Renewal Assessment Report

under Regulation (EC) 1107/2009

Zoxamide

Volume 3

Active substance
B.8 Environmental fate and behavior

Rapporteur Member State: Latvia
Co-Rapporteur Member State: France

Version history

Date	Subject
May 2001	Initial DAR. Draft Assessment Report (DAR) – prepared in the context of the application for the first inclusion of the a.s. in Annex I to Council Directive 91/414/EEC.
July 2016	Renewal Assessment Report (RAR) – prepared in the context of the application for renewal of approval of the a.s. according to Regulation (EC) No 1107/2009. Note: The RAR is a stand-alone document containing the evaluations already displayed in the original DAR dated May 2001 considered as relevant, as well as the new assessments submitted for the Renewal in 2014. These new studies are evaluated and summarized below, under the relevant points of this report, together with the previously evaluated studies. The information of the DAR considered as no more relevant is deleted with 'strikethrough' function, while the new studies, changes to the text and information provided by RMS (LV) is highlighted in yellow shading.

TABLE OF CONTENTS

B.8 ENVIRONMENTAL FATE AND BEHAVIOUR

Introduction

Zoxamide (RH-117281) is an existing active substance, for the first time included into the Annex I of the Council Directive 91/414/EEC, by means of the Commission Directive 2003/119/EC of 5 December 2003 amending Council Directive 91/414/EEC to include mesosulfuron, propoxycarbazone and zoxamide as active substances, on the 1st April 2004.
The active substance is currently approved under Commission Regulation (EC) 1107/2009 (repealing Commission Directive 91/414/EEC) as specified in Commission Implementing Regulation (EU) No. 540/2011 of 25 May 2011.

Zoxamide is a non-systemic fungicide belonging to the benzamide group of compounds. It is intended to protect against oomycete diseases such as *Phytophthora infestans* (late blight of potato) and *Plasmopara viticola* (downy mildew of grapevines). Zoxamide inhibits germ tube development and mycelium growth by inhibiting cell division. As a result, the fungal organism dies.

This document presents data and information on the environmental fate and behaviour of zoxamide submitted in support of the renewal of approval of zoxamide under Regulation (EC) 1107/2009. Most of the data presented were also submitted to secure the first inclusion of zoxamide in Annex I to Directive 91/414/EEC.

B.8.1 Fate and behaviour in soil

B.8.1.1 Route and rate of degradation in soil

B.8.1.1.1 Aerobic degradation

Zoxamide

Adequate data to assess the aerobic route and rate of degradation of zoxamide in soil were evaluated during the first EU review. The guidance for the conduct of such studies has not substantively altered since the first review, therefore no further data are considered necessary.

Under the original review DT_{50}s were available for six soils, besides one soil was investigated considering three different incubation conditions. Zoxamide degraded with DT_{50}s of 2.0-10 days and DT_{90}s of 6.7-110 day (1st order at 10-20°C and root 1st order at 25°C).

The rates of degradation in the aerobic soil degradation studies of Smalley and Reynolds (1997) and Burgerer (1998) have been re-evaluated according to the recommendations of the FOCUS Kinetics Guidance Document (FOCUS 2006).

Metabolite, breakdown and reaction products

Under the original review three metabolites were identified exceeding 10% AR in soil; RH-127450 (maximum of 15.1% AR), RH-24549 (maximum of 33.8% AR), and RH-163353 (maximum of 15% AR). Adequate data to assess the aerobic rate of degradation of the metabolites of zoxamide in soil were evaluated during the first EU review and no further data are considered necessary.

The rates of degradation in the aerobic soil degradation studies of Smalley and Reynolds (1997) and Burgerer (1998) have been re-evaluated according to the recommendations of the FOCUS Kinetics Group (FOCUS 2006).

Since the original review, the guidelines for evaluating the relevance of metabolites have changed. Data requirements under Regulation (EC) 1107/2009 require that metabolites occurring at >5% on two or more consecutive occasions should be considered as well as those which reach their maximum at the final time-point of the study. The previously submitted studies were examined for any other metabolites meeting these lower thresholds. In the soil degradation and metabolism study (Burgener, 1998a), metabolite RH-141455 was detected at levels above 5% on more than two occasions (maximum was 8% AR), therefore data on the degradation of this metabolite in soil have been provided.

Studies from the original DAR (May 2001):

Reference:	Smalley J., Reynolds JL. (1997). Aerobic soil metabolism of [14C]-RH-117281 Fungicide, XenoBiotic Laboratories, Inc., Rohm and Haas Technical Report No. 34-96-07, June 26, 1997.
Guideline(s):	US EPA guidelines (Subdivision N, 162-1, 1982)
GLP:	Yes
Previous evaluation:	In DAR (May 2001)
Validity of the study:	Considered acceptable for the first Annex I inclusion but partly acceptable for the renewal of the approval

[14C-phenyl-U]-RH-117281 (radiochemical purity 97.8%, specific activity 3.34 MBq/mg) was added in acetonitrile to two freshly-collected microbially-viable soils (see Table B.8.1.1.1-2) at a concentration of 1.49 mg/kg. Treated soils (50 g dry weight equivalent, 2 mm sieved) were incubated under aerobic conditions at 25 $\pm$ 1°C in the dark for up to 122 days. Soil was maintained at 75% FMC. Volatiles (CO_2/acidic volatiles) were trapped in potassium hydroxide solution.

Duplicate samples were analysed on days 0, 3, 7, 14, 30, 59, 90 and 122 post-treatment. Samples were extracted with acidified acetonitrile, filtered and the filtrate partitioned with dichloromethane. Unextracted material was subjected to acid hydrolysis. Further separation into humin, fulvic and humic acid fractions was achieved by centrifugation in sodium hydroxide solution followed by acidification (HCl) to pH 2 and further centrifugation (humic acid fraction precipitates). Analysis was by LSC, TLC and reverse phase radio- and UV- HPLC. Compound identification was by co-chromatography with unlabelled reference standards. Evolved CO_2 was identified by means of LSC of the barium carbonate precipitate.

Total recovery of radioactivity was 92.11-103.86% AR. The distribution of radioactivity is shown in Table B.8.1.1.1-1 CO_2 was steadily evolved from both soils and accounted for a maximum of 34.4 and 47.8% AR by day 122 for the loamy sand and silt loam soils respectively. Levels of non-extracted radioactivity were 0.7 and 3.3% AR on day 0, increasing to 39.1 and 33% AR by days 90-122.

The decline of RH-117281 did not appear to follow first order kinetics (Table B.8.1.1.1-2). DT50s and DT90s have been estimated using ✓1st order kinetics. The Rapporteur supports this approach.

A total of six metabolites were characterised. Of these, four compounds were identified as RH-129151, RH-139432, RH-127450 and RH-24549. None of the metabolites individually accounted for >7.4% AR, except for Met 4, characterised as a polar metabolite, which increased to 12.5% AR on day 14. Further analysis showed this to comprise 4-6 components, none of which exceeded 5% AR. Levels of the other unknown metabolite did not exceed 1.3% AR. Of the unextracted residues, 9.2-10.2% AR and 10.4-12.7% AR was associated with fulvic and humic acid fractions respectively and 2.8-4.8% AR was associated with humins. The remaining 10-11% unextracted residues were characterised as organo- or aqueous- soluble. The proposed metabolism pathway is shown in Figure B.8.1.1.1-1.

Table B.8.1.1.1-1: Distribution of RH-117281 and metabolites in two soils during aerobic degradation (% AR)

Day	parent RH-117281	RH-129151	Met 2	RH-139432	Met 4 Origin	RH-127450	RH-24549	CO_2	*Non-extracted
Ohio Loamy Sand									
0	102.28	0.27	0.20	0.35	0.23	ND	ND	NA	0.92
0	101.96	0.32	0.18	0.35	0.20	ND	ND	NA	0.43
Arith. mean	**102.12**	**0.30**	**0.19**	**0.35**	**0.22**	**ND**	**ND**	**NA**	**0.68**
3	82.39	1.49	0.46	1.21	3.82	1.64	1.97	0.11	7.78
3	79.26	1.70	0.30	1.01	4.76	1.70	3.00	0.19	9.14
Arith. mean	**80.83**	**1.60**	**0.38**	**1.11**	**4.29**	**1.67**	**2.49**	**0.15**	**8.46**
7	60.65	1.35	0.25	0.80	10.77	3.28	5.03	0.53	13.15
7	64.02	1.53	0.28	0.74	9.11	4.10	4.97	0.52	12.72
Arith. mean	**62.34**	**1.44**	**1.27**	**0.77**	**9.94**	**3.69**	**5.00**	**0.53**	**12.94**
14	50.68	1.34	0.50	0.99	11.75	6.25	7.58	2.10	20.69
14	51.93	1.11	0.43	1.06	13.29	5.16	7.26	1.89	18.60
Arith. mean	**51.31**	**1.23**	**0.47**	**1.03**	**12.52**	**5.71**	**7.42**	**2.00**	**19.65**
30	41.96	1.29	0.58	0.44	7.67	3.17	3.13	8.51	27.82
30	36.23	0.95	0.55	0.57	9.98	3.51	3.61	9.50	29.73
Arith. mean	**39.10**	**1.12**	**0.57**	**0.51**	**8.83**	**3.34**	**3.37**	**9.01**	**28.78**
59	20.84	0.54	0.38	0.23	8.57	2.45	2.08	21.02	36.20
59	19.18	0.38	0.26	0.21	9.17	2.02	2.18	21.20	35.81
Arith. mean	**20.01**	**0.46**	**0.32**	**0.22**	**8.87**	**2.24**	**2.13**	**21.11**	**36.01**
90	15.35	0.57	0.35	0.55	8.29	2.11	1.94	28.94	38.91
90	12.97	0.54	0.34	0.29	8.72	2.26	2.47	30.21	39.45
Arith. mean	**14.16**	**0.56**	**0.35**	**0.42**	**8.51**	**2.19**	**2.21**	**29.58**	**39.18**
122	9.43	0.28	0.28	0.14	7.80	1.53	1.38	35.51	39.31
122	10.81	0.34	0.33	0.18	7.86	1.81	1.70	33.20	37.55
Arith. mean	**10.12**	**0.31**	**0.31**	**0.16**	**7.83**	**1.67**	**1.54**	**34.36**	**38.43**
Pennsylvania Silt Loam									
0	98.17	ND	0.26	0.36	0.32	ND	ND	NA	3.47
0	98.96	0.29	0.22	0.29	0.30	ND	ND	NA	3.04
Arith. mean	**98.57**	**0.15**	**0.24**	**0.33**	**0.31**	**ND**	**ND**	**NA**	**3.26**
3	53.99	0.72	0.52	0.49	10.51	3.16	6.84	2.85	19.26
3	65.46	0.44	0.32	0.36	8.43	2.72	5.28	2.91	15.41

Arith. mean	**59.73**	**0.58**	**0.42**	**0.43**	**9.47**	**2.94**	**6.06**	**2.88**	**17.34**
7	58.67	0.30	0.27	0.16	7.49	2.07	2.35	6.42	19.85
7	39.92	0.33	0.33	0.22	10.80	2.40	3.35	10.51	27.53
Arith. mean	**49.30**	**0.32**	**0.30**	**0.19**	**9.15**	**2.24**	**2.85**	**8.47**	**23.69**
14	53.70	0.36	0.41	0.28	7.04	2.27	1.35	9.57	20.13
14	45.26	0.29	0.49	0.28	9.22	2.67	1.65	12.08	25.50
Arith. mean	**49.48**	**0.33**	**0.45**	**0.28**	**8.13**	**2.47**	**1.50**	**10.83**	**22.82**
30	24.08	0.20	0.43	0.21	5.16	1.33	0.64	27.22	33.45
30	26.79	0.22	0.47	0.18	5.69	1.57	0.60	25.19	31.78
Arith. mean	**25.44**	**0.21**	**0.45**	**0.20**	**5.43**	**1.45**	**0.62**	**26.21**	**32.62**
59	28.38	0.15	0.42	0.12	5.14	1.73	0.51	28.22	28.25
59	7.94	0.09	0.61	0.09	5.96	1.23	0.37	39.57	35.68
Arith. mean	**18.16**	**0.12**	**0.52**	**0.11**	**5.55**	**1.48**	**0.44**	**33.90**	**31.97**
90	21.52	0.19	0.36	0.13	4.35	1.28	0.42	36.06	30.17
90	13.83	0.13	0.43	0.09	5.62	1.28	0.38	39.23	32.84
Arith. mean	**17.68**	**0.16**	**0.40**	**0.11**	**4.99**	**1.28**	**0.40**	**37.65**	**31.51**
122	8.74	0.06	0.39	0.08	4.15	0.84	0.39	46.17	32.66
122	3.37	0.04	0.51	0.06	4.23	0.81	0.32	49.37	33.36
Arith. mean	**6.06**	**0.05**	**0.45**	**0.07**	**4.19**	**0.83**	**0.36**	**47.77**	**33.01**

* Not extracted with acidified acetonitrile

Table B.8.1.1.1-2: Estimated laboratory DT50 and DT90 for the aerobic degradation of RH-117281 in two soils, at 25°C

Soil type/origin	% sand	% silt	% clay	pH	% organic matter	Microbial biomass[1]	DT50[2] days	DT90[2] days	Rate[2] order / r^2
Loamy sand Ohio, USA	76	18	6	6.9	2.4	7.08	~~10~~	~~110~~	~~1st, 0.99~~
Silt loam Pennsylvania, USA	20	65	15	6.8	1.8	37.6	~~10~~	~~110~~	~~1st, 0.94~~

1: mg microbial C/kg soil dry weight.

2: 1st order fit resulted in correlation coefficients of 0.91 and 0.95 for loamy sand and silt loam soils respectively, with corresponding DT50s of 37d and 38d and DT90s of 120d and 130d respectively.

RMS comment:

The study has been previously evaluated and considered acceptable for the Annex I inclusion by United Kingdom (UK). The RMS (Latvia) believes the study is still overall acceptable except the kinetic

evaluation which is outdated. However a new kinetic evaluation of the study is provided by applicant, please see *Callow & Hilton (2013a)*.

Reference:	Burgener A. (1998a). 14C-RH-117281: Rate of degradation and metabolism in four soils incubated under aerobic conditions, RCC Umweltchemie Ag, Rohm and Haas Technical Report No. 34-98-45, September 17, 1998.
Guideline(s):	SETAC guidelines (1.1, 1995)
GLP:	Yes
Previous evaluation:	In DAR (May 2001)
Validity of the study:	Considered acceptable for the first Annex I inclusion but partly acceptable for the renewal of the approval

[14C-phenyl-U]-RH-117281 (radiochemical purity 97.8%, specific activity 3.34 MBq/mg) was added in acetonitrile to four freshly-collected, microbially-viable, soils (see Table B.8.1.1.1-4) at a concentration of 0.2 mg/kg. Treated soils (100 g dry weight equivalent, 2 mm sieved) were incubated under aerobic conditions at 20 $\pm$ 2°C and 10 $\pm$ 2°C (sandy loam only) in the dark for up to 125 days. Soil was maintained at 50% WHC and the sandy loam was additionally maintained at 100% WHC. Volatiles were trapped in sodium hydroxide (CO_2) and ethylene glycol (volatile organics) solutions.

Single samples were analysed on days 0, 1 3, 7, 14, 28, 56, and 120 or 125 days post-treatment. Samples were extracted and analysed as outlined in study of *Smalley & Reynolds (1997)*.

Total recovery of radioactivity was 96.9-100.3% AR. The distribution of radioactivity is shown in Table B.8.1.1.1-3 CO_2 was steadily evolved from all soils and accounted for a maximum of 35.5-57.8% AR by day 120. Levels of organic volatiles were <0.1% AR. Levels of non-extracted radioactivity were 0.4-0.8% AR on day 0, increasing to 25.6-38.3% AR by days 28-120.

The decline of RH-117281 did not appear to follow first order kinetics (Table B.8.1.1.1-4). DT50s and DT90s for RH-117281 were estimated by the applicant using one compartment first order kinetics with non-linear curve fitting (MicroCal 3.5). The Rapporteur has re-calculated DT50s and DT90s using best fit (✓1st order) kinetics.

A total of 23 metabolites were characterised. Of these, 9 compounds were identified as RH-139432, RH-129151, RH-127450, RH-24549, RH-141453, RH-141454, RH-141455, RH-141288, and RH-163353. None of the metabolites individually accounted for >8.4% AR, except for RH-127450, RH-24549 and RH-163353. ... In soils at 20°, levels of all three major metabolites peaked on days 3-7. Maximum concentrations were 8.1-15.1% AR (RH-127450), 5.5-33.8% AR (RH-24549) and 7.9-15.0% AR (RH163353). In sandy loam at 10°C, maximum levels were 9.4% AR (RH-127450, day 14), 12.6%AR (RH-24549, day 7) and 11.9% AR (RH163353, day 14). Of the unextracted residues, 9.5-17.5% AR and <0.1-3.2% AR was associated with fulvic and humic acid fractions respectively and 6.1-22.7% AR was associated with humins. The proposed metabolism pathway is shown in Figure B.8.1.1.1-1.

DT50s and DT90s for RH-117281 were estimated by the applicant assuming first order kinetics, using one compartment, non-linear regression analysis. DT50s and DT90s for major metabolites RH-127450, RH-24549 and RH-163353 were estimated using consecutive pairs of first order reactions for the formation and decline of each metabolite (MicroCal Origin 3.5). Whilst assuming first order decline does not give the best fit, the results (Table B.8.1.1.1-5) describe the actual degradation kinetics of each metabolite, and are in general worst case compared to results for the observed decline in soil using best fit kinetics, as estimated by the Rapporteur.

Table B.8.1.1.1-3: Distribution of RH-117281 and major metabolites in four soils during aerobic degradation under varying conditions (% AR)

	Day 0	Day 1	Day 3	Day 7	Day 14	Day 28	Day 56	Day 120
RH-117281								
Soil A, 20 °C, 50%MWC	99.7	75.0	33.9	8.7	6.6	2.3	0.6	0.7
Soil B, 20 °C, 50%MWC	98.0	79.4	46.3	13.9	6.0	2.0	0.6	1.0
Soil C, 20 °C, 50%MWC	96.0	78.6	39.3	11.1	3.0	2.2	0.5	0.7
Soil D, 20 °C, 50%MWC	97.7	89.1	58.4	30.0	10.8	5.2	1.8	2.5
Soil B, 10 °C, 50%MWC	100.2	93.2	78.0	46.1	32.3	10.6	6.1	2.7
Soil B, 20 °C, 100%FC	99.5	79.9	39.0	9.9	4.3	3.6	0.7	0.6
RH-127450								
Soil A, 20 °C, 50%MWC	0.0	5.6	10.6	8.5	3.2	0.9	0.7	0.7
Soil B, 20 °C, 50%MWC	0.0	2.2	7.7	11.2	5.2	1.6	0.7	0.3
Soil C, 20 °C, 50%MWC	0.0	3.4	8.1	3.8	1.4	0.1	0.0	0.3
Soil D, 20 °C, 50%MWC	0.0	4.2	10.5	15.1	12.7	7.3	2.9	1.9
Soil B, 10 °C, 50%MWC	0.0	0.0	3.5	8.2	9.4	7.0	3.5	2.0
Soil B, 20 °C, 100%FC	0.0	2.5	12.2	9.3	5.7	1.6	0.3	0.2
RH-24549								
Soil A, 20 °C, 50%MWC	0.0	4.3	11.9	9.7	5.7	2.1	2.3	2.9
Soil B, 20 °C, 50%MWC	0.0	6.8	5.5	8.3	5.3	0.9	2.0	0.9
Soil C, 20 °C, 50%MWC	0.0	4.8	21.4	33.8	18.1	3.6	3.0	1.9
Soil D, 20 °C, 50%MWC	0.0	1.2	3.0	5.5	2.9	3.6	2.1	2.5
Soil B, 10 °C, 50%MWC	0.0	3.2	6.3	12.6	9.6	5.3	5.6	3.0
Soil B, 20 °C, 100%FC	0.0	6.4	12.6	8.3	4.0	1.3	1.2	1.1
RH-163353								
Soil A, 20 °C, 50%MWC	0.0	3.3	13.1	10.6	5.0	3.8	1.4	1.3
Soil B, 20 °C, 50%MWC	0.0	5.1	15.0	12.5	8.7	2.8	1.7	1.1
Soil C, 20 °C, 50%MWC	0.0	6.1	10.8	12.7	6.9	2.3	0.9	0.2
Soil D, 20 °C, 50%MWC	0.0	1.8	4.9	7.9	5.0	6.4	5.0	2.4
Soil B, 10 °C, 50%MWC	0.0	1.2	3.4	8.7	11.9	8.7	7.7	5.5
Soil B, 20 °C, 100%FC	0.0	5.3	10.0	13.0	7.4	3.9	2.6	0.4
CO_2								
Soil A, 20 °C, 50%MWC	0.0	0.3	3.8	14.4	27.8	37.1	45.8	48.5
Soil B, 20 °C, 50%MWC	0.0	0.4	5.3	17.7	31.6	43.2	50.4	57.8

Soil C, 20 °C, 50%MWC	0.0	0.1	1.6	9.5	28.8	44.9	50.4	55.8
Soil D, 20 °C, 50%MWC	0.0	0.4	3.2	8.4	20.3	29.0	37.5	42.6
Soil B, 10 °C, 50%MWC	0.0	< 0.1	0.5	2.9	8.8	19.8	28.2	35.5
Soil B, 20 °C, 100%FC	0.0	0.4	5.0	17.7	31.3	41.4	46.2	56.2
Non-extractables								
Soil A, 20 °C, 50%MWC	0.7	7.9	12.5	28.1	35.3	38.3	35.4	34.7
Soil B, 20 °C, 50%MWC	0.5	5.4	10.8	22.2	26.4	30.2	28.7	29.2
Soil C, 20 °C, 50%MWC	0.8	6.3	8.2	18.8	32.1	36.0	34.7	32.2
Soil D, 20 °C, 50%MWC	0.7	4.6	6.5	12.1	19.3	21.2	25.6	23.8
Soil B, 10 °C, 50%MWC	0.4	2.8	3.5	8.9	17.4	25.5	31.4	34.1
Soil B, 20 °C, 100%FC	0.5	5.6	11.5	23.2	26.7	30.9	30.4	28.1

Table B.8.1.1.1-4: Estimated laboratory DT50 and DT90 for the aerobic degradation of RH-117281 in four soils (at 20°C and 50% WHC unless otherwise stated)

Soil type/origin	% sand	% silt	% clay	pH	% organic matter	Microbial biomass[1]	DT50 days	DT90 days	Rate order / r^2
Soil A – loam. Bordeaux, France	37	38	25	7.4	2.26	28.8-48.1	~~2.0~~	~~6.7~~	~~1~~[st] ~~0.997~~
Soil B - sandy loam. Mechthild-shausen, Germany	59	24	17	7.4	1.20	22.1-42.5	~~2.7~~	~~9.0~~	~~1~~[st] ~~0.998~~
10°C, 50% WHC							~~7.7~~	~~25.7~~	~~1~~[st] ~~0.994~~
20°C, 100% WHC							~~2.3~~	~~7.5~~	~~1~~[st] ~~0.997~~
Soil C - clay loam. St. Margherita, Italy	23	48	29	8.1	0.80	27.1-46.4	~~2.4~~	~~7.9~~	~~1~~[st] ~~0.998~~
Soil D - silt loam. Shelley, England	31	51	18	5.0	1.8	18.5-32.4	~~4.2~~	~~13.8~~	~~1~~[st] ~~0.997~~
Mean (at 20°C)							~~**2.7**~~	~~**9.0**~~	

1: mg microbial C/kg soil dry weight.

Table B.8.1.1.1-5: Estimated laboratory DT50 and DT90 for the aerobic degradation of major metabolites of RH-117281 in four soils

Soil type/origin *	Soil A – loam. Bordeaux, France 20°C, 50% WHC	Soil B - sandy loam. Mechthild-shausen, Germany 20°C, 50% WHC	 10°C, 50% WHC	 20°C, 100% WHC	Soil C - clay loam. St. Margherita, Italy 20°C, 50% WHC	Soil D - silt loam. Shelley, England 20°C, 50% WHC	**Mean** DT50/ DT90 at 20°C
RH-127450							
DT50, days	~~4.5~~	[1]~~6.8~~	~~28.3~~	~~6.1~~	[1]~~4.0~~	~~17.8~~	~~7.8~~
DT90, days	~~14.9~~	[1]~~22.6~~	~~94.0~~	~~20.4~~	[1]~~13.3~~	~~59.0~~	~~26.0~~
k_1	~~0.15~~	~~0.10~~	~~0.02~~	~~0.11~~	~~0.17~~	~~0.04~~	
k_2	~~0.41~~	-	~~0.14~~	~~0.33~~	-	~~0.28~~	
RH-24549							
DT50, days	~~8.5~~	~~19.0~~	~~43.4~~	~~5.5~~	[1]~~7.4~~	-	~~10.1~~
DT90, days	~~28.1~~	~~63.3~~	~~144.3~~	~~18.3~~	[1]~~24.5~~	-	~~33.5~~
k_1	~~0.08~~	~~0.04~~	~~0.02~~	~~0.13~~	~~0.09~~	-	
k_2	~~0.48~~	~~2.14~~	~~0.36~~	~~0.57~~	-	-	
RH-163353							
DT50, days	~~9.0~~	~~9.2~~	~~89.3~~	~~13.0~~	~~7.5~~	-	~~9.7~~
DT90, days	~~29.8~~	~~30.6~~	~~295.2~~	~~43.7~~	~~25.1~~	-	~~32.3~~
k_1	~~0.08~~	~~0.07~~	~~0.008~~	~~0.05~~	~~0.09~~	-	
k_2	~~0.46~~	~~0.44~~	~~0.17~~	~~0.43~~	~~0.35~~	-	

* 2-compartment 1st order kinetics, non-linear curve fitting, except where indicated;

k_1 and k_2 are rate constants for the formation (decline of precursor) and decline of metabolite respectively.

