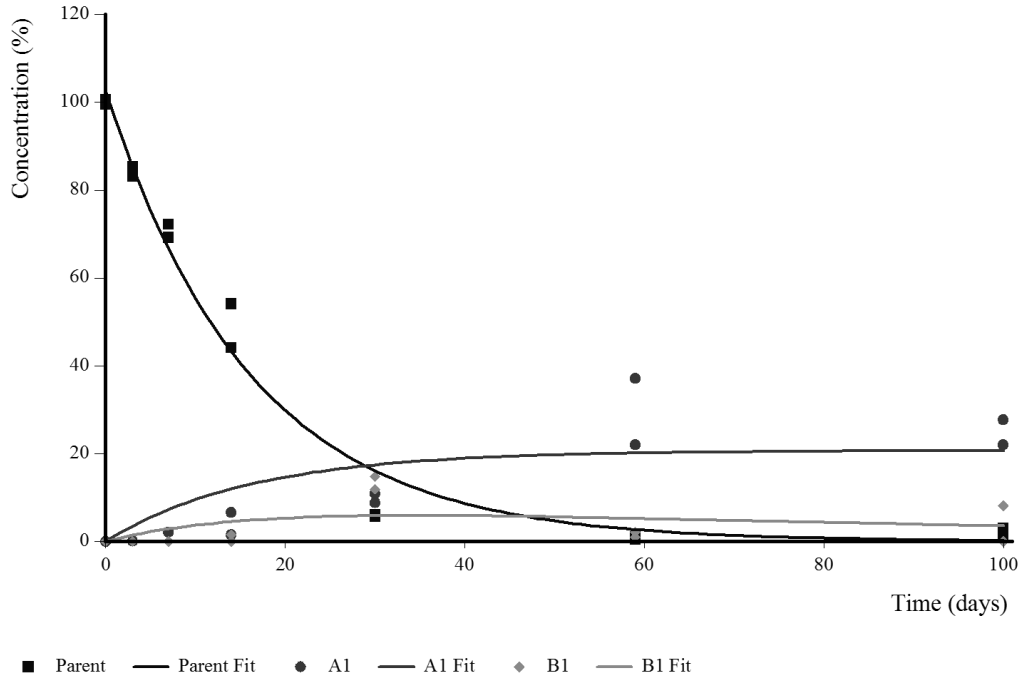
Reference Table:

Compartment	Name
Parent	Parent
A1	A1

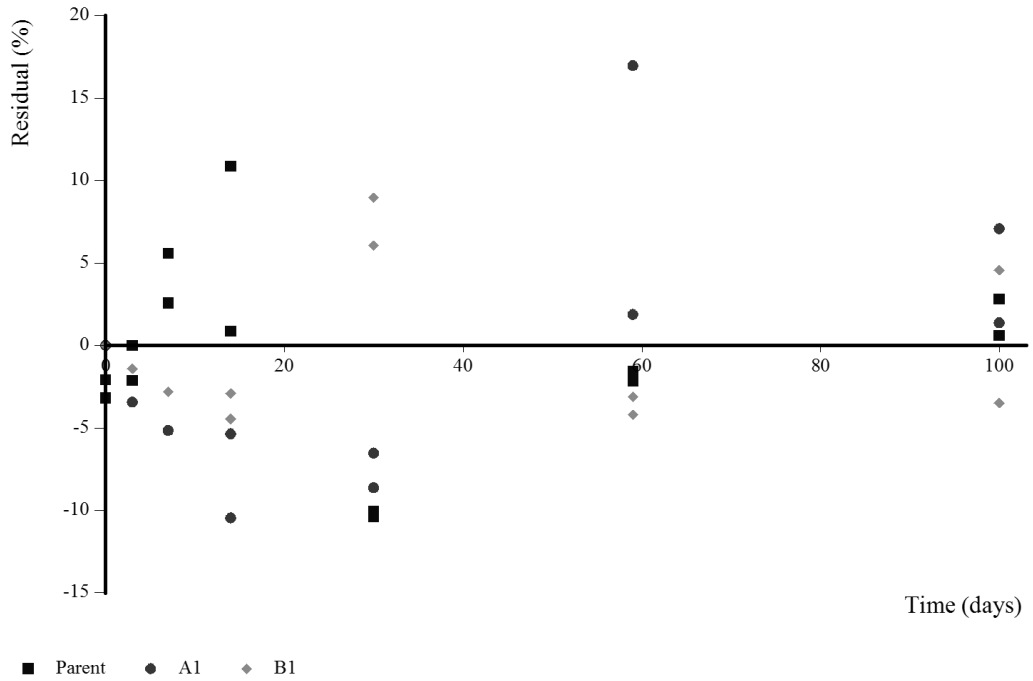
B1	B1

Graphical Summary:

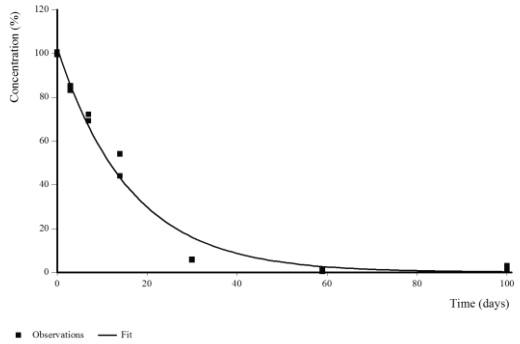
Observations and Fitted Model:

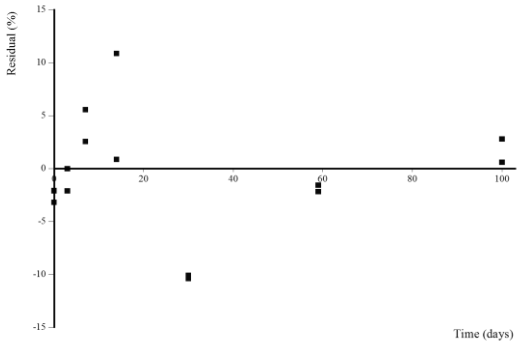


Residuals:

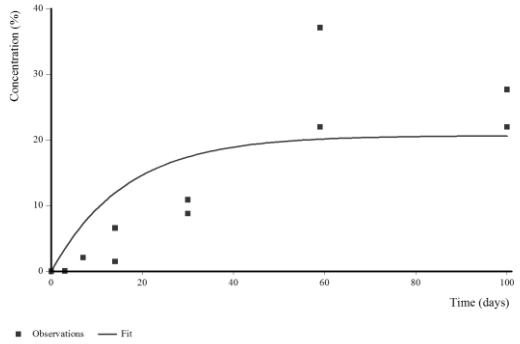


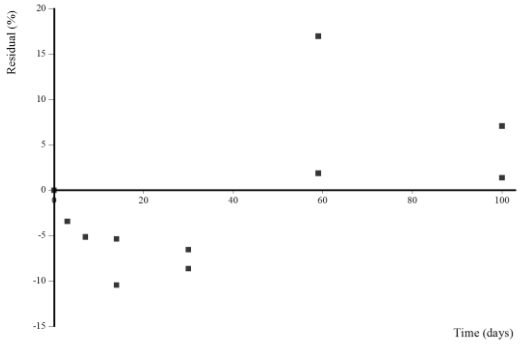
Compartment Parent:



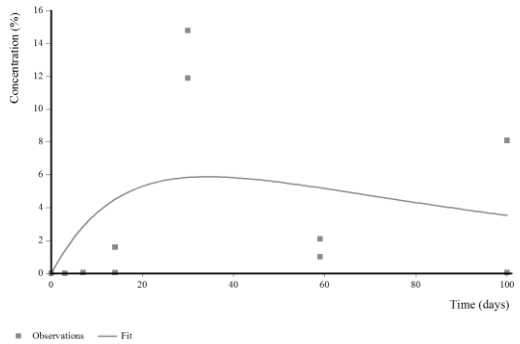


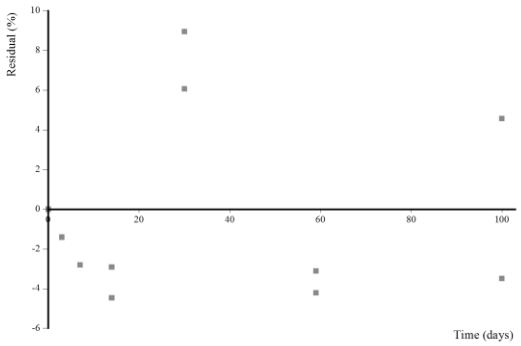
Compartment A1:





Compartment B1:





Initial Values for This Step:

Parameter	Initial Value	Bounds	Fixed
Parent_0	102.7	0 to (unbounded)	Yes
k_Parent	0.06179	0 to (unbounded)	Yes
f_Parent_to_A1	0.2013	0 to 1	No
f_Parent_to_B1	0.08251	0 to 1	No
k_A1	2.531E-11	0 to (unbounded)	No
k_B1	0.01056	0 to (unbounded)	No

Estimated Values:

Parameter	Value	σ	Prob. > t	Lower (90%) CI	Upper (90%) CI	Lower (95%) CI	Upper (95%) CI
f_Parent_to_A1	0.2013	0.09972		0.03318	0.3694	-0.0005687	
k_A1	4.48E-011	0.01301	0.5				
f_Parent_to_B1	0.08251	0.08492		-0.06067	0.2257	-0.08941	
k_B1	0.01056	0.01922	0.293				

χ^2

Parameter	Error %	Degrees of Freedom
All data	22.8	17
Parent	7.79	7
A1	48.6	5
B1	90	5

Decay Times:

Compartment	DT50 (days)	DT90 (days)
Parent	11.2	37.3
A1	1.55E+10	5.14E+10
B1	65.7	218

Additional Statistics:

Parameter	r^2 (Obs v Pred)	Efficiency
All data	0.9665	0.9653
Parent	0.9817	0.9812
A1	0.7056	0.6524
B1	0.3428	0.2989

Parameter Correlation:

	f_Parent_to_A1	k_A1	f_Parent_to_B1	k_B1
f_Parent_to_A1	1	0.9732	-0.7485	-0.5095
k_A1	0.9732	1	-0.7676	-0.5239
f_Parent_to_B1	-0.7485	-0.7676	1	0.8149
k_B1	-0.5095	-0.5239	0.8149	1

Observed v. Predicted:

Compartment Parent

Time (days)	Value (%)	Predicted Value	Residual
0	99.5	102.7	-3.2

0	100.6	102.7	-2.1
3	83.2	85.32	-2.123
3	85.3	85.32	-0.0229
7	69.2	66.64	2.562
7	72.2	66.64	5.562
14	54.1	43.24	10.86
14	44.1	43.24	0.8606
30	6	16.09	-10.09
30	5.7	16.09	-10.39
59	0.5	2.681	-2.181
59	1.1	2.681	-1.581
100	3	0.2128	2.787
100	0.8	0.2128	0.5872

Compartment A1

Time (days)	Value (%)	Predicted Value	Residual
0	0	0	0
0	0	0	0
3	0.05	3.498	-3.448
3	0.05	3.498	-3.448
7	2.1	7.26	-5.16
7	2.1	7.26	-5.16
14	1.5	11.97	-10.47
14	6.6	11.97	-5.37
30	10.9	17.44	-6.536
30	8.8	17.44	-8.636
59	22	20.14	1.865
59	37.1	20.14	16.96
100	27.7	20.63	7.068
100	22	20.63	1.368

Compartment B1

Time (days)	Value (%)	Predicted Value	Residual
0	0	0	0
0	0	0	0
3	0	1.411	-1.411
3	0	1.411	-1.411
7	0.05	2.86	-2.81

7	0.05	2.86	-2.81
14	1.6	4.513	-2.913
14	0.05	4.513	-4.463
30	14.8	5.844	8.956
30	11.9	5.844	6.056
59	1	5.215	-4.215
59	2.1	5.215	-3.115
100	8.1	3.534	4.566
100	0.05	3.534	-3.484

Sequence Creation Information:
Fit generated by CAKE version 2.0 (Release)
running on R version 3.0.0 (2013-04-03)

Report Information:
Report generated by CAKE version 2.0 (Release)
CAKE developed by Tessella Ltd, Abingdon, Oxfordshire, UK for Syngenta
Running on .Net version 4.0.30319.18444