- Concentrations too low for rate determination

1 One compartment first order kinetics, non linear curve fitting.

Since new requirements are defined for minor non transient metabolites under Regulation 1107/2009, maximum occurrence of minor metabolite should be reported, see Table B.8.1.1.1-5a.

Table B.8.1.1.1-5a: Maximum levels of minor metabolites in four soils during aerobic degradation under varying conditions (% AR)

	Soil A, 20 °C, 50%MWC	Soil B, 20 °C, 50%MWC	Soil C, 20 °C, 50%MWC	Soil D, 20 °C, 50%MWC	Soil B, 10 °C, 50%MWC	Soil B, 20 °C, 100%FC
M1 (RH-139432)	3.3 (day 3)	2.9 (day 7)	2.9 (day 7)	4.9 (day 14)	3.6 (day 7)	3.4 (day 7)
M2 (RH-129151)	0.0	0.0	1.1 (day 3)	0.0	0.0	0.0
M5 (RH-141454)	0.8 (day 3)	0.0	0.0	1.0 (day 7)	0.7 (day 7)	0.0
M6 (RH-141455)	4.0 (day 7)	8.0* (day 28)	1.9 (day 28)	3.3 (day 28)	6.0 (day 120)	8.4 (day 14)
M7 (unknown)	2.2 (day 3)	2.0 (day 3)	3.5 (day 3)	2.3 (day 3)	1.8 (day 7)	0.9 (day 7)
M8 (unknown)	0.4 (day 7)	0.0	0.4 (day 0)	4.9 (day 14)	0.0	0.2 (day 28)
M9 (unknown)	0.9 (day 14)	1.0 (day 7)	0.0	4.2 (day 7)	2.4 (day 28)	1.6 (day 3)
M10 (unknown)	1.6 (day 7)	0.6 (day 14)	0.2 (day 14)	3.3 (day 14)	0.0	0.0
M11 (unknown)	2.1 (day 7)	0.4 (day 14)	0.9 (day 7)	2.7 (day 14)	0.0	1.4 (day 7)
M12 (unknown)	1.0 (day 28)	0.9 (day 28)	0.4 (day 3)	0.0	0.0	0.9 (day 7)
M13 (unknown)	4.6 (day 28)	3.3 (day 3)	1.9 (day 3, 7, 28)	3.4 (day 28)	3.2 (day 3, 7)	5.0 (day 7)
M15 (unknown)	0.6 (day 7)	0.0	0.1 (day 3)	0.2 (day 120)	0.0	0.0
M16 (unknown)	0.4 (day 28)	1.4 (day 28)	0.0	2.4 (day 28)	1.0 (day 28)	0.4 (day 28)
M17 (unknown)	0.0	0.8 (day 56)	0.0	3.4 (day 120)	1.4 (day 120)	0.0
M18 (unknown)	0.0	1.3 (day 3)	0.0	1.2 (day 56)	0.0	0.0
M19 (unknown)	0.0	0.0	0.1 (day 7, 14)	2.5 (day 28)	1.4 (day 28)	0.0
M20 (unknown)	0.0	0.0	0.6 (day 120)	0.0	0.0	0.6 (day 7)
M21 (unknown)	0.6 (day 3)	1.3 (day 28)	0.0	1.4 (day 56)	2.8 (day 56)	0.6 (day 28)
M22 (RH-141453)	0.0	0.0	0.0	1.7 (day 3)	0.0	0.0
M23 (RH-1412530	0.1 (day 3)	0.0	0.0	1.4 (day 7)	0.0	0.2 (day 56)

* M6 (RH-141455) was detected at >5% on more than two consecutive occasions (8% - day 28, 7.4% - day 56 and 5.8% - day 120)

RMS comment:

The study has been previously evaluated and considered acceptable for the Annex I inclusion by UK. The RMS believes the study is still overall acceptable except the kinetic evaluation which is outdated.

However a new kinetic evaluation of the study is provided by applicant, please see *Callow & Hilton (2013a).*

Since the original review, the guidelines for evaluating the relevance of metabolites have changed. Data requirements under Regulation (EC) 1107/2009 require that metabolites occurring at >5% on two or more consecutive occasions should be considered as major metabolites. Consequently, metabolite RH-141455 was found to meet this condition as it was detected at >5% on three consecutive occasions.

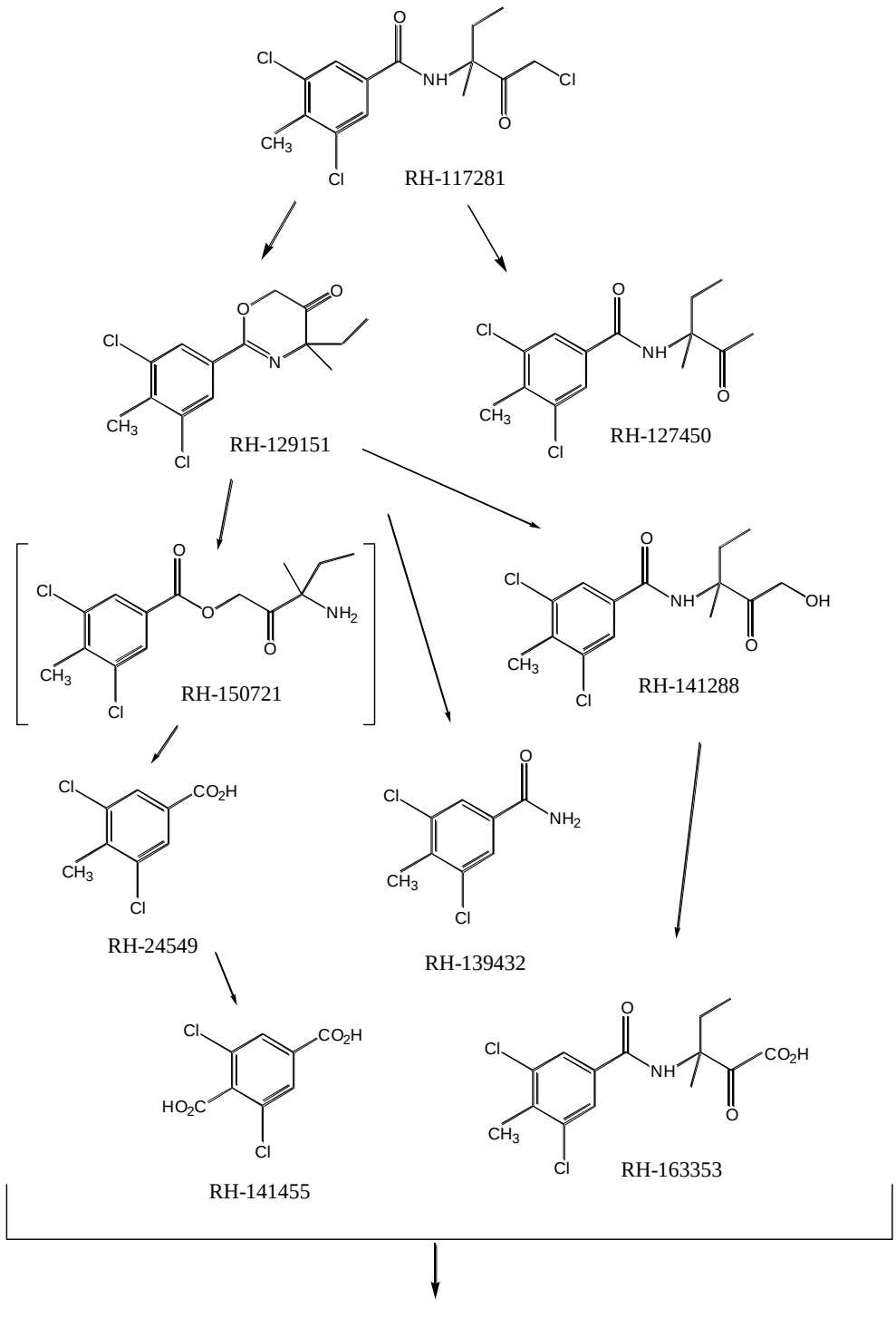


Figure B.8.1.1.1-1: The proposed metabolism pathway of RH-117281 in soil

Studies submitted with the dossier for the renewal of the approval:

Zoxamide:

Reference:	Callow B., Hilton M. (2013a). Determination of rates of decline for zoxamide and its metabolites, in soil according to the guidance within the FOCUS Kinetics Guidance Document.
Guideline(s):	FOCUS Kinetics Guidance Document (2006)
GLP:	No (calculation - GLP is not relevant)
Previous evaluation:	No (submitted for the purpose of renewal of a.s. approval)
Validity of the study:	Considered acceptable

Executive Summary

The decline of zoxamide in six laboratory soils was modelled according to the recommendations of the FOCUS Kinetics Guidance Document using a step-wise approach. The SFO model satisfactorily describes the decline of zoxamide in six of the eight incubations. The DFOP model provides the most appropriate fit in the remaining two incubations. Persistence endpoints of DT_{50}s of 2.03 to 13.75 days and modelling endpoints of DT_{50}s 2.03 to 41.3 days were obtained at 10 to 25°C.

I. MATERIAL AND METHODS

Rates of degradation were calculated according to the guidance of the FOCUS Degradation Kinetics Workgroup, using KinGui Version 2.0 (Bayer CropScience 2011).

The approach used followed that given in Chapter 7 of the FOCUS Kinetics Guidance Document. The suitability of the fit of the models was evaluated both visually and statistically by calculating the minimum % error required to pass the chi^2 test at a probability of 0.05 (acceptability criteria chi^2 error < 15%).

II. RESULTS AND DISCUSSION

The detections of zoxamide and its metabolites in the studies of Smalley and Reynolds (1997) and Burgener (1998a) are given in Tables B.8.1.1.1-6 to B.8.1.1.1-13.

Table B.8.1.1.1-6: Detections of zoxamide and its metabolites at 20°C and 50%MWHC in Burgener, 1998 – England silt loam (% AR).

Days after treatment	Soil – England silt loam			
	zoxamide	RH-127450	RH24549	RH-163353
0	97.7	nd	nd	nd
1	89.1	4.2	1.2	1.8
3	58.4	10.5	3.0	4.9
7	30.0	15.1	5.5	7.9
14	10.8	12.7	2.9	5.0
28	5.2	7.3	3.6	6.4
56	1.8	2.9	2.1	5.0
120	2.5*	1.9	2.5	2.4

nd – not detected, *data point omitted from parent kinetics calculation

Table B.8.1.1.1-7: Detections of zoxamide and its metabolites at 20°C and 50%MWHC in Burgener, 1998 – France loam (% AR).

Days after treatment	Soil – France loam			
	zoxamide	RH-127450	RH24549	RH-163353
0	99.7	nd	nd	nd
1	75	5.6	4.3	3.3
3	33.9	10.6	11.9	13.1
7	8.7	8.5	9.7	10.6
14	6.6	3.2	5.7	5.0
28	2.3	0.9	2.1	3.8
56	0.6	0.7	2.3	1.4
120	0.7*	0.7	2.9	1.3

nd – not detected, *data point omitted from parent kinetics calculation

Table B.8.1.1.1-8: Detections of zoxamide and its metabolites at 20°C and 50%MWHC in Burgener, 1998 – Germany sandy loam (% AR).

Days after treatment	Soil – Germany sandy loam				
	zoxamide	RH-127450	RH24549	RH-163353	RH-141455
0	98	nd	nd	nd	nd
1	79.4	2.2	6.8	5.1	nd
3	46.3	7.7	5.5	15.0	1.2
7	13.9	11.2	8.3	12.5	4.9
14	6.0	5.2	5.3	8.7	4.3
28	2.0	1.6	0.9	2.8	8.0
56	0.6	0.7	2.0	1.7	7.4
120	1.0*	0.3	0.9	1.1	5.8

nd – not detected, *data point omitted from parent kinetics calculation

Table B.8.1.1.1-9: Detections of zoxamide and its metabolites at 20°C and 50%MWHC in Burgener, 1998 – Italy clay loam (% AR).

Days after treatment	Soil – Italy clay loam			
	zoxamide	RH-127450	RH24549	RH-163353
0	96.0	nd	nd	nd
1	78.6	3.4	4.8	6.1
3	39.3	8.1	21.4	10.8
7	11.1	3.8	33.8	12.7
14	3.0	1.4	18.1	6.9
28	2.2	0.1	3.6	2.3
56	0.5	nd	3.0	0.9
125	0.7*	0.3**	1.9	0.2

nd – not detected, *data point omitted from parent kinetics calculation, **data point omitted

Table B.8.1.1.1-10: Detections of zoxamide and its metabolites at 10°C and 50%MWHC in Burgener, 1998 – Germany sandy loam (% AR).

Days after treatment	Soil – Germany sandy loam			
	zoxamide	RH-127450	RH24549	RH-163353
0	100.2	nd	nd	nd
1	93.2	nd	3.2	1.2
3	78.0	3.5	6.3	3.4
7	46.1	8.2	12.6	8.7
14	32.3	9.4	9.6	11.9
28	10.6	7.0	5.3	8.7
56	6.1	3.5	5.6	7.7
120	2.7	2.0	3.0	5.5

Table B.8.1.1.1-11: Detections of zoxamide and its metabolites at 20°C and 100%FC in Burgener, 1998 – German sandy loam (% AR).

Days after treatment	Soil – Germany sandy loam				
	zoxamide	RH-127450	RH24549	RH-163353	RH-141455
0	99.5	nd	nd	nd	nd
1	79.9	2.5	6.4	5.3	nd
3	39.0	12.2	12.6	10.0	1.2
7	9.9	9.3	8.3	13.0	2.6
14	4.3	5.7	4.0	7.4	8.4
28	3.6	1.6	1.3	3.9	4.1
56	0.7	0.3	1.2	2.6	3.4
120	0.6*	0.2	1.1	0.4	6.7

nd – not detected, *data point omitted from parent kinetics calculation

Table B.8.1.1.1-12: Detections of zoxamide and its metabolites at 25°C and 75%FC in Smalley *et al*, 1997 – Ohio loamy sand (% AR).

Days after treatment	Soil – Ohio loamy sand	
	zoxamide	RH24549
0	102.28	nd
0	101.96	nd
Arith. mean	**102.12**	**nd**
3	82.39	1.97
3	79.26	3.00
Arith. mean	**80.83**	**2.49**
7	60.65	5.03
7	64.02	4.97
Arith. mean	**62.34**	**5.00**
14	50.68	7.58
14	51.93	7.26
Arith. mean	**51.31**	**7.42**
30	41.96	3.13
30	36.23	3.61
Arith. mean	**39.10**	**3.37**
59	20.84	2.08
59	19.18	2.18
Arith. mean	**20.01**	**2.13**
90	15.35	1.94
90	12.97	2.47

Arith. mean	**14.16**	**2.21**
122	9.43	1.38
122	10.81	1.70
Arith. mean	**10.12**	**1.54**

Table B.8.1.1.1-13: Detections of zoxamide and its metabolites at 25°C and 75%FC in Smalley *et al*, 1997 – Pennsylvania silt loam (% AR).

Days after treatment	Soil – Pennsylvania silt loam
	zoxamide
0	98.17
0	98.96
Arith. mean	**98.57**
3	53.99
3	65.46
Arith. mean	**59.73**
7	58.67
7	39.92
Arith. mean	**49.30**
14	53.7
14	45.26
Arith. mean	**49.48**
30	24.08
30	26.79
Arith. mean	**25.44**
59	28.38
59	7.94
Arith. mean	**18.16**
90	21.52
90	13.83
Arith. mean	**17.68**
122	8.74
122	3.37
Arith. mean	**6.06**

The results of the determinations are summarised in Tables B.8.1.1.1-14 to B.8.1.1.1-16 and the plots of the decline and the residuals are given in Figures B.8.1.1.1-2 and B.8.1.1.1-3.

In Burgener (1998a) optimisation using SFO kinetics provided an acceptable statistical and visual fit to the data for soils incubated at 20°C, and SFO kinetics provided a better fit than FOMC kinetics. SFO kinetics were therefore considered appropriate to describe the degradation in these soils. For the soil incubated at 10°C SFO kinetics provided an acceptable statistical and visual fit but the fit of FOMC kinetics was better. However, looking at the visual fit, this appeared to be largely influenced by the fit beyond the DT_{90}. As it adequately described 90% of the decline, SFO kinetics were therefore concluded to be appropriate to describe the decline in this soil.

In Smalley *et al* (1997), although SFO kinetics provided an acceptable statistical fit to the Ohio loamy sand, FOMC kinetics provided the best fit to the data from both soils (both statistically and visually), therefore DFOP kinetics were also applied to the data. DFOP kinetics provided the best statistical and visual fit to the data. Although the P value for k1 in the Pennsylvania soil was marginally above 0.05 the fit was considered acceptable.

Table B.8.1.1.1-14: Summary of the results of the kinetic determinations for zoxamide in the soils incubated in Burgener (1998)

Model	Parameter	England silt loam 20°C 50%MWHC	France loam 20°C 50%MWHC	Germany sandy loam 20°C 50%MWHC	Italy clay loam 20°C 50%MWHC	Germany sandy loam 10°C 50% MWHC	Germany S loam 20°C 100%FC
SFO	χ^2 error (%)	**5.279**	**6.979**	**4.65**	**6.055**	**6.581**	**6.668**
	P	**1.9E-5**	**2.73E-5**	**5E-6**	**1.35E-5**	**2.36E-5**	**2.19E-5**
	k	**0.167**	**0.34**	**0.255**	**0.29**	**0.0897**	**0.305**
	DT_{50}	**4.16**	**2.03**	**2.7**	**2.38**	**7.726**	**2.27**
	DT_{90}	**13.8**	**6.74**	**9.01**	**7.91**	**25.665**	**7.55**
FOMC	χ^2 error (%)	5.695	7.505	5.024	6.548	5.286	7.204
	α^+	543.8 (NA)	19.369 (99.9)	775.6 (17690)	748 (7471)	2.53 (1.3)	2934 (72200)
	β^+	3257.8 (NA)	55.092 (292)	3034 (68980)	2568 (25665)	22.098 (13.8)	9613 (236700)
	DT_{50}	4.15	2.01	2.7	2.38	6.96	2.27
	DT_{90}	13.8	6.95	9.0	7.92	32.8	7.55

[+]Standard deviation given in brackets; figures in bold are those considered to provide the best fit

Table B.8.1.1.1-15: Summary of the results of the kinetic determinations for zoxamide in the soils incubated in Smalley *et al* (1997)

Model	Parameter	Pennsylvania silt loam	Ohio loamy sand
SFO	χ^2 error (%)	22.32	13.32
	P	0.057	9.17E-6
	k	0.03438	0.029
	DT_{50}	20.16	23.9
	DT_{90}	66.98	79.5
FOMC	χ^2 error (%)	11.38	5.056
	α^+	0.5103 (0.13)	0.81 (0.1)
	P	0.000983	2.17E-6
	β^+	2.62 (1.78)	10.23 (2.68)
	P	0.082060	0.00107
	DT_{50}	7.57	13.7
	DT_{90}	236.24	162.49
DFOP	χ^2 error (%)	**8.738**	**2.796**
	k1	**0.63**	**0.22**
	P	**0.079**	**3.62E-5**
	k2	**0.0177**	**0.0168**
	P	**0.000673**	**1.18E-8**
	g	**0.42**	**0.39**
	DT_{50}	**7.75**	**13.75**
	DT_{90}	**98.1**	**107.1**

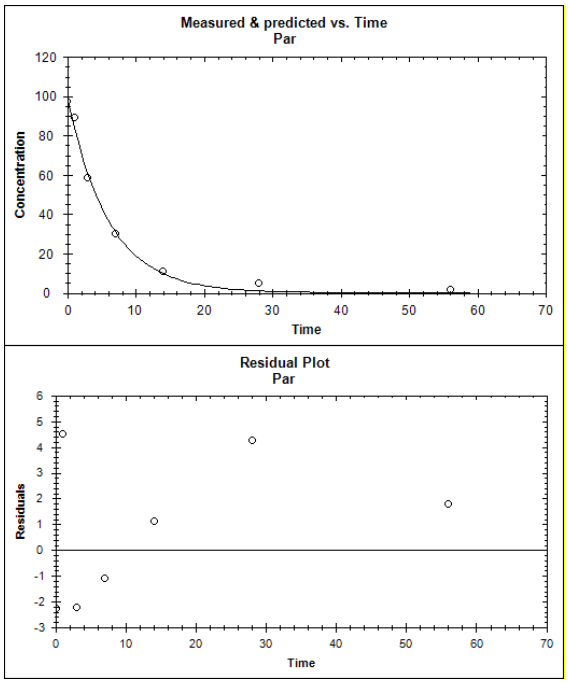
[+]Standard deviation given in brackets; figures in bold are those considered to provide the best fit

Table B.8.1.1.1-16: Summary of modelling and persistence end-points for zoxamide

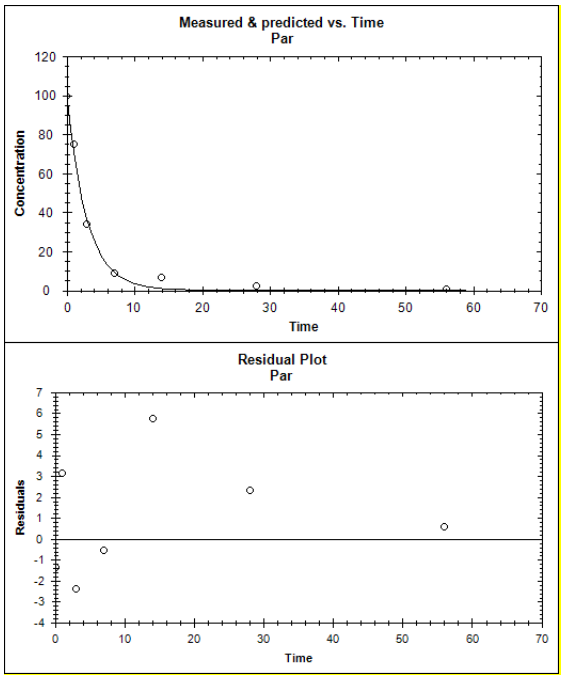
Compound	Soil	Model	DT_{50} (days)	DT_{90} (days)	χ^2 error (%)
Zoxamide	England silt loam 20°C 50%MWHC	SFO (persistence & modelling)	4.16	13.8	5.28
	France loam 20°C 50%MWHC	SFO (persistence & modelling)	2.03	6.7	6.98
	Germany sandy loam 20°C 50%MWHC	SFO (persistence & modelling)	2.7	9.0	4.65
	Italy clay loam 20°C 50%MWHC	SFO (persistence & modelling)	2.38	7.9	6.06
	Germany sandy loam 10°C 50%MWHC	SFO (persistence & modelling)	7.73	25.7	6.58
	Germany sandy loam 20°C 100%FC	SFO (persistence & modelling)	2.27	7.6	6.67
	Pennsylvania silt loam 25°C 75%FC	DFOP (persistence)	7.75	98.1	8.74
		DFOP (modelling) [a)]	39.2 [c)]	-	
	Ohio loamy sand 25°C 75%FC	DFOP (persistence)	13.75	107.1	2.80
		DFOP (modelling) [b)]	41.3 [c)]	-	

a) $k_1 = 0.63d^{-1}$, $k_2 = 0.0177d^{-1}$, g = 0.42
b) $k_1 = 0.22d^{-1}$, $k_2 = 0.0168d^{-1}$, g = 0.39
c) calculated from slow phase of DFOP modelling (= ln2/k2)

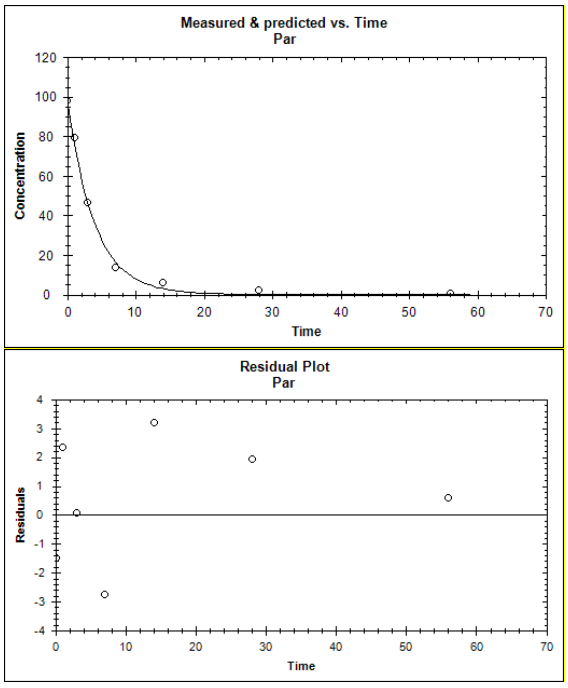
Figure B.8.1.1.1-2 Plot of the decline and the residuals (Burgener 1998)



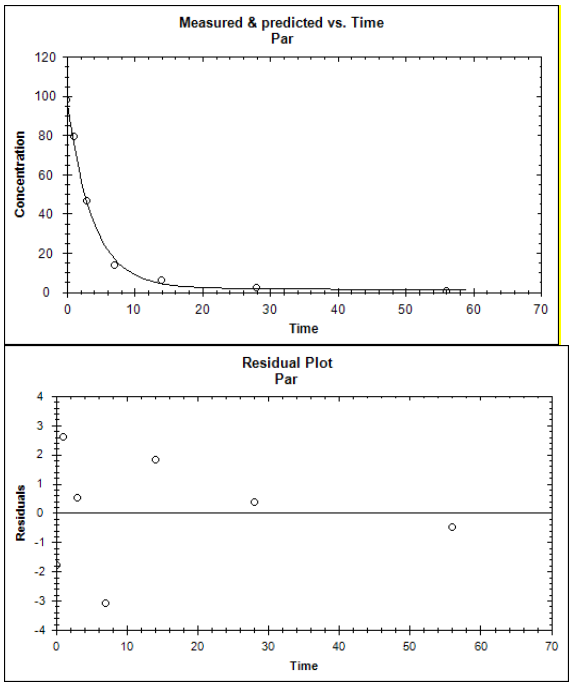
England silt loam 20°C 50%MWHC (SFO)



France loam 20°C 50%MWHC (SFO)



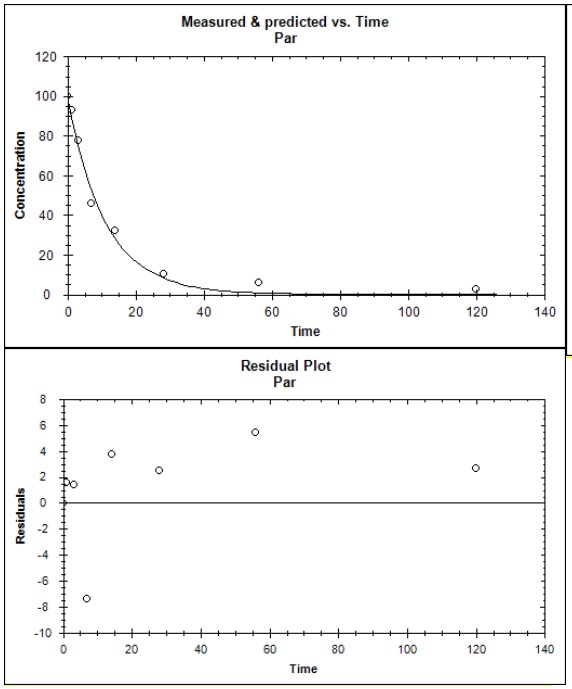
Germany sandy loam 20°C 50%MWHC (SFO)



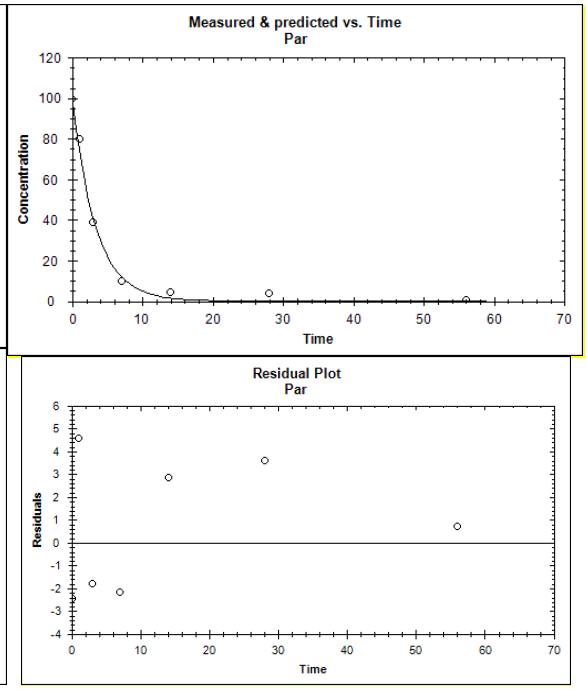
Italy clay loam 20°C 50%MWHC (SFO)

Figure B.8.1.1.1-3 Plot of the decline and the residuals (Burgener 1998 and Smalley et al 1997)

Burgener (1998)

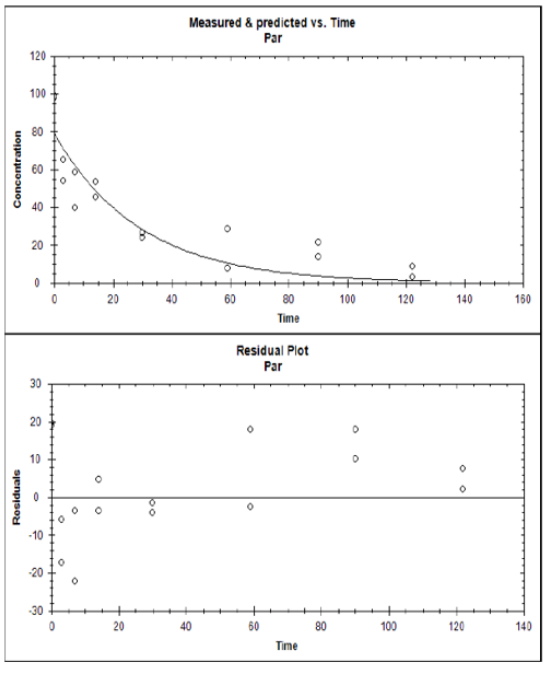


Germany sandy loam 10°C 50%MWHC (SFO)

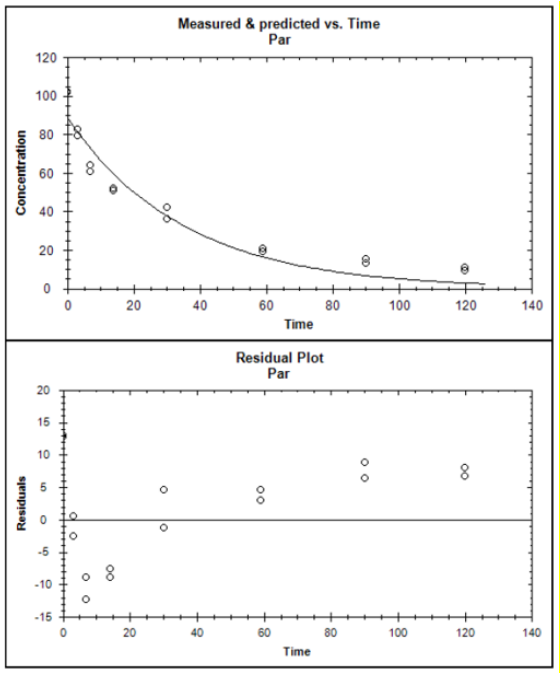


Germany sandy loam 20°C 100%FC (SFO)

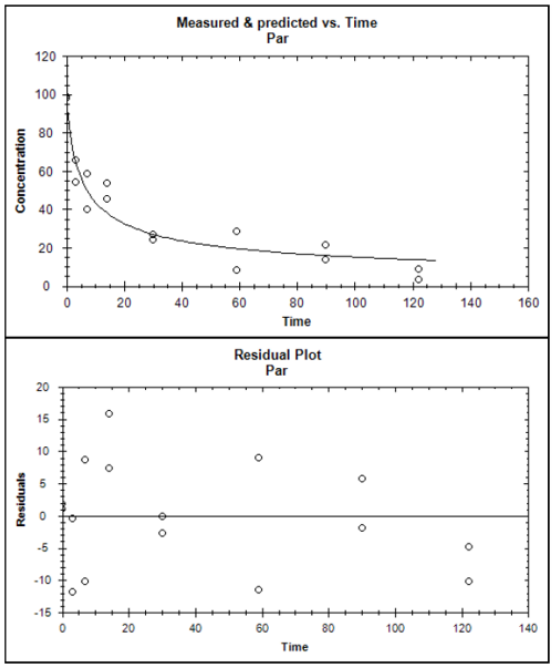
Smalley et al (1997)



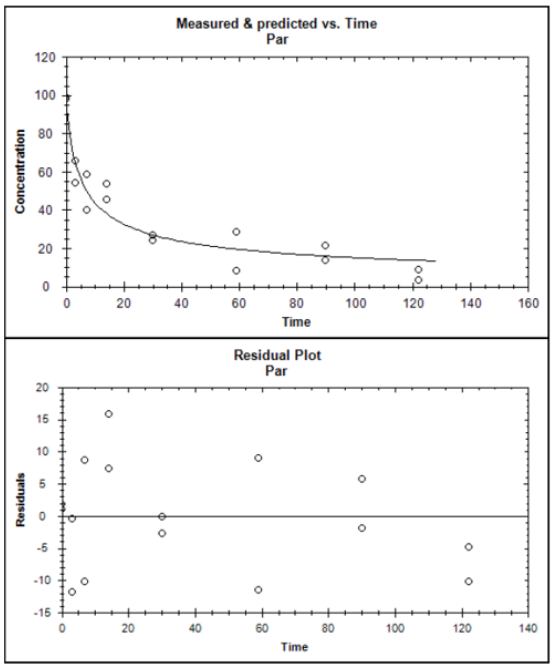
Pennsylvania silt loam 25°C 75%FC (SFO)



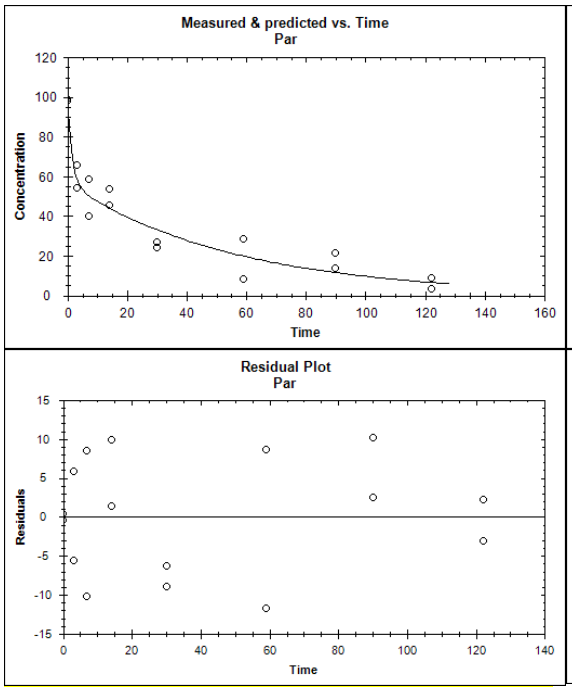
Ohio loamy sand 25°C 75%FC (SFO)



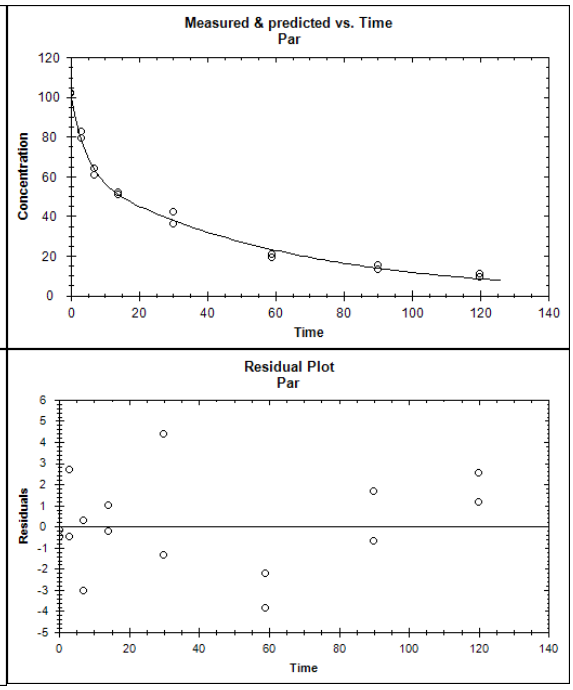
Pennsylvania silt loam 25°C 75%FC (FOMC)



Ohio loamy sand 25°C 75%FC (FOMC)



Pennsylvania silt loam 25°C 75%FC (DFOP)



Ohio loamy sand 25°C 75%FC (DFOP)

III. CONCLUSION

The SFO model satisfactorily describes the decline of zoxamide in all soils incubated in the study of Burgener (1998a). However, for soils incubated in the study of Smalley *et al* (1997) DFOP kinetics provides a better fit to the data. Persistence endpoints of DT_{50}s of 2.03 to 13.75 days at 10 to 25°C and modelling endpoints of DT_{50}s 2.03 to 41.3 days at 10 to 25°C were obtained.

RMS comment:

In general, the rates of degradation in the aerobic soil degradation studies of *Smalley & Reynolds (1997)* and *Burgerer (1998)* have been re-evaluated in line with recommendations of the FOCUS Kinetics Guidance Document (FOCUS 2006). However, according to FOCUS kinetic (2006) all data points should be included initially and the fitting repeated after exclusion of outliers. Only measurements which are clearly influenced by known analytical or procedural errors can be eliminated prior to analysis. Accordingly, a justification should be provided to explain the exclusion of the final occurrence of zoxamide (at day 120) in order to derive aerobic DT_{50} according to the FOCUS kinetic (2006).

Given the above, following justification was provided by Notifier: *Please note that for zoxamide the removed datapoints correspond to over 99% degradation in the majority of soils and ≥ 97.5% in all cases. Over 90% degradation was confirmed in at least 2 intervals used in the kinetics prior to the omitted data points. Even if the study had been continued for 1 year, the same amount would have been extracted as it corresponds only to the released bound residues. A biphasic model might give a better Chi2 value, but will almost give the same DT50 values.*

The SFO fits show clearly that the degradation ***is not*** *biphasic. According to FOCUS guidance the use of SFO is appropriate. For example see page 147 (FOCUS 2011): The use of a bi-phasic model may help improve the fit for the parent substance, but considering that >90% of its degradation is appropriately described by the SFO fit, and considering the very low Chi2 error value of 5, the SFO model can be considered appropriate.*

The same can be concluded for the one case where the RH-127450 metabolite data point was omitted (Italy clay loam soil – day 125). In this case the metabolite was observed at 0.1 % ARon day 28 and not detected on day 56 day.

The arguments of Notifier seem to be reasonable, hence RMS considers the study as acceptable.

Metabolite, breakdown and reaction products:

Reference:	Callow B., Hilton M. (2013a). Determination of rates of decline for zoxamide and its metabolites, in soil according to the guidance within the FOCUS Kinetics Guidance Document.
Guideline(s):	FOCUS Kinetics Guidance Document (2006)
GLP:	No (calculation - GLP is not relevant)
Previous evaluation:	No (submitted for the purpose of renewal of a.s. approval)
Validity of the study:	Considered acceptable

Executive Summary

The decline of the metabolites of zoxamide in six laboratory soils was modelled according to the recommendations of the FOCUS Kinetics Guidance Document using a step-wise approach. Although in many instances the chi^2 % error is >15%, P values are generally <0.1 and visual fits are acceptable with only an unacceptable fit identified for two of the metabolites in two soils. Persistence and modelling DT_{50}s were 1.99 to 11.69 days at 20°C (17.8 days at 10°C) for RH-127450, 3.05 to 16.23 days at 20 to 25°C for RH-24549, 5.62 to 53.65 days at 20°C (55.5 days at 10°C) for RH-163353 and 195.2 days at 20°C for RH-141455.

I. MATERIAL AND METHODS

Rates of degradation were calculated according to the guidance of the FOCUS Degradation Kinetics Workgroup, using KinGui Version 2.0 (Bayer CropScience 2011).

The approach used followed that given in Chapter 7 of the FOCUS Kinetics Guidance Document. The suitability of the fit of the models was evaluated both visually and statistically by calculating the minimum % error required to pass the chi^2 test at a probability of 0.05 (acceptability criteria chi^2 error < 15%).

The parent compound and any metabolites detected above the thresholds given in Sanco/221/2000 (European Commission 2003) were included in the kinetic evaluations, although RH-127450 and RH-141455 were included in the determinations for the Italian clay loam soil and German sandy loam soil (100%FC) respectively where there were only single detections >5%.

Prior to running the kinetic evaluation the appropriate metabolism scheme was investigated. In the Draft Assessment Report for zoxamide (2001) the degradation scheme given in Figure B.8.1.1.1-1 is proposed. However, the metabolites RH-127450, RH-24549 and RH-163353 are very rapidly formed peaking at around the same time (3 to 7 days at 20°C in Burgener (1998a)); therefore direct formation from the parent was assumed for these three metabolites for the purposes of this kinetic evaluation. The metabolite RH-141455 is formed slightly later peaking at 14 to 28 days, therefore this was assumed to be formed from RH-24549.

II. RESULTS AND DISCUSSION

The detections of zoxamide and its metabolites in the studies of Smalley and Reynolds (1997) and Burgener (1998a) are given in Tables B.8.1.1.1-6 to B.8.1.1.1-13.

The results of the determinations are summarised in Table B.8.1.1.1-17 and B.8.1.1.1-20 and the plots of the decline and the residuals are given in Figures B.8.1.1.1-4 to B.8.1.1.1-10.

For RH-127450 an acceptable statistical and visual fit is obtained in the England, France, and Germany 50%WHC/10°C soils. In the Italy, Germany 50%WHC/20°C and Germany 100%FC/20°C soils the chi^2 % error was >15%, however in all instances P <0.05 and the visual fit to the data was considered acceptable. In the England soil the last two time-points were slightly underestimated and in the Italian, German 20°C/100%FC and German 20°C/50%MWHC soils the peak was underestimated but these were not considered severe enough to rule the visual fit unacceptable.

For RH-24549 chi^2 % errors in all instances were above 15%, although for the Germany 100%FC/20°C soil only marginally so; however, P<0.05 in all except the England and Germany 50%WHC/10°C soils. In the case of the England soil the visual fit is poor due to the variability of the data, underestimating the peak and final time-point. The DT_{50} from the England soil is therefore not reliable. For the Germany 50%MWHC/10°C soil P <0.1 and the initial peak is well predicted although the final two time-points are significantly underestimated. The DT_{50} from this soil is therefore considered unreliable. For the France, Germany 50%MWHC/20°C, Italy and Germany 100%FC/20°C soils the visual fit is considered acceptable though there is a slight underestimation of the final two time-points in the former two soils. For the Ohio soil the chi^2 % error was >15%, however P<0.05 and the visual fit was considered acceptable.

For RH-163353 an acceptable visual and statistical fit is obtained for the Italy and Germany 100%FC/20°C soils. In the other soils the chi^2 % error was >15%, however for all soils P<0.05 and the visual fit is considered acceptable although there is a slight underestimation of the final time-points in the France and Germany 50%MWHC/20°C soils. In the England soil the fit was probably affected by the day 14 value.

For RH-141455 an acceptable fit could not be obtained for the Germany100%FC /20°C soil largely as no degradation was predicted for this metabolite. Therefore the kinetics included in the report are those fitted to the data for this soil without this metabolite. For the Germany 50%MWHC/20°C soil an

acceptable chi^2 % error was obtained and the visual fit was acceptable. P>0.05, however although it was marginally >0.1 the visual fit was considered acceptable.

For many of the metabolites, although the formation and the majority of the decline were generally well represented, the goodness of fit was affected by low residue values persisting at the final time-points. Such low level values for metabolites are not unusual in soil metabolism studies.

Table B.8.1.1.1-17: Summary of the results of the kinetic determinations for RH-127450, RH-24549, RH-163353 and RH-141455 in the soils incubated in Burgener (1998), all SFO

Compound	Parameter	England silt loam 20°C 50%MWHC	France loam 20°C 50%MWHC	Germany sandy loam 20°C 50%MWHC	Italy clay loam 20°C 50%MWHC	Germany sandy loam 10°C 50% MWHC	Germany S loam 20°C 100%FC
RH-127450	FF	0.26	0.21	0.18	0.21	0.1721	0.196
	χ2 error (%)	10.16	8.103	18.093	16.762	11.945	22.43
	P	1.45E-5	3.39e-8	8.23E-5	0.0005	0.0007	0.001
	k	0.059	0.183	0.104	0.349	0.03899	0.12
	DT50	11.69	3.78	6.66	1.99	17.8	5.76
	DT90	38.84	12.57	22.14	6.60	59.05	19.13
RH-24549	FF	0.054	0.19	0.173	0.47	0.3117	0.276
	χ2 error (%)	28.15	21.764	30.797	22.71	26.698	15.05
	P	0.123	0.0064	0.008	0.001	0.0696	0.0001
	k	0.012	0.110	0.13	0.082	0.094	0.227
	DT50	54.22	6.29	5.35	8.44	7.36	3.05
	DT90	180.13	20.89	17.77	28.03	24.48	10.15
RH-163353	FF	0.089	0.20	0.293	0.23	0.1503	0.185
	χ2 error (%)	19.39	23.634	16.144	6.754	16.459	12.97
	P	0.0163	0.0114	0.0003	1.48e-8	0.0078	0.0002
	k	0.013	0.105	0.123	0.108	0.0125	0.067
	DT50	53.65	6.62	5.62	6.39	55.5	9.9
	DT90	178.23	21.99	18.67	21.24	184.36	32.9
RH-141455	FF*	-	-	0.50	-	-	-
	χ2 error (%)	-	-	14.271	-	-	-
	P	-	-	0.12	-	-	-
	k	-	-	0.0036	-	-	-
	DT50	-	-	195.2	-	-	-
	DT90	-	-	648.5	-	-	-

Table B.8.1.1.1-18: Summary of the results of the kinetic determination for RH-24549 in the soils incubated in Smalley *et al* (1997), all SFO

Compound	Parameter	England silt loam 20°C 50%MWHC
RH-24549	FF	0.17
	χ2 error (%)	21.052
	P	5.28E-5
	k	0.0427
	DT50	16.23
	DT90	53.9

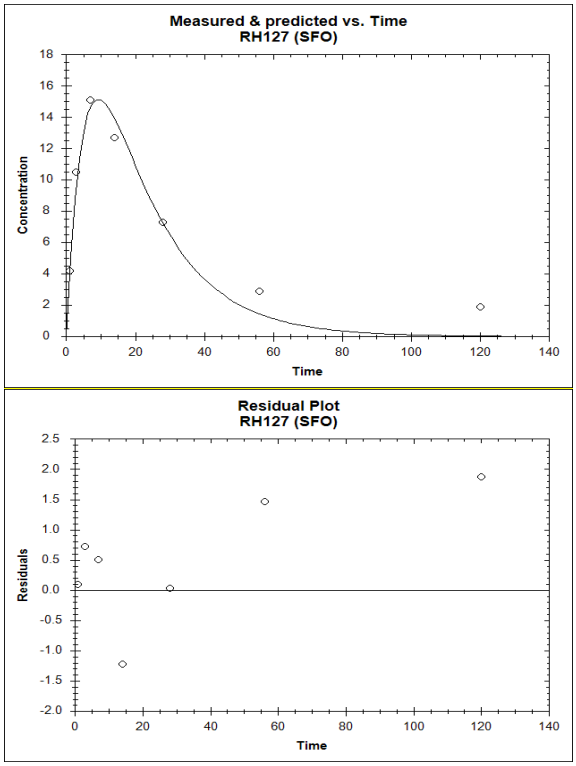
Table B.8.1.1.1-19: Calculated degradation parameters for the metabolites of zoxamide

Compound	Soil	Model	DT_{50} (days)	DT_{90} (days)	χ^2 error (%)
RH-127450	England silt loam 20°C 50%MWHC	SFO (persistence & modelling)	11.69	38.84	10.16
	France loam 20°C 50%MWHC	SFO (persistence & modelling))	3.78	12.57	8.10
	Germany sandy loam 20°C 50%MWHC	SFO (persistence & modelling)	6.66	22.14	18.093
	Italy clay loam 20°C 50%MWHC	SFO (persistence & modelling)	1.99	6.6	16.76
	Germany sandy loam 10°C 50%MWHC	SFO (persistence & modelling)	17.8	59.05	11.95
	Germany sandy loam 20°C 100%FC	SFO (persistence & modelling)	5.76	19.13	22.43
RH-24549	England silt loam 20°C 50%MWHC	Fit unacceptable			
	France loam 20°C 50%MWHC	SFO (persistence & modelling)	6.29	20.89	21.76
	Germany sandy loam 20°C 50%MWHC	SFO (persistence & modelling)	5.35	17.77	30.797
	Italy clay loam 20°C 50%MWHC	SFO (persistence & modelling)	8.44	28.03	22.71
	Germany sandy loam 10°C 50%MWHC	Fit unacceptable			
	Germany sandy loam 20°C 100%FC	SFO (persistence & modelling)	3.05	10.15	15.05
	Ohio loamy sand 25°C 75%FC	SFO (persistence & modelling)	16.23	53.9	21.05
RH-163353	England silt loam 20°C 50%MWHC	SFO (persistence & modelling)	53.65	178.23	19.39
	France loam 20°C 50%MWHC	SFO (persistence & modelling)	6.62	21.99	23.63
	Germany sandy loam 20°C 50%MWHC	SFO (persistence & modelling)	5.62	18.67	16.144
	Italy clay loam 20°C 50%MWHC	SFO (persistence & modelling)	6.39	21.24	6.75
	Germany sandy loam 10°C 50%MWHC	SFO (persistence & modelling)	55.5	184.36	16.46
	Germany sandy loam 20°C 100%FC	SFO (persistence & modelling)	9.9	32.9	12.97
RH-141455	Germany sandy loam 20°C 50%MWHC	SFO (persistence & modelling)	195.2	648.5	14.271
	Germany sandy loam 20°C 100%FC	Fit unacceptable			

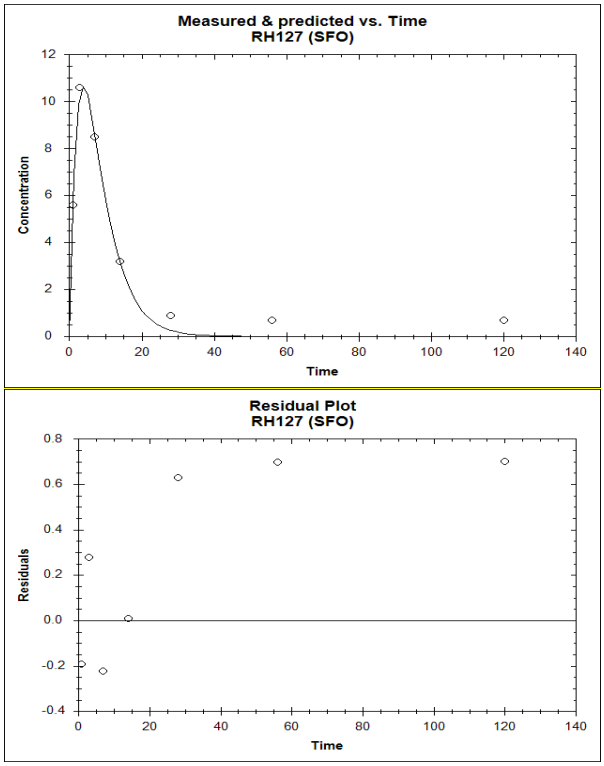
Table B.8.1.1.1-20: Calculated formation fractions for the metabolites of zoxamide

Compound	Soil	FF
RH-127450	England silt loam 20°C 50%MWHC	0.26
	France loam 20°C 50%MWHC	0.21
	Germany sandy loam 20°C 50%MWHC	0.18
	Italy clay loam 20°C 50%MWHC	0.21
	Germany sandy loam 10°C 50%MWHC	0.17
	Germany sandy loam 20°C 100%FC	0.20
RH-24549	France loam 20°C 50%MWHC	0.19
	Germany sandy loam 20°C 50%MWHC	0.17
	Italy clay loam 20°C 50%MWHC	0.47
	Germany sandy loam 20°C 100%FC	0.28
	Ohio loamy sand	0.17
RH-163353	England silt loam 20°C 50%MWHC	0.09
	France loam 20°C 50%MWHC	0.20
	Germany sandy loam 20°C 50%MWHC	0.29
	Italy clay loam 20°C 50%MWHC	0.23
	Germany sandy loam 10°C 50%MWHC	0.15
	Germany sandy loam 20°C 100%FC	0.185
RH-141455	Germany sandy loam 20°C 50%MWHC	0.50

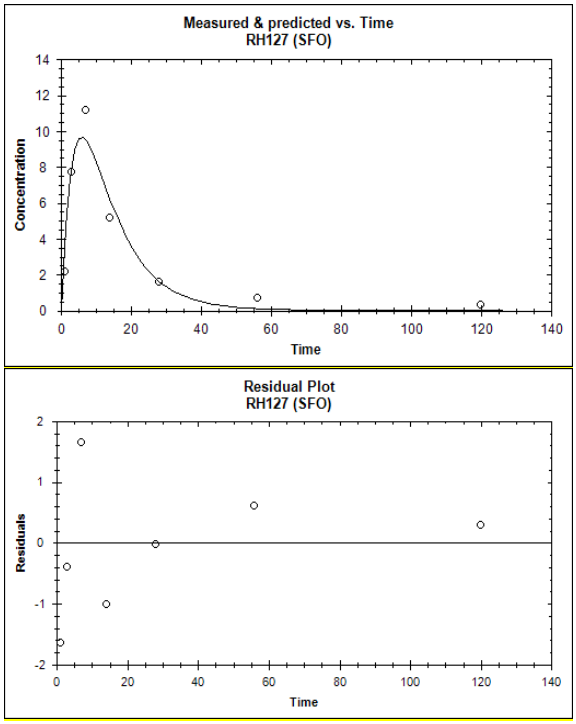
Figure B.8.1.1.1-4 Plot of the decline and the residuals –RH-127450



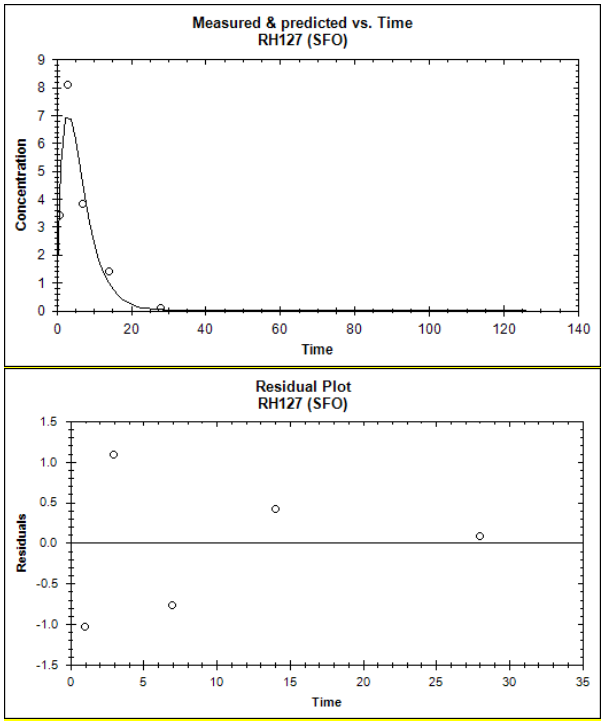
England silt loam 20°C 50%MWHC (Burgener 1998)



France loam 20°C 50%MWHC (Burgener 1998)

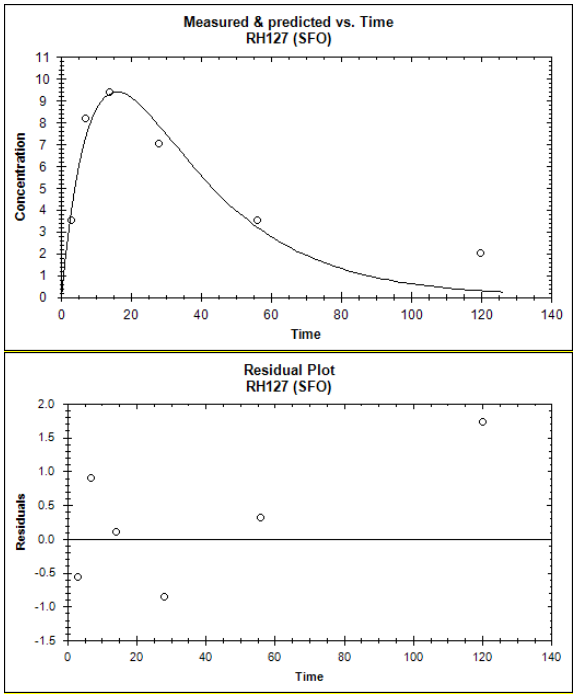


Germany sandy loam 20°C 50%MWHC (Burgener 1998)

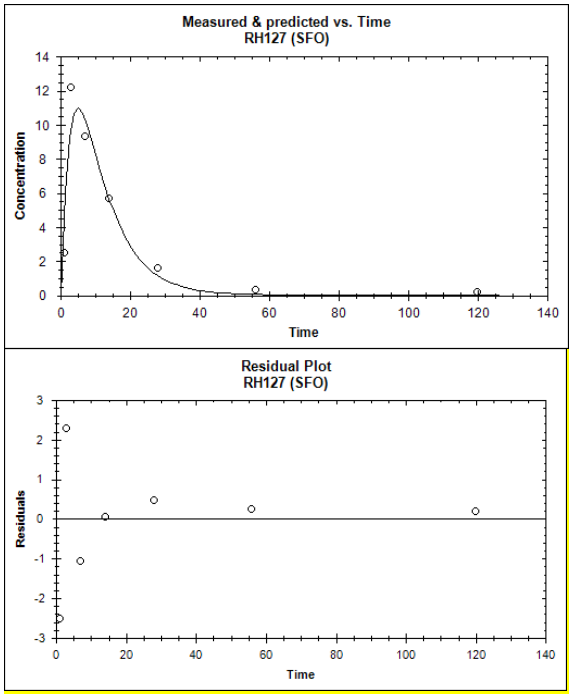


Italy clay loam 20°C 50%MWHC (Burgener 1998)

Figure B.8.1.1.1-5 Plot of the decline and the residuals –RH-127450

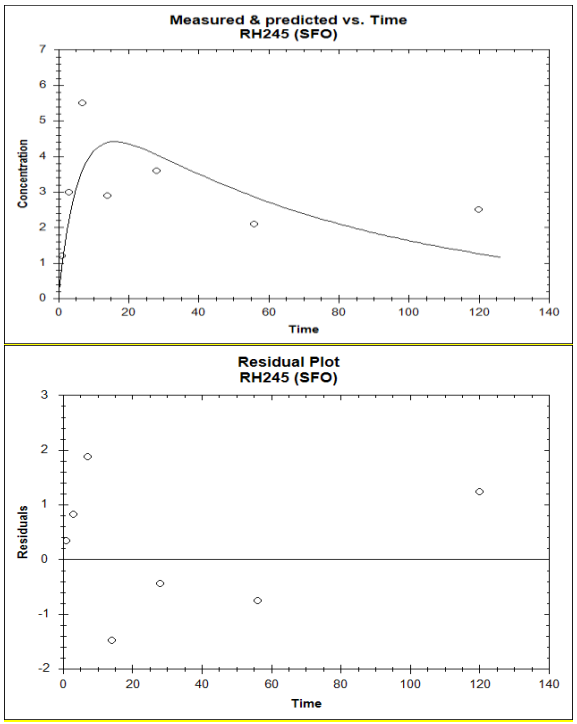


Germany sandy loam 10°C 50%MWHC (Burgener 1998)

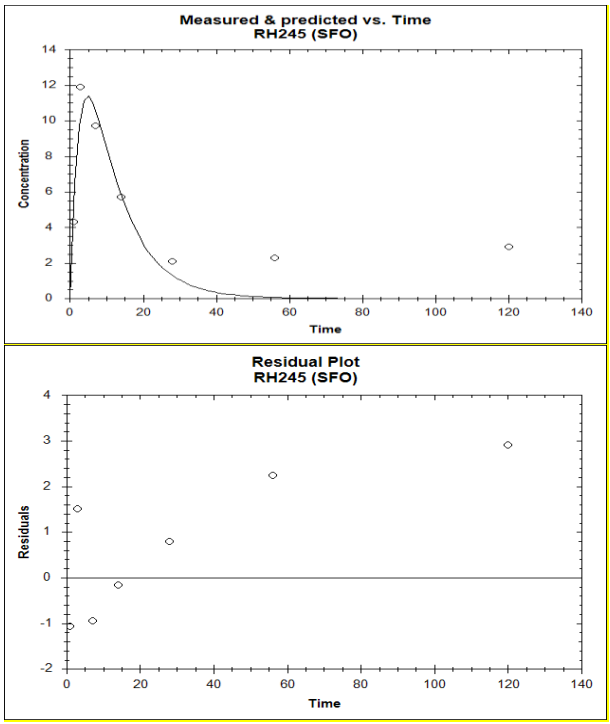


Germany sandy loam 20°C 100%FC (Burgener 1998)

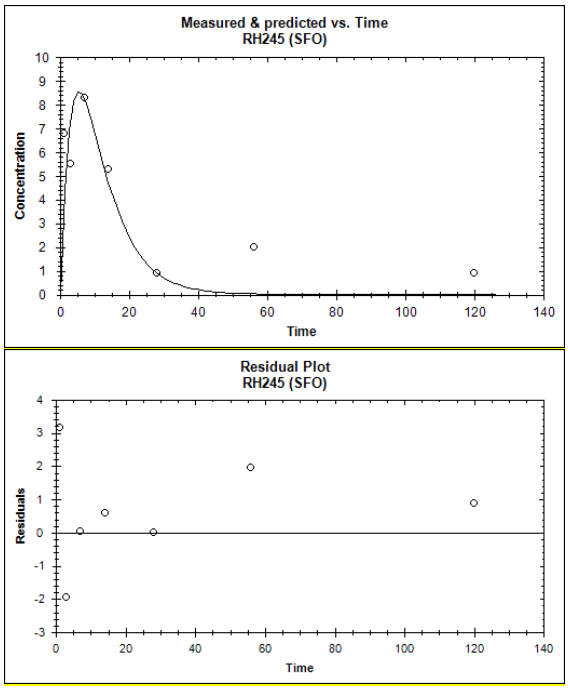
Figure B.8.1.1.1-6 Plot of the decline and the residuals –RH-24549



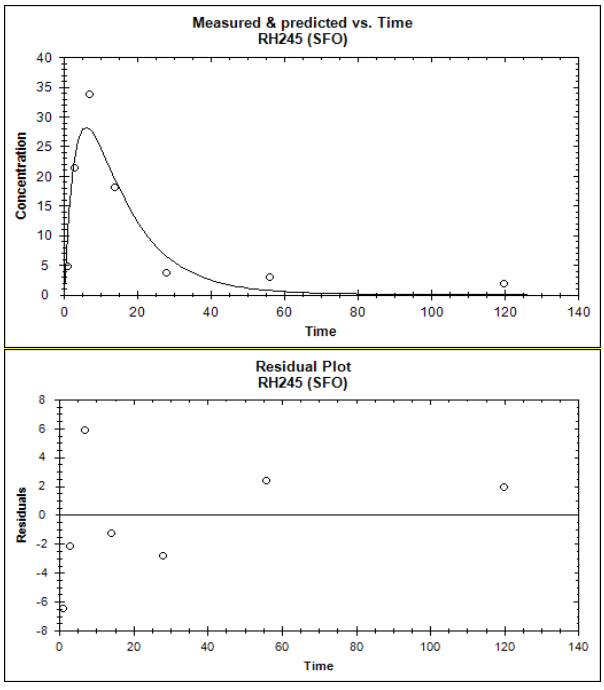
England silt loam 20°C 50%MWHC (Burgener 1998)



France loam 20°C 50%MWHC (Burgener 1998)

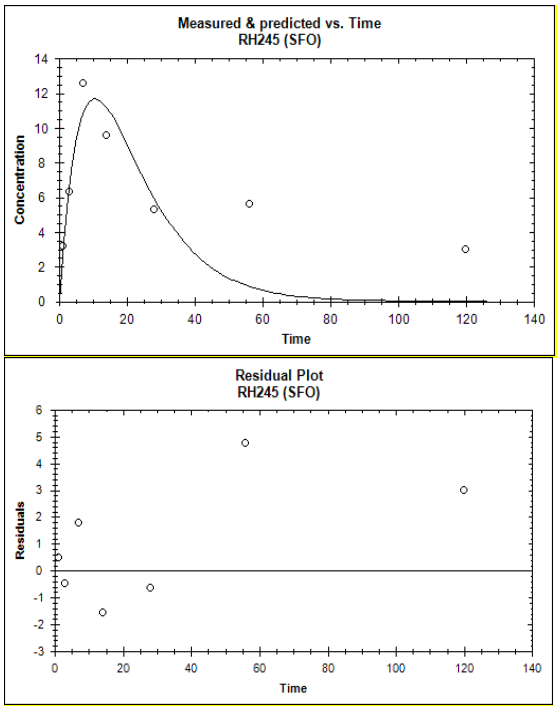


Germany sandy loam 20°C 50%MWHC (Burgener 1998)

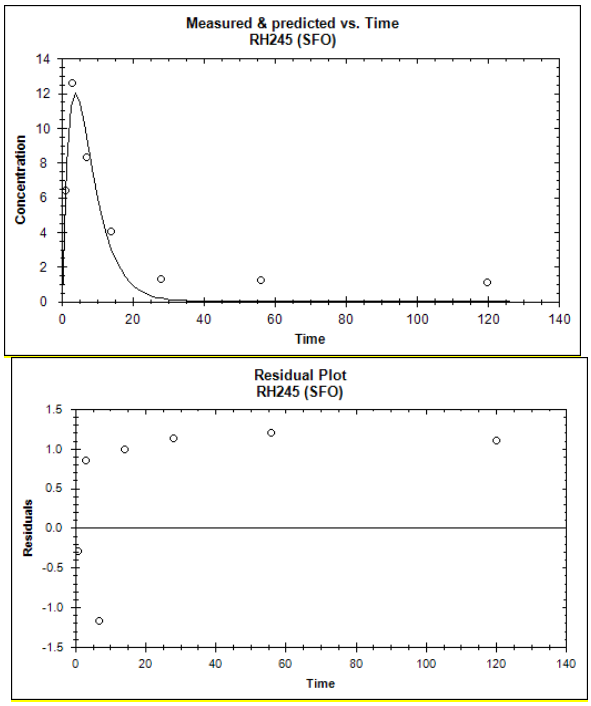


Italy clay loam 20°C 50%MWHC (Burgener 1998)

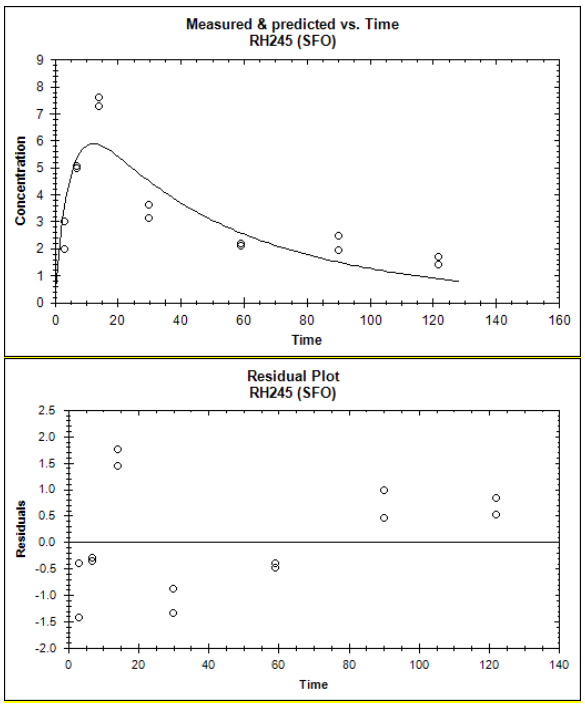
Figure B.8.1.1.1-7 Plot of the decline and the residuals –RH-24549



Germany sandy loam 10°C 50%MWHC (Burgener 1998)

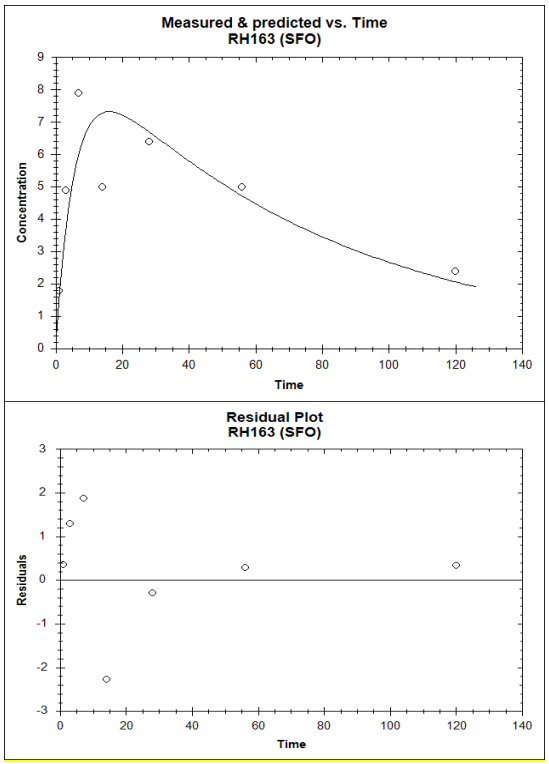


Germany sandy loam 20°C 100%FC (Burgener 1998)

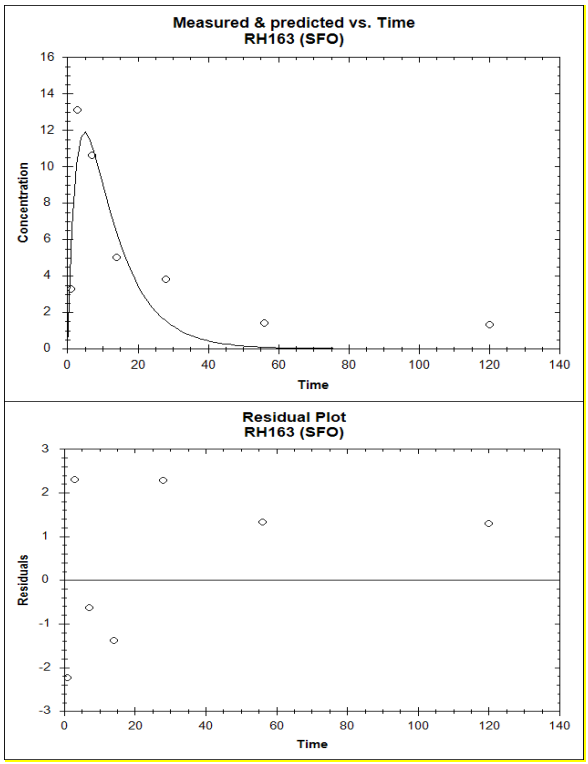


Ohio loamy sand 25°C 75%FC (Smalley et al 1997)

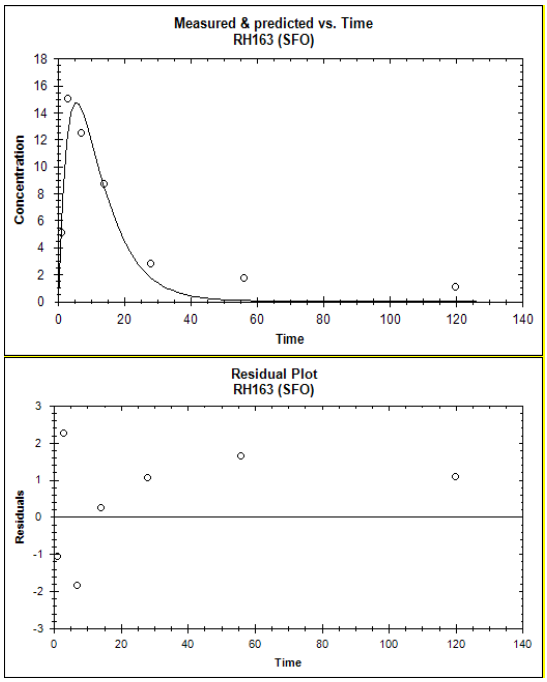
Figure B.8.1.1.1-8 Plot of the decline and the residuals –RH-163353



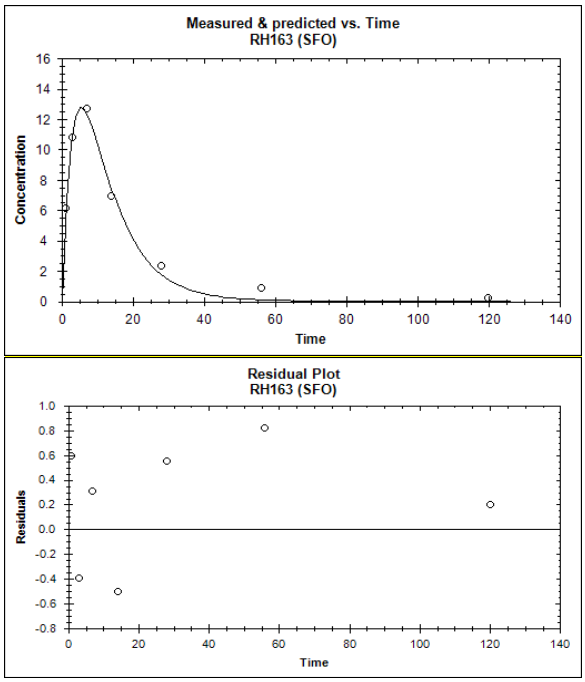
England silt loam 20°C 50%MWHC (Burgener 1998)



France loam 20°C 50%MWHC (Burgener 1998)

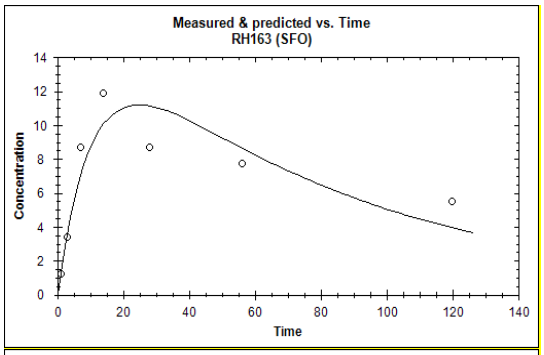


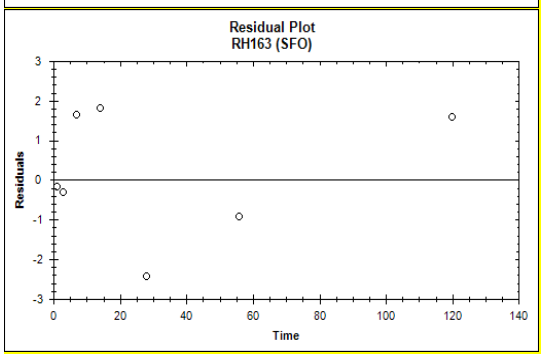
Germany sandy loam 20°C 50%MWHC (Burgener 1998)



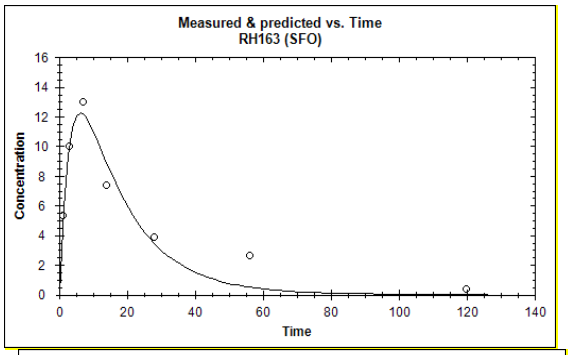
Italy clay loam 20°C 50%MWHC (Burgener 1998)

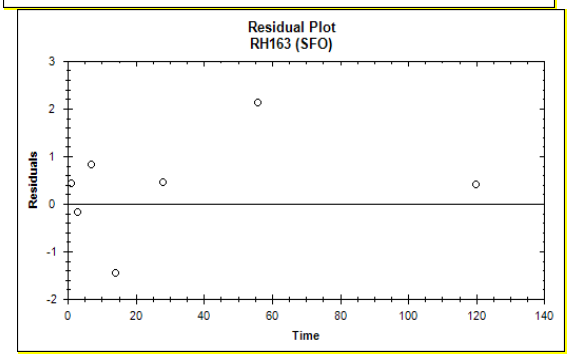
Figure B.8.1.1.1-9 Plot of the decline and the residuals –RH-163353





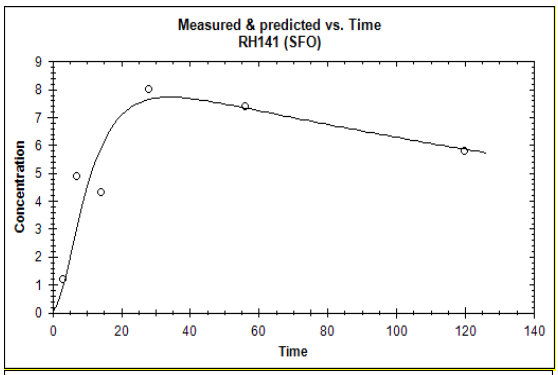
Germany sandy loam 10°C 50%MWHC (Burgener 1998)

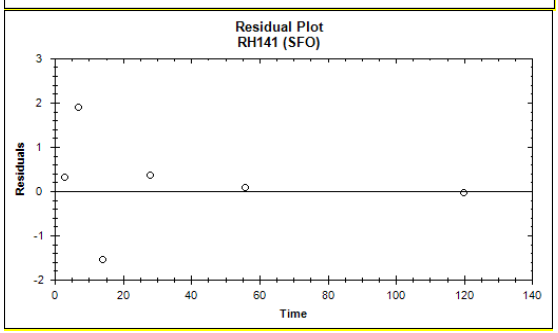




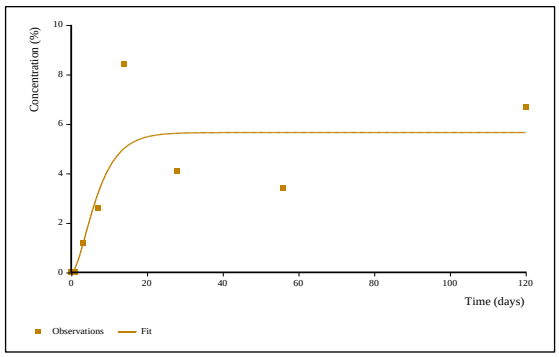
Germany sandy loam 20°C 100%FC (Burgener 1998)

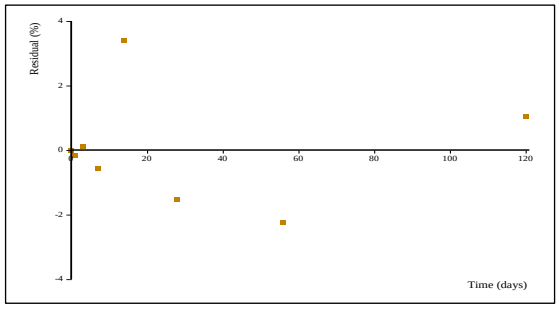
Figure B.8.1.1.1-10 Plot of the decline and the residuals –RH-141455





Germany sandy loam 20°C 50%MWHC(Burgener 1998)





Germany sandy loam 20°C 100%FC (Burgener 1998)

III. CONCLUSION

The decline of the metabolites of zoxamide in six laboratory soils was modelled according to the recommendations of the FOCUS Kinetics Guidance Document. Although in many instances the chi^2 % error is >15%, P values are generally <0.1 and visual fits are acceptable with only an unacceptable fit identified for two of the metabolites in two soils. Persistence and modelling DT_{50}s were 1.99 to 11.69 days at 20°C (17.8 days at 10°C) for RH-127450, 3.05 to 16.23 days at 20 to 25°C for RH-24549, 5.62 to 53.65 days at 20°C (55.5 days at 10°C) for RH-163353 and 195.2 days at 20°C for RH-141455.

RMS comment:

The rates of degradation in the aerobic soil degradation studies of *Smalley & Reynolds (1997)* and *Burgerer (1998)* have been re-evaluated according to the recommendations of the FOCUS Kinetics Guidance Document (FOCUS 2006). The study is acceptable and the results can be used for risk assessment purposes.

Reference:	Van den Bosch M.M.H. (2013a). Determination of the aerobic degradation rate of RH-141455 in soil.
Guideline(s):	OECD Guideline 307 "Aerobic and Anaerobic Transformation in Soil" (April 2002)
GLP:	Yes
Previous evaluation:	Submitted for the purpose of renewal
Validity of the study:	Considered acceptable

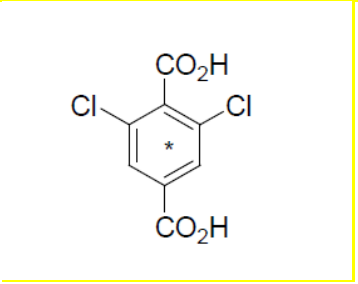
Executive Summary

The degradation rate (DT_{50} and DT_{90}) of RH-141455 was determined in three different soils (Speyer 2.2, Speyer 2.3 and Speyer 6S) according to OECD 307 guidelines. ^{14}C-labelled RH-141455 was incubated aerobically in the three soils in the dark at 20°C ± 2°C and 40% MWHC. The duration of the incubation was 63 days for Speyer 2.2 and Speyer 2.3 and 120 days for Speyer 6S. ^{14}C-RH-141455 was applied at a rate of 0.2 mg/kg dry weight of soil, corresponding to 150 g/ha assuming 100% formation from parent. The concentration of ^{14}C RH-141455 in each soil was determined at day 0, 3, 7, 14, 28, 42 (Speyer 2.2 and Speyer 2.3), 63 and 120 (Speyer 6S). Overall mass balances were between 91% and 118% of applied radioactivity (AR). ^{14}C-labelled RH-141455 gradually degraded. At the end of the incubation period 6%AR (Speyer 2.2), 5%AR (Speyer 2.3) and 11%AR (Speyer 6S) was in the soil extracts, and levels of the test item were not detectable. Significant levels of $^{14}CO_2$ were detected in NaOH traps, with a maximum of 56%AR (Speyer 2.2), 50%AR (Speyer 2.3) and 51%AR (Speyer 6S) at study termination. Unextractable residues occurred at maximums of 31%AR (Speyer 2.2), 40%AR (Speyer 2.3) and 47%AR (Speyer 6S) after 42 to 120 days incubation. The DT_{50} and DT_{90} were calculated according to the FOCUS Kinetics Guidance Document (2006, 2011), and DT_{50}s were 12.0 days (SFO, DT_{90} of 40.0 days) for Speyer 2.2 , 11.1 days for Speyer 2.3 (SFO, DT_{90} of 36.9 days) and 31.7 days for Speyer 6S (SFO, DT_{90} of 105.3 days).

I. MATERIAL AND METHODS

The test material was [Phenyl-UL-^{14}C]-labelled RH-141455 (Figure B.8.1.1.1-11) with a Radiochemical purity of 99.9 %, Specific activity of 1998 MBq/mmol (54 mCi/mmol) and Batch Number – 76045-5-20.

Figure B.8.1.1.1-11: Structure of radiolabelled metabolite [Phenyl-UL-^{14}C]RH-141455



*position of ^{14}C

Table B.8.1.1.1-21: Soil characteristics

Soil	**Speyer 2.2**	**Speyer 2.3**	**Speyer 6S**
Location	Hanhofen, Rheinland-Pfalz, Germany	Offenbach, Rheinland-Pfalz, Germany	Siebeldingen, Rheinland-Pfalz, Germany
Texture (USDA)	Loamy sand	Sandy loam	Clay
Organic carbon (%)	1.74	1.00	1.66
pH (0.01 M $CaCl_2$)	5.5	6.8	7.1
CEC (meq/100g)	10.2	10.7	26.9
Sampling date	15.05.2013	15.05.2013	15.05.2013
Field history (2009-2013):			
Pesticides	none	none	none
Fertilizers	none	none	none
Vegetation	meadow	uncultivated	uncultivated
Microbial biomass (µg C/g):			
Prior to incubation	312	158	253
End of incubation	Not determined*		402
Particle size distribution:			
% clay (<0.002 mm)	8.2	8.7	40.7
% silt (0.002-0.05 mm)	15.3	28.2	34.5
% sand (> 0.05 mm)	76.5	63.1	24.8
Maximum water holding capacity (g water/100 g soil)	42.5	38.2	40.1

* flasks for determination of biomass were accidentally discarded. However, as almost the same amounts of $^{14}CO_2$ were detected for all soils and degradation was rapid and first order no significant decrease of the microbial biomass during the incubations is likely.

Sieved soil samples (2mm) were stored at 4°C until use and brought to 40% maximum water holding capacity.

100 g soil samples were treated with ^{14}C-RH-141455 at an application rate of 0.2 mg/kg dwt dissolved in methanol. This was based on application rate of the parent substance zoxamide of 150 g a.s./ha, assuming 100% metabolite formation without taking into account differences in molecular weight and an even distribution in the top soil layer of 5 cm and bulk density of 1500 kg/m^3.

The soils were incubated in the dark at 17.5 - 22°C in cylindrical metabolism flasks, which were aerated continuously with CO_2 reduced humidified air. Volatiles were trapped by polyurethane foam, ethylene glycol monoethyl ether and NaOH traps.

On each sampling day (day 0, 3, 7, 14, 28, 42 (Speyer 2.2 and Speyer 2.3), 63 and 120 days (Speyer 6S)), one metabolism flask per soil was removed. The NaOH traps were replaced after 8, 15, 29, 41, 57, 72 and 90 days of incubation.

Different extraction methods were used for both soils Speyer 2.2/Speyer 2.3 and Speyer 6S. Based on the preliminary test and the extractions of time 0, soil Speyer 6S showed a much higher formation of bound

residues when compared to the other soils, as it is a clay soil. Therefore, a much harsher extraction solvent was used for all soils, although the the clay soil contained slightly more acid and was extracted 5 times with the extraction solvent compared to 3 times for the other two soils. Soil samples of Speyer 2.2 and Speyer 2.3 were extracted with 70/30/0.5 ((v/v/v) acetonitrile/milli-Q water/HCl) and Speyer 6S soil was extracted with 70/30/1.0 ((v/v/v) acetonitrile/milli-Q water/HCl). Radioactivity in the extracts was quantified by LSC and ^{14}C-RH-141455 residues were determined by HPLC co-chromatography.

The radioactivity trapped in the tested NaOH traps was confirmed to be CO_2 after precipitation with barium hydroxide. Bound residues were determined by combustion and LSC.

The rate of degradation of RH-141455 was estimated using KinGui Software Version 2.0 according to the FOCUS Guidance Document on estimating degradation kinetics (FOCUS 2006, 2011).

Test substance data were fitted to single first order kinetics (SFO) and to the Gustafson and Holden model (FOMC). If the SFO fit was acceptable and better than the FOMC fit (based on visual assessment, t-test, and χ^2 test), no further work was done. If the SFO fit was not acceptable or the FOMC fit was better, the data were also fitted to the hockey stick model (HS) and the bi-exponential model (DFOP).

II. RESULTS AND DISCUSSION

The distribution of radioactivity in the three soils is given in Tables B.8.1.1.1-22 to B.8.1.1.1-24. Overall mass balances were between 91% and 118%.

In all the soils negligible amounts of radioactivity (0%) were found in the polyurethane foam plugs and ethylene glycol monoethyl ether traps (organic volatiles). In the NaOH traps significant amounts of radioactivity were detected (confirmed as CO_2), increasing to a maximum of 56%AR (Speyer 2.2), 50%AR (Speyer 2.3) and 51%AR (Speyer 6S) at the end of the incubation period (63 days for Speyer 2.2 and Speyer 2.3 and 120 days for Speyer 6S).

Throughout the incubation period the extractable activity gradually decreased. After 14 days of incubation the radioactivity recovered in the soil extracts decreased to 53% AR (Speyer 2.2), 41%AR (Speyer 2.3) and 83%AR (Speyer 6S), whereas the unextractable activity, after 14 days of incubation, had increased to 22%AR (Speyer 2.2), 26%AR (Speyer 2.3) and 14%AR (Speyer 6S). At the end of the incubation period radioactivity recovered in the soil extracts had decreased to 6%AR (Speyer 2.2), 5%AR (Speyer 2.3) and 11%AR (Speyer 6S), whereas the unextractable activity, at the end of the incubation, was 31%AR (Speyer 2.2), 40%AR (Speyer 2.3) and 47%AR (Speyer 6S).

Table B.8.1.1.1-22: Distribution of radioactivity in Speyer 2.2 soil (% of applied radioactivity)

Time (days)	Organic volatiles	CO_2	Soil extract	Unextractable residues	Mass balance
0	na	na	99.1	1.4	100.4
3	0.0	2.1	92.3	5.6	100.0
7	0.0	6.5	80.0	11.6	98.1
14	0.0	21.9	53.3	21.6	96.8
28	0.0	44.2	22.0	27.0	93.2
42	0.0	51.8	13.3	31.6	96.7
63	0.0	55.5	6.2	30.5	92.2

na: not analysed

Table B.8.1.1.1-23: Distribution of radioactivity in Speyer 2.3 soil (% of applied radioactivity)

Time (days)	Organic volatiles	CO_2	Soil extract	Unextractable residues	Mass balance
0	na	na	98.4	1.0	99.4
3	0.0	6.6	78.7	14.0	99.3
7	0.0	11.4	69.8	19.9	101.0
14	0.0	23.8	40.8	26.1	90.7
28	0.0	39.7	22.3	35.9	98.0
42	0.0	43.7	15.0	42.6	101.3
63	0.0	49.7	4.6	39.7	94.0

na: not analysed

Table B.8.1.1.1-24: Distribution of radioactivity in Speyer 6S soil (% of applied radioactivity)

Time (days)	Organic volatiles	CO_2	Soil extract	Unextactable residues	Mass balance
0	na	na	93.6	7.5	101.2
3	0.0	0.5	104.6	13.0	118.2*
7	0.0	2.2	83.4	16.5	102.1
14	0.0	3.5	83.3	14.0	100.8
28	0.0	18.4	57.5	24.6	100.4
63	0.0	40.1	30.9	28.0	99.0
120	0.0	50.5	11.3	46.5	108.3

na: not analysed
* exceeds 110% AR due to unrealistic high activity measured in the soil extract (105%) as it was not well homogenised. After extraction with ethyl acetate and redissolving in methanol, 89% of activity was recovered. This latter value is used for calculation of the % RH-141455 in Speyer 6S soil at day 3. If 89% is used instead of 105%, the mass balance is 102%.

After 14 days of incubation RH-141455 had declined to 46% (Speyer 2.2), 38% (Speyer 2.3) and 83% (Speyer 6S) of applied radioactivity in the soils. At the end of the incubation period (63 days for Speyer 2.2 and Speyer 2.3 and 120 days for Speyer 6S) RH-141455 was not detectable in any of the soils.

Table B.8.1.1.1-25: HPLC detections of RH-141455 in soil extract (% of applied radioactivity)

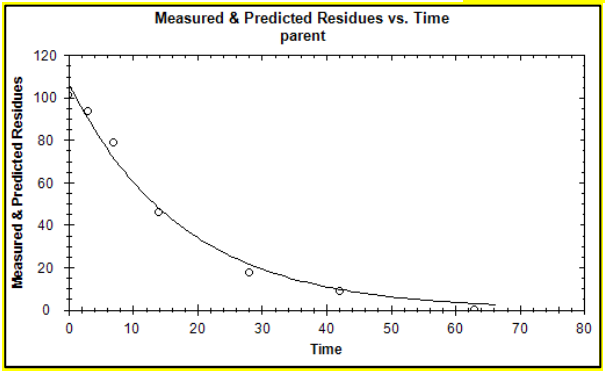
Time (days)	Speyer 2.2	Speyer 2.3	Speyer 6S
0	101.2	100.5	93.9
3	93.8	79.6	89.0
7	78.9	68.6	84.4
14	45.8	38.4	82.5
28	17.5	15.8	52.3
42	8.5	12.8	n.a*
63	0.0**	0.0**	22.9
120	n.a	na	0.0**

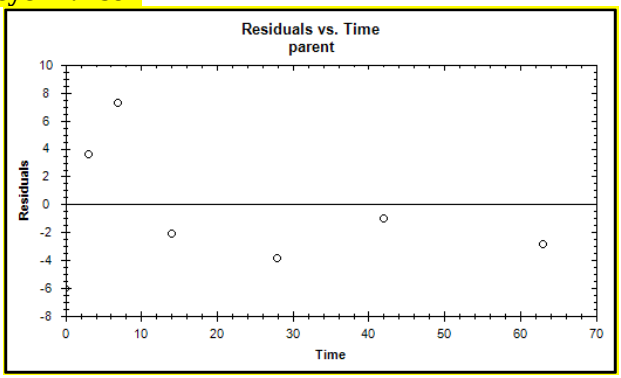
na: not analysed
* as the degradation was very slow taking a sample on day 42 would not be the optimum timing for kinetics calculation.
** <LOQ

For all soils the fits obtained by the SFO model were acceptable and better than for the FOMC model, based on visual assessment, t-test and χ^2 (error%). The plots of the decline and residuals are given in Figure B.8.1.1.1-12. The DT_{50} and DT_{90} values are shown in Table B.8.1.1.1-26.

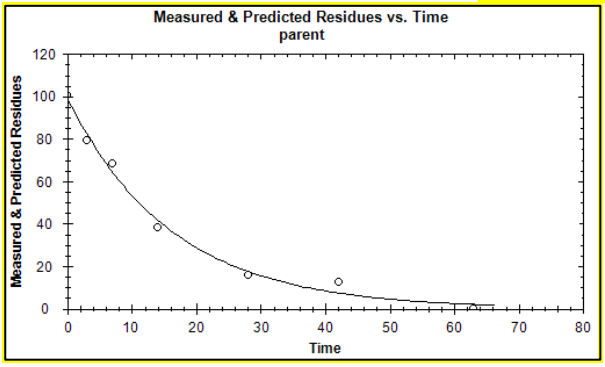
Figure B.8.1.1.1-12: SFO and FOMC fit for dissipation in all soils

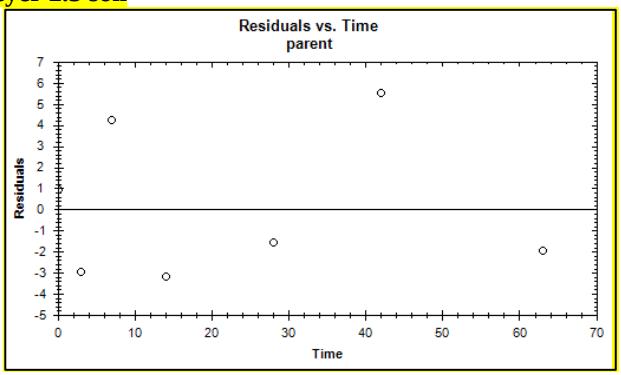
SFO fit for Speyer 2.2 soil



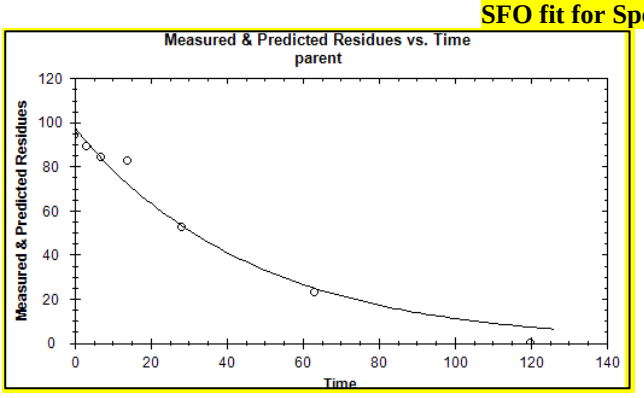


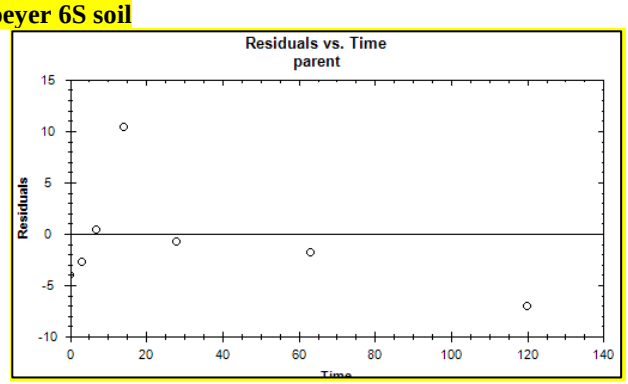
SFO fit for Speyer 2.3 soil



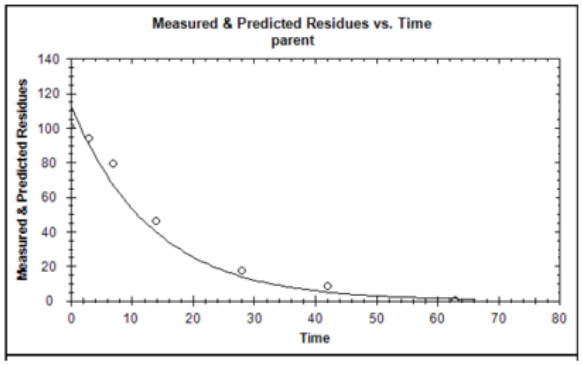


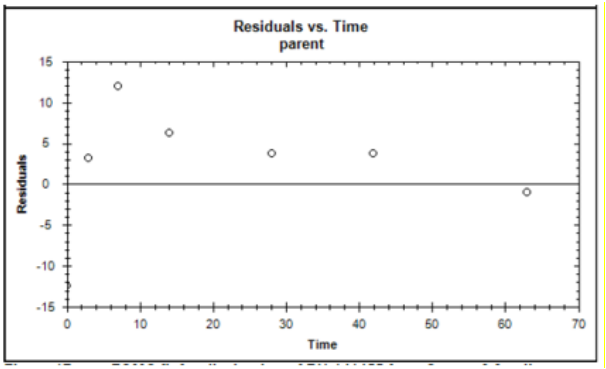
SFO fit for Speyer 6S soil



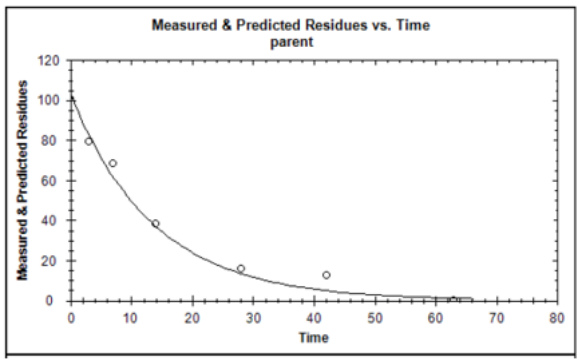


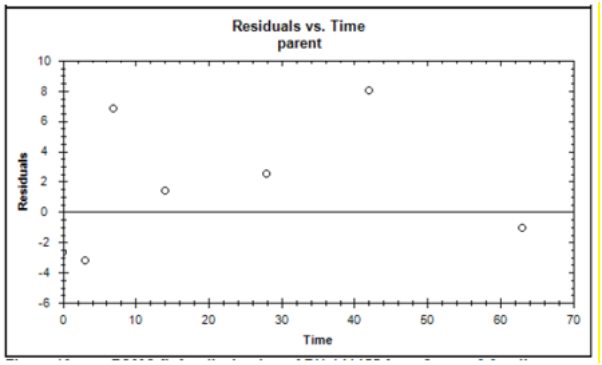
FOMC fit for Speyer 2.2 soil



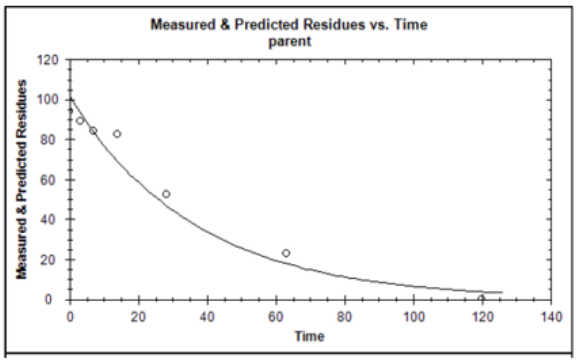


FOMC fit for Speyer 2.3 soil





FOMC fit for Speyer 6S soil



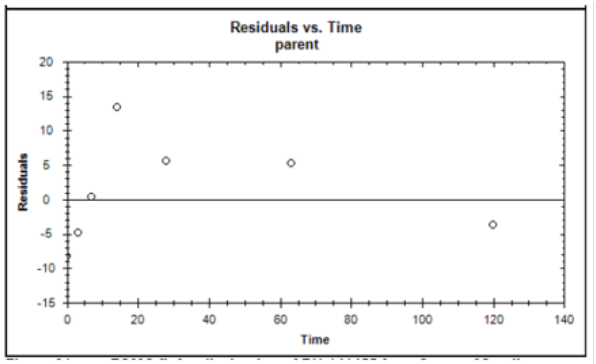


Table B.8.1.1.1-26: DT_{50} and DT_{90} of RH-141455 in soil

Soil	Kinetics	Visual fit	χ^2 (error%)	p-value[1]	Residuals	DT_{50} (days)	DT_{90}(days)
Speyer 2.2	SFO	good	6.95	<0.05	good	12.0	40.0
	FOMC	good	12.8	>0.1 (α, β)	good	9.18	30.5
Speyer 2.3	SFO	good	5.77	<0.05	good	11.1	36.9
	FOMC	good	8.47	>0.1 (α, β)	good	9.45	31.4
Speyer 6S	SFO	acceptable	6.8	<0.05	good	31.7	105.3
	FOMC	moderate	9.9	>0.1 (α, β)	moderate	24.9	82.7

[1] p<0.05: estimated parameter(s) statistically significant (t-test) at 0.05 level. p<0.1: estimated parameter(s) statistically significant (t-test) at 0.1 level. p>0.1: estimated parameter(s) statistically not significant (t-test) at 0.1 level.

III. CONCLUSION

The degradation of ^{14}C-labelled RH-141455 was studied in a test performed to the OECD 307 guideline. At the end of the incubation period 6%AR (Speyer 2.2), 5%AR (Speyer 2.3) and 11%AR (Speyer 6S) was extractable from the soils, however levels of RH-141455 were non-detectable. Significant levels of $^{14}CO_2$ were detected in NaOH traps, with a maximum of 56%AR (Speyer 2.2), 50%AR (Speyer 2.3) and 51%AR (Speyer 6S) at the study end. Unextractable residues occurred at maximums of 31%AR (Speyer 2.2), 40%AR (Speyer 2.3) and 47%AR (Speyer 6S). RH-141455 declined with DT_{50}s of 11.1 to 31.7 days (SFO, DT_{90}s 36.9 to 105.3 days).

RMS comment:

The degradation rate study and DT_{50}, DT_{90} calculations for RH-141455 have been performed according to the recommendations of current guidelines (OECD Guideline 307 and FOCUS Kinetics Guidance Document) with some deviations – the microbial biomass at the end of the incubation was not detected for Speyer 2.2 and Speyer 2.3 soil (metabolism flasks for determination of biomass were accidentally discarded for both soils). However biomass of both Speyer 2.2 and Speyer 2.3 soil was determined at the start of the study and proven to be viable. Since the test substance degraded within 63 days to <10% of applied the soils were proven to be still viable at the end of the incubation.

The study is acceptable and the results can be used for risk assessment purposes.

B.8.1.1.2 Anaerobic degradation

Zoxamide

Adequate data to assess the anaerobic route and rate of degradation of zoxamide in soil were evaluated during the first EU review. The guidance for the conduct of such studies has not substantively altered since the first review, therefore no further data are considered necessary.

Under the original review first order DT_{50}s of 7-14 days were calculated for degradation under anaerobic conditions.

The possibility that anaerobic conditions are encountered after application is unlikely as the proposed applications to potatoes and grapes will occur during the summer months.

Metabolite, breakdown and reaction products

No data are submitted. Zoxamide is applied during the summer months and therefore it is highly unlikely that it will encounter prolonged anaerobic conditions.

Studies from the original DAR (May 2001):

Reference:	Volkel W. (1998a). 14C-RH-117281: degradation in one soil incubated under anaerobic conditions, RCC Umweltchemie Ag, Rohm and Haas Technical Report No. 34-98-46, September 3, 1998.
Guideline(s):	SETAC guidelines (1.1, 1995)
GLP:	Yes
Previous evaluation:	In DAR (May 2001)
Validity of the study:	Considered acceptable

[14C-phenyl-U]-RH-117281 (radiochemical purity 97.81%, specific activity 90.2 mCi/g, 3.34 MBq/mg) was added in acetone/water to Mechthildshausen sandy loam (see Table B.8.1.1.2-1) at a concentration of 0.23 mg/kg dry weight (228µg/kg). The freshly-collected microbially-viable soil (soil moisture 75% of the water content at 0.33 bar, 37% WHC) was flooded with bi-distilled water (200 ml) and equilibrated under nitrogen for 20 days to establish anaerobic conditions. Treated soils (100 g dry weight equivalent, 2 mm sieved) were then incubated under anaerobic conditions at 20 $\pm$ 1°C in the dark for up to 120 days. Volatiles were trapped in sodium hydroxide (CO_2) and ethylene glycol (volatile organics) solutions.

Duplicate samples were analysed on days 0, 1 3, 7, 14, 28, 56, and 120 days post-treatment. Water samples were extracted with acidified ethyl acetate. Soil samples were extracted with acidified

acetonitrile. Unextracted material was further extracted, and extracts were analysed, as described in study of *Smalley & Reynolds (1997)*, see section B.8.1.1.1.

Total recovery of radioactivity was 97.3-102.4% AR. The distribution of radioactivity is shown in Table B.8.1.1.2-1. CO_2 was not detected (<0.1% AR) during anaerobic incubation, indicating mineralisation to be slow under these conditions. Levels of organic volatiles were also <0.1% AR. Levels of non-extracted radioactivity were 0.1% AR on day 0, increasing to 26.4% AR by day 120.

Table B.8.1.1.2-1: Distribution of RH-117281 and major metabolites in two soils during anaerobic degradation (% AR).

Day	parent RH-117281	RH-127450	RH-24549	CO_2	PES
Sandy loam, Germany					
0	101.3	-	-	< 0.1	0.1
1	94.5	0.2	0.3	< 0.1	1.4
3	77.4	4.7	2.4	< 0.1	5.3
7	50.0	13.4	6.6	< 0.1	9.2
14	17.4	27.7	9.5	< 0.1	19.7
28	1.7	30.2	14.5	< 0.1	26.2
56	0.6	30.1	21.4	< 0.1	25.4
120	-	23.7	23.7	< 0.1	26.4

The decline of RH-117281 under anaerobic conditions appeared to follow first order kinetics (Table B.8.1.1.2-2). DT50s and DT90s for RH-117281 were estimated by the applicant using one compartment first order kinetics with non-linear curve fitting (MicroCal 3.5). The Rapporteur (UK) has re-calculated DT50s and DT90s using linear regression.

Table B.8.1.1.2-2: Estimated laboratory DT50 and DT90 for the anaerobic degradation of RH-117281 in two soils

Soil type/origin	% sand	% silt	% clay	pH	% organic matter	Microbial viability	DT50 days	DT90 days	Rate order / r^2
Sandy loam. Mechthildshausen, Germany	59	24	17	7.4	1.20	298-356[1]	7	23	1st 0.93

1: mg microbial C/kg soil dry weight

A total of 22 metabolites were characterised. Of these, 7 compounds were identified as RH-127450, RH-24549, RH-129151, RH-139432, RH-141288, RH-141455, RH-141643. None of the metabolites individually accounted for >7% AR, except for RH-127450 and RH-24549. Maximum concentrations of RH-127450 and RH-24549 were 30.2% AR (day 28) and 23.7% AR (day 120) respectively. Of the unextracted residues, 11.7% AR and 6.7% AR was associated with fulvic and humic acid fractions respectively and 8% AR was associated with humins. The proposed anaerobic and aerobic metabolism pathways are the same (Figure B.8.1.1.1-1).

RMS comment:

The study has been previously evaluated and considered acceptable for the Annex I inclusion by UK. The RMS believes the study is still overall acceptable. In addition, the RMS would like to note that aerobic conditions are dominant in surface soils and even in sub-surface soils and anaerobic conditions may occur only on rare occasions during flooding of soils after heavy rainfalls.

Reference:	Kim-Kang H. (1997). Anaerobic soil metabolism of [14C]RH-117281, XenoBiotic Laboratories, Inc., Rohm and Haas Technical Report No. 34-97-43, April 9, 1997.
Guideline(s):	US EPA guidelines (Subdivision N, 162-1)
GLP:	Yes
Previous evaluation:	In DAR (May 2001)
Validity of the study:	Considered acceptable

[14C-phenyl-U]-RH-117281 (radiochemical purity 96. 5%, specific activity 90.2 mCi/g) was added in acetonitrile to Ohio loamy sand (see Table B.8.1.1.2-2) at a concentration of 1.5 mg/kg dry weight. The freshly-collected microbially-viable soil was used (soil moisture 75% of the water content at 0.33 bar). Treated soils (50 g dry weight equivalent, 2 mm sieved) were incubated first under aerobic conditions for 21 days. Flasks were then purged with nitrogen and soils flooded with degassed 1% glucose solution and incubated under anaerobic conditions at 25 $\pm$ 1°C in the dark for up to 59 days. CO_2 and other acid volatiles were trapped in sodium hydroxide solution.

Duplicate samples were analysed on days 0, 7, 14, 30 and 59 days of the anaerobic phase. Water samples were extracted with acidified dichloromethane. Soil samples were extracted with acidified acetonitrile and partitioned with dichloromethane. Unextracted material (day 59) was further extracted, and extracts were analysed, as described in study of *Smalley & Reynolds (1997)*, see section B.8.1.1.1. Evolved CO_2 was identified by means of LSC of the barium carbonate precipitate.

Total recovery of radioactivity was 94.5-104.8% AR. The distribution of radioactivity is shown in Table B.8.1.1.2-3. CO_2 accounted for similar levels of 4.6-5.1% AR (day 0-59), indicating that mineralisation occurred under aerobic conditions, however that under anaerobic conditions mineralisation was slow. Levels of non-extracted radioactivity were 27.26% AR on day 0, increasing to 53.5% AR by day 59.

Table B.8.1.1.2-3: Distribution of RH-117281 and major metabolites in two soils during anaerobic degradation (% AR).

Day	parent RH-117281	RH-127450	RH-24549	CO_2	PES
Loamy sand, USA					
0	47.39	3.00	7.28	4.60	27.26
7	28.07	10.65	4.51	5.11	38.02
15	18.95	10.40	4.67	4.71	44.74
30	9.62	9.77	7.23	4.79	56.35
59	2.43	13.45	16.87	4.98	53.50

The decline of RH-117281 under anaerobic conditions appeared to follow first order kinetics. DT50s and DT90s for RH-117281 are shown in Table B.8.1.1.2-4. It was not possible to estimate degradation rates for the metabolites.

Table B.8.1.1.2-4: Estimated laboratory DT50 and DT90 for the anaerobic degradation of RH-117281 in two soils

Soil type/origin	% sand	% silt	% clay	pH	% organic matter	Microbial viability	DT50 days	DT90 days	Rate order / r^2
Loamy sand Ohio, USA	76	18	6	6.9	2.4	5700,000[1]	14	47	1st 1.0

1: Plate count, CFU/ml (colony forming unit)

A total of 11 metabolites were characterised. Of these, 6 compounds were identified as RH-127450, RH-24549, RH-129151, RH-139432, RH-141288, RH-141452 and RH-141288. None of the metabolites individually accounted for >5% AR, except for RH-127450 and RH-24549. Maximum concentrations of RH-127450 and RH-24549 were 13.4% AR and 16.9% AR respectively on day 120. Of the day 59 unextracted residues, following hydrolysis 9.8% (AR) was organosoluble and contained unspecified levels of RH-127450 and RH-2454922. In addition, 3% AR and 10.2% AR was associated with fulvic and humic acid fractions respectively and 5.5% AR was associated with humins. The anaerobic and aerobic metabolism pathways are the same (Figure B.8.1.1.1-1).

RMS comment:

The study has been previously evaluated and considered acceptable for the Annex I inclusion by UK. The RMS believes the study is still overall acceptable.

B.8.1.1.3 Photolysis in soil

Adequate data to assess the soil photolysis of zoxamide in soil were evaluated during the first EU review. The guidance for the conduct of such studies has not substantively altered since the first review, therefore no further data are considered necessary.

Studies from the original DAR (May 2001):

Reference:	Reynolds JL. (1997). Soil photolysis of [14C]RH-117281, XenoBiotic Laboratories, Inc., Rohm and Haas Technical Report No. 34-96-214, July 31, 1997.
Guideline(s):	EPA guidelines (Pesticides Assessment Guidelines Subdivision N, Series 161-3)
GLP:	Yes
Previous evaluation:	In DAR (May 2001)
Validity of the study:	Considered acceptable

Portions of Ohio loamy sand soil (microbially viable, 2mm sieved, equivalent to 2g soil dry weight) were weighed into glass vessels of base area 10.4 cm^2. [14C-phenyl-U]-RH-117281 (radiochemical purity 97.7%, specific activity 90.2 mCi/g) was added in acetonitrile to give concentrations of 1 and 5 mg/kg (for compound identification). The water content of the samples was then adjusted to 75% of the 1/3 bar moisture of the soil. Volatiles were trapped in sodium hydroxide solution (CO_2/acidic volatiles),

polyurethane foam (neutral volatiles) and dilute sulphuric acid (basic volatiles). Replicate flasks were incubated at 25°C for up to 30 days, either in the dark or in 12 hour alternating light and dark cycles by irradiation in a Suntest unit (xenon lamp, cut-off <290 nm). The average light intensity (over the range 330-800 nm) and spectral distribution of the xenon lamp and of natural New Jersey summer sunlight (*ca* 42° N) were comparable (xenon lamp and natural sunlight average fluxes were 147 and 138 W/m^2 respectively).

Duplicate samples were taken for analysis 0, 3, 5, 7, 14, 21 and 30 days post-treatment (14 and 30 days only for dark controls). Soil samples were extracted with acidified acetonitrile/water and partitioned with dichloromethane. Unextracted material was subjected to acid hydrolysis and subsequent partitioning of the hydrolysate with ethyl acetate. Remaining solid material was further separated into humin, fulvic and humic acid fractions, achieved by centrifugation in sodium hydroxide solution followed by acidification (HCl) and further centrifugation. Analysis was by LSC, TLC, reverse phase radio- and UV- HPLC (254 nm) and LC-MS-ESI. Compound identification was by co-chromatography with unlabelled reference standards.

Under irradiated conditions, total recovery of radioactivity was 93.9-107.9% AR. The distribution of radioactivity is shown in Table B.8.1.1.3-1. CO_2 accounted for 0.5% AR (day 3), however subsequently, levels declined to 0.09-0.32% AR on days 5-30. Similar levels of CO_2 were evolved from dark control samples (0.17 and 0.2% AR, days 14 and 30), indicating the rate of degradation to be similar to that of irradiated samples. Levels of non-extracted radioactivity were 0.33% AR on day 0, increasing to 30.77% AR by day 30. Similarly, levels of non-extracted radioactivity in dark controls were 27.2 and 31.9% AR).

The decline of RH-117281 in irradiated soil appeared to follow 1.5st order kinetics. DT50s and DT90s for RH-117281 are shown in Table B.8.1.1.3-2. It was not possible to accurately estimate degradation rates for the metabolites.

A total of 12 metabolites were characterised. Of these, 6 compounds were identified as RH-127450, RH-24549, RH-129151, RH-139432, RH-141288, RH-163353. None of the metabolites individually accounted for >7% AR, except for RH-127450 and RH-24549. Maximum concentrations of RH-127450 and RH-24549 were 10.9% AR (day 14) and 22.2% AR (day 30) respectively. Of the day 30 unextracted residues, following hydrolysis 12% (AR) was organosoluble and contained a range of the metabolites outlined above, individually at levels of <5% AR. In addition, 8% AR and 5.2% AR was associated with fulvic and humic acid fractions respectively and 3% AR was associated with humins. The nature and levels of metabolites occurring in irradiated and dark controls was similar. The metabolic pattern was similar to that occurring in aerobic and anaerobic metabolism studies. Results suggest degradation to be primarily hydrolytic or microbial, rather than photolytic.

The applicant has stated '*This study was conducted on moist soil as required by the US EPA guidance. SETAC guidance for the soil photolysis study requires that the soils be dried at 35 °C prior to dosing and exposure, in order to insure that hydrolysis and biodegradation are suppressed. Because there was little difference between exposed samples and dark controls under moist conditions, it is reasonable to assume, as a worst case, that RH-117281 will not photodegrade on dry soil*.' This is acceptable.

Table B.8.1.1.3-1: Distribution of RH-117281 and metabolites in soil during soil photolytic degradation (% AR)

Day	Parent	M1	M2	M3	M4 (RH-141288)	M5 (RH-139432)	M6	M7 (RH-24549)	M8 (RH-127450)	M9 (RH-129151)	M10	M11	M12
0	101.63	0.16	ND	ND	ND	0.44	ND	ND	ND	1.11	ND	ND	ND
3	74.60	2.82	2.38	0.43	1.49	2.05	ND	4.33	2.84	2.84	ND	ND	ND
5	87.52	1.22	0.35	0.26	0.17	1.87	ND	0.82	ND	3.97	ND	0.71	ND
7	36.94	2.95	3.56	1.02	3.37	3.39	2.68	8.50	6.88	2.49	ND	ND	ND
14	32.05	2.71	0.61	1.02	2.38	1.96	0.76	4.27	10.94	2.06	0.70	0.90	2.68
21	18.28	6.73	1.83	1.74	2.47	3.45	1.10	19.21	7.64	1.51	1.01	3.20	1.01
30	14.00	3.71	2.32	2.17	2.15	2.06	1.22	22.23	8.52	1.63	1.51	1.88	1.34
14 Dark	28.51	4.10	5.16	1.28	2.91	1.36	1.02	8.65	8.58	2.60	0.86	0.84	3.29
30 Dark	16.78	6.12	8.14	1.21	2.45	1.20	1.26	13.57	9.03	2.16	0.87	1.52	0.69

ND = Not Detected

Table B.8.1.1.3-2: Estimated laboratory DT50 and DT90 for the photolytic degradation of RH-117281 in Soil (12 hour photoperiod, 42°N summer sunlight)

Soil type/origin	% sand	% silt	% clay	pH	% organic matter	Microbial viability	DT50 days	DT90 days	Rate order / r^2
Loamy sand Ohio, USA	76	18	6	6.9	2.4	5700,000[1]	7	37	1.5st 0.95

1: Plate count, CFU/ml (colony forming unit)

RMS comment:

The study has been previously evaluated and considered acceptable for the Annex I inclusion by UK. The RMS believes the study is still overall acceptable.

B.8.1.1.4 Field studies

Dissipation studies

Laboratory DT_{50} values for the parent zoxamide and its metabolites RH-127450, RH-24549 and RH-163353 are less than 60 days. A DT_{50} of >60 days is obtained for the metabolite RH-141455 in a single soil treated with the parent compound. This DT_{50} may have been influenced by the low levels detected

and the DT_{50}s in the study performed with the metabolite itself are significantly shorter and are <60 days. Therefore field studies are not triggered with either zoxamide or its metabolites.

Accumulation studies

Laboratory DT_{90}s for zoxamide and its metabolites presented in study of *Callow & Hilton (2013a)* (see section B.8.1.1.1) are all below the threshold of 365 days, except for metabolite RH-141455. DT_{90} of >365 days was obtained for metabolite RH-141455 in a single soil treated with the parent compound. This DT_{90} may have been influenced by the low levels detected and the DT_{90}s in the study performed with the metabolite itself are significantly shorter and are <365 days. Field accumulation testing is therefore not necessary.

Summary of route and rate of degradation in soil

Route of degradation

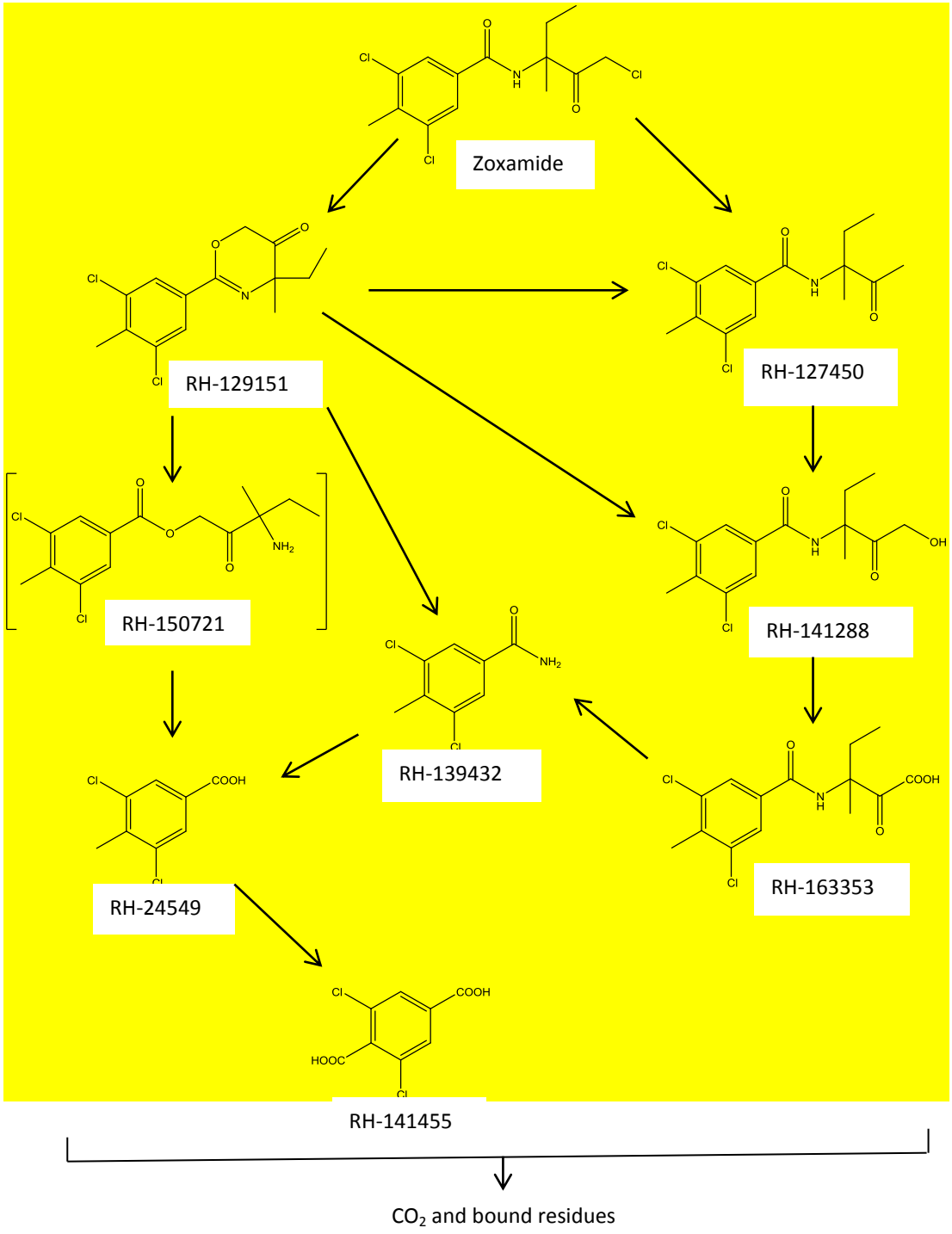
In the original review the route of degradation of zoxamide in aerobic soil was studied in six soils (pH 5.0-8.1, 0.8-2.4 %oc), besides one soil (Germany, sandy loam) was investigated considering three different incubation conditions. Up to 23 degradates were detected. Major metabolites exceeding 10% AR were RH-127450 (de-chlorinated product, 8.1-15.1% AR), RH-24549 (benzoic acid derivative, 5.5-33.8% AR) and RH-163353 (acid, 7.9-15% AR). Maximum levels of these metabolites at 20°C and 10°C were found on days 3-7 and 7-14 respectively. Mineralisation of zoxamide was significant (34.4-57.8% AR by days 120-122), although non-extractable residues accounted for maximums of 25.6-39.1-% AR (days 28-120). Of the unextracted residues, 9.2-17.5% AR and <0.1-12.7% AR was associated with fulvic and humic acid fractions respectively and 2.8-22.7% AR was associated with humins. Although there was no significant difference in metabolite levels found at different temperatures, mineralisation was slower at 10°C. Mineralisation of non-extracted residues over the study period was slow. Aerobic metabolism of zoxamide in soil is similar to that observed during hydrolysis studies in water and proceeds by cyclisation and de-chlorination, followed by oxazine ring-opening by hydrolysis leading to amination or hydroxylation and subsequent cleavage at the epoxyimino- and epidioxy- bridges (Figure B.8.1.1-1).

Since the original review, the guidelines for evaluating the relevance of metabolites have changed. Metabolites occurring at >5% on two or more consecutive occasions should be considered as well as those which reach their maximum at the final time-point of the study. The previously submitted studies were examined for any other metabolites meeting these lower thresholds. In the soil degradation and metabolism study (Burgener, 1998a), metabolite RH-141455 was detected at levels above 5% on more than two occasions (Maximum was 8%), therefore this metabolite also requires further consideration.

The anaerobic degradation of zoxamide was examined in two soils (soil pH 6.9-7.4, 1.2-2.4 %oc, 20-25°C). In the first study, anaerobic conditions were established before introduction of the test material, however in the second study, aerobic metabolism was allowed to take place for 21 days before anaerobic conditions were introduced. Up to 22 degradates were identified. Major metabolites exceeding 10% AR were RH-127450 and RH-24549. Near-maximum concentrations occurred in soils around days 14-56 and were continually maintained under anaerobic conditions, as were levels of CO_2 (4.6-5.1% AR), indicating that although metabolites were formed under both aerobic and anaerobic conditions, their degradation was slower under anaerobic conditions. Levels of RH-127450 increased from 0.2% AR (day 1) to 30.2% AR (day 28), declining slowly to 23.7% AR by day 120. Levels of RH-24549 increased from 0.3% AR (day 1) to 23.7% AR (day 120). Non-extractable residues accounted for maximums of 25.6-39.1-% AR and were associated primarily with humic and fulvic acids and humins. The possibility that anaerobic conditions are encountered after application is unlikely as the proposed applications to potatoes and grapes will occur during the summer months.

In a soil photolysis study, degradation was observed in both the irradiated and dark control samples. Similar metabolites were identified as in the aerobic and anaerobic soil degradation studies, suggesting that degradation is primarily hydrolytic or microbial, rather than photolytic.

Figure B.8.1.1-1: Proposed route of degradation of zoxamide in soil



Rate of degradation

Under the original review DT_{50}s of 2.0-10 days and DT_{90}s of 6.7- 110 days (1st order at 10-20°C and root1st order at 25°C) were calculated for zoxamide. It was concluded that there was no clear effect of temperature on degradation rate within the range of studies undertaken and no significant effect of soil type/properties on degradation rates. DT_{50}s under anaerobic conditions were 7 to 14 days. Degradation rates for the metabolites, RH-127450, RH-24549 and RH-163353 were calculated to be 4.0-17.8 days, 5.5-19 days and 7.5-13 days respectively under aerobic conditions at 20°C. Results suggest their degradation may be slower under anaerobic conditions, however it was not possible to estimate DT_{50}s.

Since the original review, the guidelines for evaluating the relevance of metabolites have changed. Metabolites occurring at >5% on two or more consecutive occasions should be considered as well as those which reach their maximum at the final time-point of the study. In the soil degradation and metabolism study (Burgener, 1998a), metabolite RH-141455 was detected at levels above 5% on more than two occasions (Maximum was 8%), therefore data on the degradation of this metabolite in soil are provided.

The rates of degradation in the aerobic soil degradation studies of Smalley and Reynolds (1997) and Burgerer (1998) have been re-evaluated according to the recommendations of the FOCUS Kinetics Group (FOCUS 2006). The SFO model satisfactorily describes the decline of zoxamide in all soils incubated in the study of Burgener (1998a). However, for soils incubated in the study of Smalley *et al* (1997) DFOP kinetics provides a better fit to the data. Persistence endpoints of DT_{50}s of 2.03 to 13.75 days at 10 to 25°C and modelling endpoints of DT_{50}s 2.03 to 41.3 days at 10 to 25°C were obtained.

For the metabolites, although in many instances the chi^2 % error is >15%, P values are generally <0.1 and visual fits are acceptable with only an unacceptable fit identified for two of the metabolites in two soils. Persistence and modelling DT_{50}s were 1.99 to 11.69 days at 20°C (17.8 days at 10°C) for RH-127450, 3.05 to 16.23 days at 20 to 25°C for RH-24549, 5.62 to 53.65 days at 20°C (55.5 days at 10°C) for RH-163353 and 195.2 days at 20°C for RH-141455. In the new study with RH-141455 DT_{50}s were 11.1 to 31.7 days at 20°C. The slower DT_{50} for RH-141455 in the study with the parent is largely due to the low detections of the metabolite in this study.

The rates of degradation of zoxamide and its metabolites are summarised in Table B.8.1.1-1. DT_{50}s for modelling were normalised to a temperature of 20°C and a soil moisture of pF 2 according to FOCUS (2000) guidance. Details of the normalisation are given in Table B.8.1.1-2.

Table B.8.1.1-1: Summary of the rates of degradation of zoxamide and its metabolites

Study	Soil	Model	DT_{50} (days)	DT_{90} (days)	FF[1]
Zoxamide					
Burgener 1998	England silt loam 20°C 50%MWHC	SFO (persistence & modelling)	4.16	13.8	-
	France loam 20°C 50%MWHC	SFO (persistence & modelling)	2.03	6.7	-
	Germany sandy loam 20°C 50%MWHC	SFO (persistence & modelling)	2.7	9.0	-
	Italy clay loam 20°C 50%MWHC	SFO (persistence & modelling)	2.38	7.9	-
	Germany sandy loam 10°C 50%MWHC	SFO (persistence & modelling)	7.73	25.7	-
	Germany sandy loam 20°C 100%FC	SFO (persistence & modelling)	2.27	7.6	-
Smalley et al (1997)	Pennsylvania silt loam 25°C 75%FC	DFOP (persistence)	7.75	98.1	-
		DFOP (modelling)*	39.2	-	-
	Ohio loamy sand 25°C 75%FC	DFOP (persistence)	13.75	107.1	-
		DFOP (modelling)*	41.3	-	-
RH-127450					
Burgener 1998	England silt loam 20°C 50%MWHC	SFO (persistence & modelling)	11.69	38.84	0.26
	France loam 20°C 50%MWHC	SFO (persistence & modelling))	3.78	12.57	0.21
	Germany sandy loam 20°C 50%MWHC	SFO (persistence & modelling)	6.66	22.14	0.18
	Italy clay loam 20°C 50%MWHC	SFO (persistence & modelling)	1.99	6.6	0.21
	Germany sandy loam 10°C 50%MWHC	SFO (persistence & modelling)	17.8	59.05	0.17
	Germany sandy loam 20°C 100%FC	SFO (persistence & modelling)	5.76	19.13	0.20
RH-24549					
Burgener 1998	France loam 20°C 50%MWHC	SFO (persistence & modelling)	6.29	20.89	0.19
	Germany sandy loam 20°C 50%MWHC	SFO (persistence & modelling)	5.35	17.77	0.17
	Italy clay loam 20°C 50%MWHC	SFO (persistence & modelling)	8.44	28.03	0.47
	Germany sandy loam 20°C 100%FC	SFO (persistence & modelling)	3.05	10.15	0.28
Smalley et al (1997)	Ohio loamy sand 25°C 75%FC	SFO (persistence & modelling)	16.23	53.9	0.17
RH-163353					
Burgener 1998	England silt loam 20°C 50%MWHC	SFO (persistence & modelling)	53.65	178.23	0.09
	France loam 20°C 50%MWHC	SFO (persistence & modelling)	6.62	21.99	0.20
	Germany sandy loam 20°C 50%MWHC	SFO (persistence & modelling)	5.62	18.67	0.29
	Italy clay loam 20°C 50%MWHC	SFO (persistence & modelling)	6.39	21.24	0.23
	Germany sandy loam 10°C 50%MWHC	SFO (persistence & modelling)	55.5	184.36	0.15
	Germany sandy loam 20°C 100%FC	SFO (persistence & modelling)	9.9	32.9	0.185
RH-141455					
Burgener 1998	Germany sandy loam 20°C 50%MWHC	SFO (persistence & modelling)	195.2	648.5	0.50[2]
Van den Bosch (2013)	Speyer 2.2 20°C 40%MWHC	SFO (persistence & modelling)	12.0	40.0	-[3]
	Speyer 2.3 20°C 40%MWHC	SFO (persistence & modelling)	11.1	36.9	-[3]
	Speyer 6S 20°C 40%MWHC	SFO (persistence & modelling)	31.7	105.3	-[3]

[1] formation fraction [2] from RH-24549 [3] study conducted with metabolite (RH-141455)

Table B.8.1.1-2: Summary of the modelling rates of degradation of zoxamide and its metabolites normalised to 20°C and pF2

Compound	Soil	DT_{50} (days)	Temp	T-Corr	Moist. Cont. (%)	Moist. Corr	*DT_{50} normal-ised to 20°C & pF2
Zoxamide	England silt loam 20°C 50%MWHC	4.16	20˚C	1.00	20.4	0.84	3.51
	France loam 20°C 50%MWHC	2.03	20˚C	1.00	22.8	0.94	1.90
	Italy clay loam 20°C 50%MWHC	2.38	20˚C	1.00	21.3	0.83	1.97
	Germany sandy loam 20°C 50%MWHC	2.7	20˚C	1.00	18.7	0.99	2.67
	Germany sandy loam 20°C 100%FC	2.27	20˚C	1.00	22.7	1.00	2.27
	Germany sandy loam 10°C 50%MWHC	7.73	10˚C	0.39	18.7	0.99	2.96
	Pennsylvania silt loam 25°C 75%FC	39.2	25˚C	1.57	16.95	0.74	45.60
	Ohio loamy sand 25°C 75%FC	41.3	25˚C	1.57	7.35	0.71	45.99
	Geometric mean						**6.4**
RH-127450	England silt loam 20°C 50%MWHC	11.69	20˚C	1.00	20.4	0.84	9.86
	France loam 20°C 50%MWHC	3.78	20˚C	1.00	22.8	0.94	3.54
	Italy clay loam 20°C 50%MWHC	1.99	20˚C	1.00	21.3	0.83	1.64
	Germany sandy loam 20°C 50%MWHC	6.66	20˚C	1.00	18.7	0.99	6.59
	Germany sandy loam 20°C 100%FC	5.76	20˚C	1.00	22.7	1.00	5.76
	Germany sandy loam 10°C 50%MWHC	17.8	10˚C	0.39	18.7	0.99	6.81
	Geometric mean						**4.3**
RH-24549	France loam 20°C 50%MWHC	6.29	20˚C	1.00	22.8	0.94	5.90
	Italy clay loam 20°C 50%MWHC	8.44	20˚C	1.00	21.3	0.83	6.97
	Germany sandy loam 20°C 50%MWHC	5.35	20˚C	1.00	18.7	0.99	5.29
	Germany sandy loam 20°C 100%FC	3.05	20˚C	1.00	22.7	1.00	3.05
	Ohio loamy sand 25°C 75%FC	16.23	25˚C	1.57	7.35	0.71	18.07
	Geometric mean						**7.5**
RH-163353	England silt loam 20°C 50%MWHC	53.65	20˚C	1.00	20.4	0.84	45.27
	France loam 20°C 50%MWHC	6.62	20˚C	1.00	22.8	0.94	6.21
	Italy clay loam 20°C 50%MWHC	6.39	20˚C	1.00	21.3	0.83	5.28
	Germany sandy loam 20°C 50%MWHC	5.62	20˚C	1.00	18.7	0.99	5.56
	Germany sandy loam 20°C 100%FC	9.9	20˚C	1.00	22.7	1.00	9.90
	Germany sandy loam 10°C 50%MWHC	55.5	10˚C	0.39	18.7	0.99	21.24
	Geometric mean						**10.3**
RH-141455	Germany sandy loam 20°C 50%MWHC	195.2	20˚C	1.00	18.7	0.99	193.04
	Speyer 2.2	12	20˚C	1.00	17	1.00	12.00
	Speyer 2.3	11.1	20˚C	1.00	15.3	0.86	9.54
	Speyer 6S	31.7	20˚C	1.00	16.1	0.46	14.72
	Geometric mean of soils at 20/25°C						**23.9**

*Geometric mean was calculated for the soils incubated at 20/25°C; where a number of DT_{50}s were obtained from the same soil (German sandy loam), the arithmetic mean DT_{50} was first calculated for this soil before calculating the geometric mean for all the soils

RMS comment:

DT_{50} values at three different incubation conditions were presented for sandy loam soil (Germany), current practice at EU Level is preferably to only use the DT_{50} values derived from soil with standard incubation conditions (20°C, pF2). In particula case, since no significant impact on the derived geomean DT_{50} values and following risk assessment is foreseen, we suggest to keep all the proposed DT_{50} values.

B.8.1.2 Adsorption and desorption in soil

Zoxamide

Adequate data to assess the adsorption/desorption of zoxamide were evaluated during the first EU review.

Studies from the original DAR (May 2001):

Reference:	Shelby DJ. (1996). Adsorption and desorption of RH-117281 to soil, Ricerca, Inc., Rohm and Haas Technical Report No. 34-96-01, February 9, 1996.
Guideline(s):	US EPA guidelines (Subdivision N, 161-1) and Directive 95/36/EC
GLP:	Yes
Previous evaluation:	In DAR (May 2001)
Validity of the study:	Considered acceptable

[14C-phenyl-U]-RH-117281 (radiochemical purity 97.7%, specific activity 52.9 mCi/g) in 0.01 M calcium nitrate was added to triplicate air-dried samples (equivalent to 1 g dry weight, 2mm sieved) of five soils at concentrations of 0.05, 0.1, 0.25 and 0.5 mg a.s./l. Pre-tests indicated that the a.s. was stable in non-sterile 0.01 M calcium nitrate over 48 hours and stable in the presence of soils for 24 hours, except for the Newtown silty loam, where degradation was detected at 24 hours. Treated slurries were shaken in borosilicate glass tubes at 25+1°C for 24 hours (Newtown silty loam, 2 hours only) in the dark. After equilibration, the supernatant was removed by centrifugation and radioactivity was quantified by LSC. Selected samples treated at 0.5 mg/l were also analysed by radio-HPLC.

As radio-HPLC analysis showed RH-117281 to be stable under the test conditions, the adsorbed RH-117281 was calculated by difference. After centrifugation, soil pellets were re-suspended in 0.01 M calcium nitrate (equal to decanted volume) and again shaken for 24 hours under the same conditions (Newtown silty loam, 2 hours only), prior to analysis as detailed above. In addition, radioactivity in the remaining soil pellet was quantified by combustion LSC.

Total recovery of radioactivity was 91-107% AR (except for three samples <90% due to faulty soil combustion). The adsorption and desorption isotherms for each concentration were used to calculate Freundlich coefficients (Kf) and Kfoc values for each soil (Table B.8.1.2-1). Adsorption Kfoc constants were 815-1431, desorption Kfoc constants were 927-1671.

Table B.8.1.2-1: Adsorption and desorption coefficients for RH-117281

Soil type	% sand	% silt	% clay	pH	% oc	1/n	Adsorption Kf ml/g	Adsorption Kfoc ml/g	Desorption Kfoc ml/g
Loam, Huntsburg, Ohio, USA	34	40	26	7.2	1.27	0.896 0.859	10.35	815 -	- 927
Sandy loam, Madera, CA. , USA	69	24	7	5.6	0.26	0.986	3.36	1294	-

						0.966		-	1618
Silty clay loam, Concord, Ohio, USA	11	50	39	4.8	1.77	0.963 0.948	25.33	1431 -	- 1671
Sandy loam, Madison, Ohio, USA	73	18	9	6.7	1.10	0.953 0.938	15.23	1385 -	- 1415
Silty loam, Newtown, Pennsylvania, USA	20	65	15	6.8	1.04	1.067 0.956	12.44	1196 -	- 1032

RMS comment:

The study has been previously evaluated and considered acceptable for the Annex I inclusion by UK. The RMS believes the study is still overall acceptable and the results are appropriate for risk assessment purposes.

Metabolite, breakdown and reaction products

Adequate data to assess the adsorption/desorption of the potentially relevant metabolites of zoxamide occurring at >10% (RH-127450, RH-24549 and RH-163353) were evaluated during the first EU review.

Since the original review, the guidelines for evaluating the relevance of metabolites have changed. Data requirements under Regulation (EC) 1107/2009 require that metabolites occurring at >5% on two or more consecutive occasions should be considered as well as those which reach their maximum at the final time-point of the study. In the soil degradation and metabolism study (Burgener, 1998a), metabolite RH-141455 was detected at levels above 5% on more than two occasions (Maximum was 8%), therefore data on the adsorption of this metabolite in soil are provided.

Studies from the original DAR (May 2001):

Reference:	Reynolds JL. (1998a). Adsorption and desorption of 14C-RH-24549 in three soils, XenoBiotic Laboratories, Inc., Rohm and Haas Technical Report No. 34-98-53, October 14, 1998.
Guideline(s):	OECD guidelines (Section 106, 1989) and OPPTS (Section 835.1220, 1996)
GLP:	Yes
Previous evaluation:	In DAR (May 2001)
Validity of the study:	Considered acceptable

[14C-phenyl-U]-RH-24549 (radiochemical purity 100%, specific activity 19.5 mCi/g) in 0.01 M calcium chloride was added in acetonitrile duplicate air-dried samples (equivalent to 5 g dry weight, 2mm sieved) of three soils at concentrations of 0.1, 0.25, 0.46 and 0.9 mg metabolite/l. Treated slurries (soil/water

ratio 1:5) were shaken in Teflon tubes at ambient temperature for 4 and 8 hours in the dark. Optimum test conditions had previously been determined from preliminary adsorption kinetics studies. After equilibration, the supernatant was removed by centrifugation and radioactivity was quantified by LSC of aliquots. The supernatant was extracted by C18 SPE (methanol/dichloromethane) and extracts were analysed by LSC and HPLC with radio- and UV (254 nm) detection.

Total recovery of radioactivity, during determination of isotherms, was 96-102% AR. HPLC analyses indicated that the metabolites were relatively stable in the presence of soils for 48 hours. A minor degradate was detected at up to 6.6% of the level of RH-24549, identified by co-chromatography as RH-141452. The adsorption isotherms for each concentration were used to calculate Freundlich coefficients (Kf) and Kfoc values for each soil. Adsorption Kfoc constants were 90.5-307.4 (Table B.8.1.2-2).

Table B.8.1.2-2: Adsorption coefficients for RH-24549

Soil type	% sand	% silt	% clay	pH	% oc	1/n	Adsorption Kf ml/g	Adsorption Kfoc ml/g
Sandy loam Iowa/USA	64	19	17	5.2	1.3	0.791	4.0	307.43
Silty clay loam Illinois/USA	19	50	31	7.3	2.4	0.833	3.6	150.16
Silt loam Ohio/USA	23	52	25	7.6	2.0	0.811	1.8	90.55

RMS comment:

The study has been previously evaluated and considered acceptable for the Annex I inclusion by UK. The RMS believes the study is still overall acceptable and the results are appropriate for risk assessment purposes.

In addition, the RMS would like to note that although RH-24549 is considered as pH dependent only three reliable Kfoc values are available. Hence position (comments) regarding this issue from other Member States would be highly appreciated.

Reference:	Volkel W. (1998b). Adsorption/Desorption of RH-127450 on Three Soils, RCC Ltd., Rohm and Haas Technical Report No. 34-98-54, December 15, 1998.
Guideline(s):	OECD guidelines (Section 106, 1981)
GLP:	Yes
Previous evaluation:	In DAR (May 2001)
Validity of the study:	Considered acceptable

RH-127450 (purity 98.9 %) in 0.01 M calcium chloride was added in acetonitrile to duplicate air-dried samples (equivalent to 5 g dry weight, 2mm sieved; soil/water ratio 1:5) of three soils at concentrations of

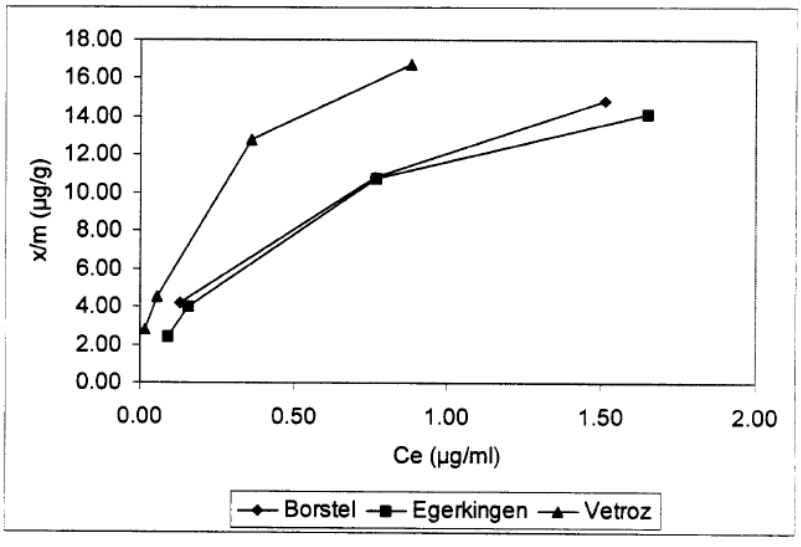
0.6, 1.0, 2.9 and 4.5 mg metabolite/l. Treated slurries were shaken in Teflon tubes at 20 ± 1°C for 16 hours in the dark. Optimum test conditions had previously been determined from preliminary adsorption kinetics studies. After equilibration, the supernatant was removed by centrifugation and either analysed directly or acidified and extracted with ethyl acetate. Analysis was by RP-HPLC-UV (240 nm). A mass balance was performed for the highest concentration. Soil was extracted with acidified acetonitrile and analysed by HPLC.

Total recovery of residues, for the highest concentration samples were 4.9-15.5% (soil) and 31.6-53.4% (aqueous) of the amount applied. The remainder (46.6-68.4% of applied) was calculated to be non-extractable by difference. HPLC analyses indicated that the metabolite was stable in 0.01M calcium chloride for at least 24 hours, therefore the adsorbed RH-127450 was calculated by difference. The adsorption isotherms for each concentration were used to calculate Freundlich coefficients (Kf) and Kfoc values for each soil. Adsorption Kfoc constants were 404-1156 (Table B.8.1.2-3), Freundlich exponents were 0.488-0.519 (Table B.8.1.2-3 and Figure B.8.1.2-1).

Table B.8.1.2-3: Adsorption coefficients for RH-127450

Soil type	% sand	% silt	% clay	pH	% oc	1/n	Adsorption Kf ml/g	Adsorption Kfoc ml/g
Loamy sand Borstel/Germany	83.5	10.9	5.6	6.1	1.05	0.519	12.14	1156
Clay Egerkingen/ Switzerland	20.1	37.7	42.2	5.0	2.82	0.603	11.4	404
Silt loam Vetroz/Switzerland	26.7	53.9	19.4	7.3	4.05	0.448	18.12	447

Figure B.8.1.2-1: Adsorption isotherms for RH-127450



RMS comment:

The study has been previously evaluated and considered acceptable for the Annex I inclusion by UK. The RMS believes the study is still overall acceptable and the results are appropriate for risk assessment purposes.

Reference:	Volkel W. (2000). Adsorption/Desorption of RH-163,353 In Three Soils, RCC Ltd, Rohm and Haas Technical Report No. 34-00-06, January 31, 2000.
Guideline(s):	OECD guidelines (Section 106, 1981)
GLP:	Yes
Previous evaluation:	In DAR (May 2001)
Validity of the study:	Considered acceptable

RH-163353 (purity 93.9 %) in 0.01 M calcium chloride was added in acetonitrile to duplicate air-dried samples (equivalent to 10 g dry weight, 2mm sieved; soil/solution ratio 2:5) of two soils at concentrations of 1.4, 4.1, 6.8 and 13.4 mg metabolite/l. Treated slurries were shaken in Teflon tubes at 20 $\pm$ 1°C for 16 hours in the dark. Optimum test conditions had previously been determined from preliminary adsorption/desorption kinetics studies on three soils (concentrations 4.5 and 4.8 mg metabolite/l, soil/water ratios 1:5 and 2:5). After equilibration, the supernatant was removed by centrifugation and analysed directly by RP-HPLC-UV (240 nm). A mass balance was performed for the highest concentration. Soil was extracted with acetonitrile/water and analysed by HPLC.

Total recovery of residues (soil + water), for the highest concentration samples were 104-109% of the amount applied. HPLC analyses indicated that the a.s. was stable in 0.01M calcium chloride for at least 16 hours, therefore the adsorbed RH-163353 was calculated by difference. The adsorption isotherms for each concentration were used to calculate Freundlich coefficients (Kf) and Kfoc values for each soil. Adsorption Kfoc constants were 75-79 (Table B.8.1.2-4).

Table B.8.1.2-4: Adsorption coefficients for RH-163353

Soil type	% sand	% silt	% clay	pH	% oc	1/n	Adsorption Kf ml/g	Adsorption Kfoc ml/g
Loamy sand Borstel/Germany	78.1	15.5	6.4	6.1	1.22		0.6	50[1]
Clay Egerkingen/ Switzerland	10.1	38.1	51.8	5.4	3.17	0.833	2.4	75
Silt loam Vetroz/Switzerland	18.2	57.2	24.6	7.2	4.79	0.844	3.8	79

1. Adsorption constant (K‘oc) from screening study only, soil/solution ratio 2:5, concentration 4.8 mg as./l.

NB. K'ocs from screening study with soil/solution ratio 1:5, concentration 4.5 mg as./l for loamy sand, clay and silt loam soils were 44, 42 and 61 respectively.

RMS comment:

The study has been previously evaluated and considered acceptable for the Annex I inclusion by UK. The RMS believes the study is still overall acceptable and the results are appropriate for risk assessment purposes.

Reference:	Volkel W. (1998c). Determination of the Adsorption Coefficient of 14C-RH-163353 on Soil and its Octanol/Water Partition Coefficient Using High Performance Liquid Chromatography (HPLC), RCC Ltd., Rohm and Haas Technical Report No. 34-98-55, November 9, 1998.
Guideline(s):	OECD guidelines (Section 106, 1989) and OPPTS (Section 835.1220, 1996)
GLP:	Yes
Previous evaluation:	In DAR (May 2001)
Validity of the study:	Considered not acceptable

A study estimating the adsorption coefficient of RH-163353 using the OECD HPLC screening method was submitted but was not considered acceptable for regulatory use and was therefore not evaluated.

RMS comment:

The study was not considered acceptable for regulatory use and was therefore not evaluated for the Annex I inclusion by UK.

Studies submitted with the dossier for the renewal of the approval:

Reference:	Van den Bosch M.M.H. (2013b). Adsorption/desorption of RH-141455 on three soils.
Guideline(s):	OECD 106
GLP:	Yes
Previous evaluation:	Submitted for the purpose of renewal
Validity of the study:	Considered acceptable

Executive Summary

The adsorption behaviour of RH-141455 was studied on three soils (Speyer 2.2, 1.87%oc, pH 5.5; Speyer 2.3, 0.94%oc, pH 6.8; Speyer 6S, 1.64%oc, pH 7.1). Adsorption of RH-141455 on soil was very low (Kd x test system/solution < 0.3) and therefore desorption kinetics and adsorption/desorption isotherms were not determined. Based upon the results of the 48 hours samples taken in the adsorption kinetics experiment, adsorption coefficients (Kd) for RH-141455 ranged from 0.03 mL/g for Speyer 6S and Speyer 2.3 to 0.06 mL/g for Speyer 2.2 soil; Koc values ranged from 2.1 mL/g (Speyer 6S) to 3.3 mL/g (Speyer 2.3).

I. MATERIAL AND METHODS

[phenyl-UL-^{14}C]-RH-141455
Batch: 76045-5-20
Radiochemical purity: 99.9%
Specific activity: 1998 MBq/mmol (54 mCi/mmol)

Three soils were used in the study (soil characteristics given in Table B.8.1.2-5).

Table B.8.1.2-5: Soil characteristics

Name	**Speyer 2.2 soil**	**Speyer 2.3 soil**	**Speyer 6S soil**
Location	Hanhofen, Rheinland-Pfalz, Germany	Offenbach, Rheinland-Pfalz, Germany	Siebeldingen, Rheinland-Pfalz, Germany
Batch number	F223711	F233711	F6S3711
Sampling date	15 September 2011	13 September 2011	15 September 2011
Characteristics			
pH-$CaCl_2$	5.5	6.8	7.1
Organic carbon %	1.87	0.94	1.64
Organic matter %	3.24	1.62	2.84
Cation exchange capacity (meq/100 g soil)	10	11	24
Water holding capacity (g water/100 g soil)	44.4	35.6	38.9
Particle size distribution (USDA)			
% clay (< 2 µm; w/w)	6.8	8.7	41.0
% silt (2-50 µm; w/w)	12.6	27.6	36.8
% sand (50-2000 µm; w/w)	80.6	63.7	22.2
Texture (USDA)	Loamy sand	Sandy loam	Clay

Prior to the definitive test the stability of the test item in the test system, possible sorption of the test item to the test vessels and the soil/solution ratio were determined. Based on these results soil:solution ratio of 1:1 was chosen for the determination of the equilibration time. 13.5 ml of 0.01 M $CaCl_2$ was added to 15 g soil. Soil slurries were equilibrated in the dark for 4 days and then 1.5 ml of spike solution was added to give a final concentration of 0.5 mg/L. Samples were equilibrated for 3, 6, 24 and 48 hours at which times samples were centrifuged and aliquots of the supernatant were taken and radioactivity determined by LSC. After the final sampling time the supernatants were analysed by HPLC and a mass balance was determined.

Due to the low observed adsorption Freundlich isotherms and desorption were not determined.

II. RESULTS AND DISCUSSION

Mass balances were 98%, 99% and 91% in the Speyer 2.2, 2.3 and 6S soil, respectively.

The results of the initial tests demonstrated that RH-141455 was stable in the test system after 48 hours contact time with no sorption to the walls of the containers. The degree of adsorption after 3, 6, 24 and 48 hours equilibration time is given in Table 8.1.2-6.

Table 8.1.2-6: Percentage adsorption of RH-141455 to soil

Soil	Replicate	% adsorption			
		3 hours	6 hours	24 hours	48 hours
Speyer 2.2	A	4.57	3.86	2.00	5.76
	B	0.22	0.13	0.62	5.04
Speyer 2.3	A	-3.80	-1.01	-2.31	4.75
	B	-2.63	-3.66	-1.51	1.06
Speyer 6S	A	-4.58	-5.77	-3.95	2.95
	B	-3.52	-6.11	-3.66	-3.56*

*negative sorption, replicate excluded from adsorption calculations

The calculated soil, adsorption coefficients after 48 hours equilibration are given in Table 8.1.2-7. For detailed results please see Table 8.1.2-8 to Table 8.1.2-11.

Table 8.1.2-7: Soil adsorption coefficients of RH-141455

Soil	K_d (ml/g)	K_{oc} (ml/g)
Speyer 2.2	0.06	3.1
Speyer 2.3	0.03	3.3
Speyer 6S	0.03*	2.1*

*based on replicate A only

Table 8.1.2-8: Detailed results

Test system	Code	% Moisture	Weighed test system (g)	Dry mass of soil m_{soil} (g)	Water volume in weighed test system (mL)	Spike volume [1] (mL)	m_0 (µg)	0.01 M $CaCl_2$ added (mL)	V_0 (mL)	C_0 (µg/mL)
Speyer 2.2	A	1.09	15.0889	14.9247	0.1642	1.4897	7.7226	13.5925	15.2464	0.5065
	B	1.09	15.0012	14.8380	0.1632	1.4861	7.7039	13.5253	15.1746	0.5077
	Bl	1.09	15.0013	14.8381	0.1632	1.4909	n.a.	13.5539	15.2080	n.a.
Speyer 2.3	A	1.98	15.0033	14.7067	0.2966	1.4853	7.6998	13.4952	15.2771	0.5040
	B	1.98	15.0051	14.7085	0.2966	1.4861	7.7039	13.5150	15.2977	0.5036
	Bl	1.98	15.0073	14.7106	0.2967	1.4834	7.6899	13.5227	15.3028	0.5025
Speyer 6S	A	5.38	15.0010	14.1942	0.8068	1.4859	7.7029	13.5930	15.8857	0.4849
	B	5.38	15.0066	14.1995	0.8071	1.4899	7.7236	13.5031	15.8001	0.4888
	Bl	5.38	15.0004	14.1937	0.8067	1.4920	n.a.	13.5707	15.8694	n.a.

[1] to the blanks, 0.01 M CaCl2 solution was spiked instead of spike solution n.a. not applicable

Table 8.1.2-9: Detailed results

Test system	Code	Activity in V_a^A (in DPM)				m_m^{ads} (t_n) (in µg)			
		t_1	t_2	t_3	t_4	t_1	t_2	t_3	t_4
Speyer 2.2	A	24681.30	25023.89	25673.66	24882.55	0.0483	0.0490	0.0503	0.0487
	B	25865.01	26053.74	26094.35	25130.68	0.0507	0.0510	0.0511	0.0492
	Bl	26.04	19.27	16.09	44.67	n.a.	n.a.	n.a.	n.a.
Speyer 2.3	A	26701.08	26169.64	26669.19	25154.60	0.0523	0.0512	0.0522	0.0490
	B	26378.50	26832.63	26439.89	26098.51	0.0517	0.0525	0.0518	0.0508
	Bl	15.67	29.70	18.63	175.60	n.a.	n.a.	n.a.	n.a.
Speyer 6S	A	25881.96	26347.33	26055.67	24503.36	0.0507	0.0516	0.0510	0.0480
	B	25828.29	26645.66	26194.64	26359.38	0.0506	0.0522	0.0513	0.0516
	Bl	15.86	19.91	16.71	36.84	n.a.	n.a.	n.a.	n.a.

t_1: 3 h, t_2: 6 h, t_3: 24 h, t_4: 48 h
n.a. not applicable

Table 8.1.2-10: Detailed results

Test system	repl.	m_{aq}^{ads} (in µg)				m_s^{ads} (in µg)				At_i (in %)			
		t_1	t_2	t_3	t_4	Δt_1	Δt_2	Δt_3	Δt_4	t_1	t_2	t_3	t_4
Speyer 2.2	A	7.369	7.425	7.568	7.278	0.353	-0.055	-0.144	0.291	4.573	3.856	1.996	5.758
	B	7.687	7.694	7.656	7.315	0.017	-0.007	0.038	0.341	0.221	0.129	0.624	5.044
Speyer 2.3	A	7.992	7.778	7.877	7.334	-0.293	0.215	-0.100	0.543	-3.799	-1.012	-2.307	4.746
	B	7.906	7.986	7.820	7.622	-0.202	-0.079	0.166	0.198	-2.628	-3.659	-1.510	1.064
Speyer 6S	A	8.056	8.148	8.007	7.476	-0.353	-0.092	0.140	0.532	-4.578	-5.773	-3.952	2.949
	B	7.996	8.195	8.006	7.999	-0.272	-0.200	0.189	0.007	-3.520	-6.106	-3.657	-3.561

t_1: 3 h, t_2: 6 h, t_3: 24 h, t_4: 48 h

Table 8.1.2-11: Detailed results

Test system	repl.	m_s^{ads}(eq) (µg)	m_{aq}^{ads}(eq) (µg)	K_d (mL/g)	% om	K_{om} (mL/g)	% oc	K_{oc} (mL/g)	Average (mL/g)		
									K_d	K_{om}	K_{oc}
Speyer 2.2	A	0.4447	7.2779	0.062	3.24	1.926	1.87	3.338	0.058	1.801	3.121
	B	0.3886	7.3154	0.054	3.24	1.677	1.87	2.905			
Speyer 2.3	A	0.3655	7.3343	0.052	1.62	3.195	0.94	5.506	0.031	1.943	3.348
	B	0.0820	7.6220	0.011	1.62	0.690	0.94	1.190			
Speyer 6S	A	0.2271	7.4758	0.034	2.84	1.197	1.64	2.073	-0.002	-0.075	2.073
	B	-0.2751	7.9987	-0.038	2.84	-1.347	1.64	-2.333			

III. CONCLUSION

The adsorption of RH-141455 was examined in 3 soils. Due to the low adsorption, desorption was not investigated and Freundlich adsorption isotherms were not calculated. Adsorption of RH-141455 was very low with K_{oc} values of 2.1 to 3.3 ml/g.

RMS comment:

The study is overall acceptable and the results are appropriate for risk assessment purposes.

B.8.1.2.1 Aged sorption

No data on the aged sorption of zoxamide or its metabolites were considered necessary for the first EU review, neither for the renewal of the approval.

B.8.1.3 Mobility in soil

B.8.1.3.1 Column leaching studies

Adequate data to assess the column leaching of zoxamide were evaluated during the first EU review and no further data are considered necessary.

Studies from the original DAR (May 2001):

Reference:	Volkel W. (1998d). 14C-RH-117281: Leaching characteristics of aged residues in one soil, RCC Umweltchemie Ag, Rohm and Haas Technical Report No. 34-98-48, September 15, 1998.
Guideline(s):	Commission Dir. 95/36/EC and SETAC guidelines (Procedures for assessing the environmental fate and ecotoxicity of pesticides, Section 6, 1995)
GLP:	Yes
Previous evaluation:	In DAR (May 2001)
Validity of the study:	Considered acceptable

[14C-phenyl-U]-RH-117281 (radiochemical purity 100%, specific activity 90.2 mCi/g) in acetone was added to duplicate samples of microbially-viable Mechthildshausen sandy loam soil (59% sand; 24% silt; 17% clay; pH 7.4; %oc 1.2; equivalent to 100 g dry weight, 2mm sieved) at a concentration equivalent to 0.2 kg a.s./ha. Soil was maintained at 50% WHC (equiv. 82.4% field capacity at 0.33 bar). Treated soils were incubated under aerobic conditions at 20 $\pm$ 1°C in the dark for 3 days. Volatiles were trapped in sodium hydroxide (CO_2) and ethylene glycol (volatile organics) solutions.

Samples were taken for analysis at 0, 3 and 4 days during incubation. Radioactivity was extracted with acidified acetonitrile, with additional Soxhlet extraction. Analysis was by combustion/LSC, one- and two-dimensional TLC and reverse phase radio- and UV- HPLC. Compound identification was by co-chromatography with unlabelled reference standards. Total recovery of radioactivity was 95-100.6% AR. RH-117281 and the three major soil metabolites RH-127450, RH-24549 and RH-163353 were present at >10% AR (Table B.8.1.3.1-1). CO_2 accounted for 2.4% AR by day 3 and levels of organic volatiles were <0.1% AR. The levels of non-extracted radioactivity was 11.6% AR on day 3.

Table B.8.1.3.1-1: Distribution and characterisation of radioactivity in [14C-phenyl-U]-RH-117281 treated 3-day aged soil.

Radioactive Fraction	% Applied Radioactivity
Parent, RH-117281	41.9
RH-139432	2.3
RH-127450	10.9
RH-24549	10.4
RH-163353	14.2
CO_2	2.4
Organic volatiles	<0.1
Non-extracted	11.6

Replicate 30 cm columns (5 cm i.d.) were packed with the same untreated soil (750g air-dried, 2mm sieved, resulting bulk density 1.27 g/cm^3), which was then saturated from below with water. Samples of the 3-day incubated treated soil were than added to the column to a depth of 5 cm. Columns were eluted with bi-distilled water (393 ml, corresponding to *ca* 200 mm) over 2 days and the leachate collected. Leached soil columns were divided into 6cm segments, extracted with acidified acetonitrile/ Soxhlet. Analysis was by LSC, TLC and HPLC as detailed above.

Recovery following elution is summarised in Table B.8.1.3.1-2. The majority of radioactivity applied (85.9-90.8% AR) was retained by the soil column and of this, the majority remained in the upper soil layer (68.6-74.4% AR). Compounds present at >10% AR were RH-117281 and RH-127450 (Table B.8.1.3.1-3). Parent compound was only detectable in the 0-5 cm layer and RH-127450 was detectable down to 10 cm. Metabolites RH-24549 and RH-163353 were detected at <10% AR in soil layers down to 20 cm. A further 8 metabolites were present, including RH 139432, RH-129151 and RH-141288, which individually accounted for $\leq$ 6%AR. Non-extractable radioactivity accounted for 22.4-23.1% AR, the majority being present in the 0-5 cm layer. No characterisation of radioactivity in the leachate was carried out.

Table B.8.1.3.1-2: Distribution of applied radioactivity (%AR) from eluted aged residue columns

Soil layer (cm)	Column 1	Column 2	Column 3
0-5	73.5	74.4	68.6
5-10	6.4	8.4	9.9
10-15	3.3	4.8	4.3
15-20	1.5	2.1	3.1
20-25	0.6	0.7	0.8
25-30	0.6	0.4	0.4

Total leached soil	85.9	90.8	87.1
Leachate	2.3	1.8	1.9

Table B.8.1.3.1-3: Characterisation of applied radioactivity (%AR) from eluted aged residue columns

Column Segment	parent RH-117281	RH-127450	RH-24549	RH-163353	PES
Column 1					
0-5	12.3	8.0	5.6	5.3	20.7
5-10	ND	ND	4.2[1]		1.4
10-15	ND	ND	1.2	1.2	0.4
15-20	ND	ND	0.3	0.5	0.2
Column 2					
0-5	13.7	6.9	8.8	6.7	19.4
5-10	ND	ND	6.4[1]		1.4
10-15	ND	ND	4.2[1]		0.8
15-20[2]	---	---	---	---	0.4
Column 3					
0-5	16.5	11.9	8.1	4.0	20.4
5-10	ND	0.3	5.7[1]		1.5
10-15	ND	ND	3.6[1]		0.4
15-20	ND	ND	1.9	0.7	0.2

1. HPLC peak for RH-24549 not fully resolved from RH-163353.

2. Extractable residue too low for analysis. ND Not detected.

RMS comment:

The study has been previously evaluated and considered acceptable for the Annex I inclusion by UK. The RMS believes the study is still overall acceptable. Moreover the results of this study confirm the results of the adsorption/desorption studies.

B.8.1.3.2 Lysimeter studies

On the basis of the available data lysimeter studies are not necessary.

B.8.1.3.3 Field leaching studies

On the basis of the available data field leaching studies are not necessary.

Summary of adsorption/desorption and mobility in soil

Under the original review adsorption studies for zoxamide on a range of five soils (pH4.8-7.2, 0.26-1.77%oc) gave Kfoc values of 815-1431, classifying zoxamide as slightly mobile using the SSLRC scale.

The adsorption behaviour for the metabolites RH-24549, RH-127450 and RH-163353, was also examined, each in a range of 3 soils (RH-24549: pH 5.2-7.6, 1.3-2.4%oc; RH-127450: pH 5-7.3, 1.0-4.0%oc; RH-163353: pH 5.4-7.2, 1.2-4.8 %oc). Kfoc values obtained were as follows: RH-24549: 90.5-307.4 ml/g; RH-127450: 404-1156 ml/g and RH-163353: 75-79 ml/g. RH-24549 and RH-163353 are therefore classified as moderately mobile and RH-127450 as slightly to moderately mobile (BCPC monograph 47).

No clear relationships were observed between pH and Kfoc or between % clay content and Kfoc for zoxamide or any of the metabolites, except for RH-24549 which showed tendency of pH dependence. The role of pH or % clay content in determining the mobility of zoxamide is limited, as expected from the lack of a pKa and the non-dependency of water solubility on pH under environmentally relevant conditions.

A 3-day aged column leaching study, performed in one sandy loam soil with 59.1% sand content and relatively low %oc content (pH 7.4, 1.2 %oc) showed 68.6-74.4 %AR in the top 0-5 cm layer and only 1.8-2.3 %AR in the leachate. Zoxamide and major metabolite RH-127450 were only detectable in the 0-5 and 0-10 cm layers respectively. Results indicate a slightly greater potential for leaching of metabolites RH-24549 and RH-163353, however levels were <10% AR in the 0-5 cm layer and non-detectable in the 20-30cm layers.

Due to the short half lives of zoxamide and major soil metabolites RH-24549, RH-127450 and RH-163353, and the low to moderate mobility through soil, it is considered highly unlikely that these compounds will leach to groundwater.

Since the original review, the guidelines for evaluating the relevance of metabolites have changed. Metabolites occurring at >5% on two or more consecutive occasions should be considered as well as those which reach their maximum at the final time-point of the study. In the soil degradation and metabolism study (Burgener, 1998a), metabolite RH-141455 was detected at levels above 5% on more than two occasions (Maximum was 8%), therefore data on the adsorption of this metabolite have been provided. Adsorption of RH-141455 was examined in three soils (0.94 to 1.87%oc, pH 5.5 to 7.1) and was found to be very low with Koc values of 2.1 to 3.3 ml/g (Kd 0.03 to 0.06). As adsorption was so low, Freundlich isotherms were not determined.

The data on the adsorption of zoxamide and its metabolites are summarised in Table B.8.1.2/3-1.

Table B.8.1.2/3-1: Kfoc values for zoxamide and its metabolites

Compound	**Soil**	**%oc**	**pH**	**Kf**	**1/n**	**Kfoc**	**Kom**
Zoxamide	Loam, Huntsburg, Ohio, USA	1.27	7.2	10.35	0.896	815	473
	Sandy loam, Madera, CA., USA	0.26	5.6	3.36	0.986	1294	751
	Silty clay loam, Concord, Ohio, USA	1.77	4.8	25.33	0.963	1431	830
	Sandy loam, Madison, Ohio, USA	1.1	6.7	15.23	0.953	1385	803
	Silty loam, Newtown, Pennsylvania, USA	1.04	6.8	12.44	1.067	1196	694
	Mean / Geometric mean				**0.973**	**1224 / 1201**	**710 / 697**
RH-127450	Loamy sand, Borstel/Germany	1.05	6.1	12.14	0.519	1156	671
	Clay, Egerkingen/ Switzerland	2.82	5.0	11.4	0.603	404	234
	Silt loam, Vetroz/Switzerland	4.05	7.3	18.12	0.448	447	259
	Mean / Geometric mean				**0.523**[1]	**669 /**	**388 /**

						593	**344**
RH-24549	Sandy loam, Iowa/USA	1.3	5.2	4.0	0.791	307.43	178
	Silty clay loam, Illinois/USA	2.4	7.3	3.6	0.833	150.16	87
	Silt loam, Ohio/USA	2.0	7.6	1.8	0.811	90.55	53
	Mean / Geometric mean				**0.811**	**183 / 161**	**106 / 94**
RH-163353	Loamy sand, Borstel/Germany	1.22	6.1	0.6	1.0[2]	50[2]	29
	Clay, Egerkingen/ Switzerland	3.17	5.4	2.4	0.833	75	44
	Silt loam, Vetroz/Switzerland	4.79	7.2	3.8	0.844	79	46
	Mean / Geometric mean				**0.892**	**68 / 67**	**39 / 39**
RH-141455	Speyer 2.2, loamy sand	1.87	5.5	0.06[3]	1.0[2]	3.1[2,4]	~~2~~ 1.8
	Speyer 2.3, sandy loam	0.94	6.8	0.03[3]	1.0[2]	3.3[2,4]	~~2~~ 1.9
	Speyer 6S, clay	1.64	7.1	0.03[3]	1.0[2]	2.1[2,4]	~~1~~ 1.2
	Mean / Geometric mean				**1.0**	**2.8 / 2.8**	**~~2~~ 1.6 / 1.6**

[1]low value not considered reliable [2]Kfoc/Koc derived from a Kf/Kd from the screening study, therefore a 1/n value of 1.0 was assumed [3]represents Kd [4]represents Koc

B.8.2 Fate and behaviour in water and sediment

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

B.8.2.1.1 Hydrolytic degradation

Studies from the original DAR (May 2001):

Reference:	Reynolds JL. (1998b). Hydrolysis of [14C]RH-117281 in Water at pH 4, 7, and 9, XenoBiotic Laboratories, Inc., Rohm and Haas Technical Report Number 34-98-39, September 29, 1998.
Guideline(s):	US EPA guidelines (Subdivision N, 161-1, 1982)
GLP:	Yes
Previous evaluation:	In DAR (May 2001)
Validity of the study:	Considered acceptable

Sterile aqueous buffer solutions (pH 4, 7 and 9) were prepared containing [14C-phenyl-U] RH-117281 (radiochemical purity ≥97.5, specific activity 25.4-90.2 mCi/g (depending on test), ca 0.5 mg a.s./litre). Samples were incubated under sterile conditions in the dark at either 50 ± 0.5°C or 25 ± 1°C for up to 30 days. Duplicate samples were taken at minimum of six time points between 0-30 days after application and the radioactivity quantified directly by LSC and in SPE extracts by one and two-dimensional TLC and reverse phase HPLC. Analyte identity was confirmed by LC-MS-MS using reference and synthesised standard compounds.

At 25°C, total recoveries (mean ± SD) of radioactivity were 93.4 ± 1.7% (pH4), 93.8 ± 1.2% (pH 7) and 94.3 ± 1.4% (pH 9). RH-117281 comprised 90-92% at day 0, decreasing to 7-32% after 30 days and degraded with first-order half lives of 16, 16 and 8 days (r^2 1.0) at pHs 4, 7 and 9 respectively. Major metabolites exceeding 10% AR were identified as RH-129151 (re-arranged cyclic product), RH-150721 (amine), RH-24549 (benzoic acid derivative), and RH-141288 (alcohol) (see Table B.8.2.1.1-1). The proposed hydrolytic degradation pathway is shown in Figure B.8.2.1.1-1. Minor metabolites included

RH-127450, RH-139432 and six other unidentified metabolites which individually did not account for >7% AR. At 50°C, DT50s were 14.3, 14.8 and 4.0 hours at pHs 4, 7 and 9 respectively.

Table B.8.2.1.1-1: Identification and mass balance of radioactivity in buffer solutions during hydrolysis at 25°C (% AR)

Day	parent RH-117281	RH-150721	RH-24549	RH-141288	RH-129151	RH-127450	RH-139432	Total (identified + non-identified)
pH 4								
0	90.25	ND	ND	ND	ND	0.51	ND	90.76
3	84.39	7.50	0.36	ND	0.67	0.86	ND	93.78
7	68.05	22.59	2.61	ND	0.39	0.66	ND	94.30
14	51.17	28.96	9.67	0.17	0.26	0.90	ND	92.25
21	37.04	37.61	18.04	ND	ND	0.59	ND	94.83
30	24.02	32.06	30.94	0.64	0.20	0.73	0.30	94.32
pH 7								
0	91.87	ND	ND	ND	0.17	0.44	ND	92.48
3	83.61	ND	0.60	0.50	8.23	0.99	ND	93.92
7	69.58	0.45	2.38	2.52	16.98	0.85	ND	92.75
14	53.47	0.82	6.92	9.10	22.75	0.64	0.13	94.19
21	37.15	1.24	14.11	16.29	24.50	1.06	0.69	95.60
30	24.70	1.52	20.75	21.86	21.26	0.73	0.61	93.81
pH 9								
0	92.17	ND	ND	ND	0.22	0.78	ND	93.16
3	75.15	ND	ND	8.86	9.17	0.36	0.20	94.14
5	61.94	ND	1.26	16.44	14.68	0.81	ND	95.61
7	45.43	ND	1.37	29.26	16.37	1.01	1.91	95.88
14	26.93	ND	7.31	36.35	14.48	0.68	2.41	93.67
30	7.08	0.13	11.54	50.22	7.65	0.66	5.82	92.69

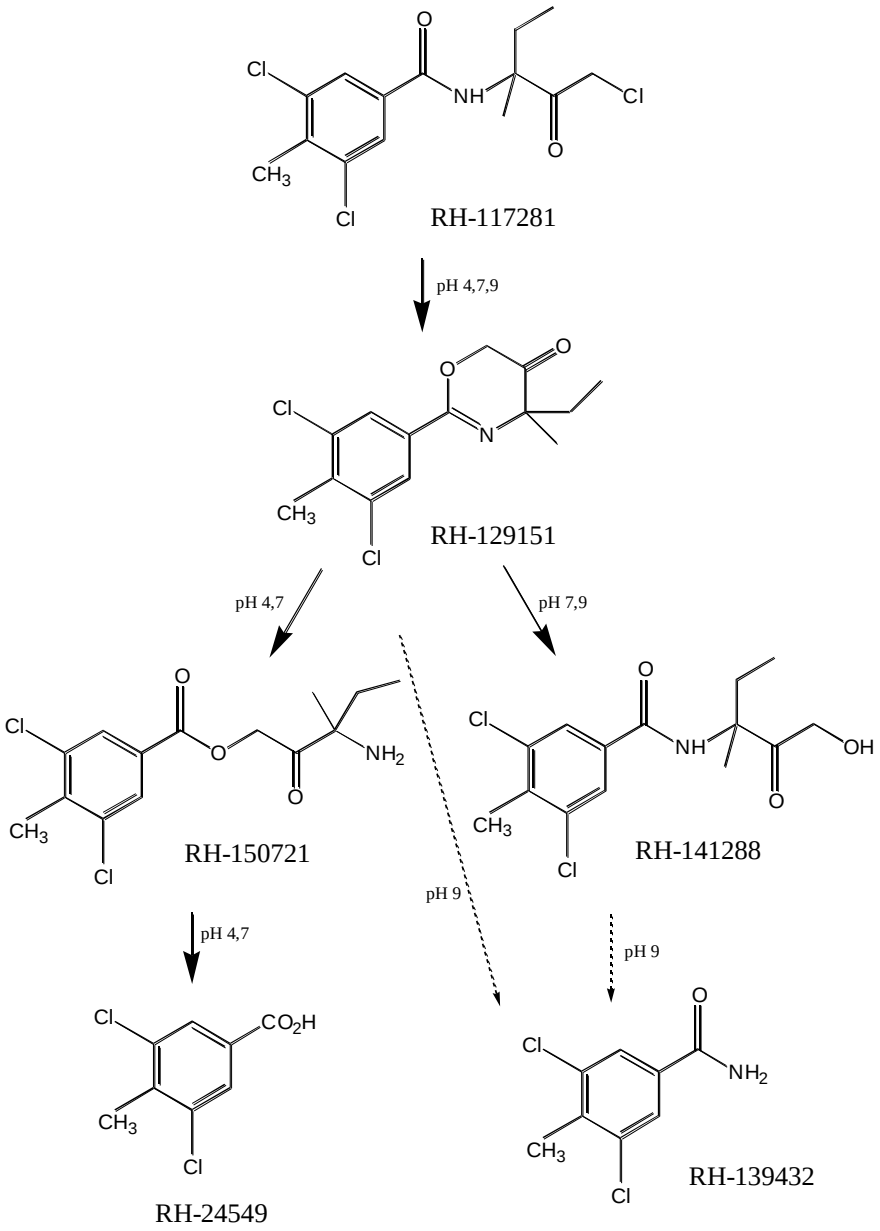


Figure B.8.2.1.1-1 Proposed Hydrolytic Degradation Pathway

RMS comment:

The study has been previously evaluated and considered acceptable for the Annex I inclusion by UK. The RMS believes the study overall complies with the requirements of existing guidance document – OECD guideline 111 "Hydrolysis as a Function of pH" (April 2004) and therefore is still considered acceptable.

Reference:	Chong BP. (1998). RH-117281 Fungicide: hydrolysis rates of relevant degradation products, Rohm and Haas Technical Report No. 34-98-26, September 30, 1998.
Guideline(s):	US EPA guidelines (Subdivision N, 161-1, 1982)
GLP:	No (calculation - GLP is not relevant)
Previous evaluation:	In DAR (May 2001)
Validity of the study:	Considered acceptable

The hydrolytic degradation rates of relevant metabolites in each pH regime were calculated from the hydrolysis study detailed above (Reynolds JL., 1998b).

Degradation rate constants were calculated, assuming first order kinetics, using linear and non-linear compartmental regression analysis (SAS JMP Version 3.2). Full details of the differential equation calculations were submitted. The proposed metabolism pathway and statistical approach are acceptable.

DT50s (Table B.8.2.1.1-2) were calculated from the rate constants (K) as follows: DT50 = ln(2) / K.

Table B.8.2.1.1-2: Hydrolytic DT50s of relevant metabolites of [14C-phenyl-U] RH-117281.

Metabolite	pH 4	pH 7	pH 9
RH-129151	<10%AR	9.1 days	2.4 days
RH-150721	18.3 days	<10% AR	<10% AR
RH-24549	stable	stable	stable
RH-141288	<10% AR	stable	stable

RMS comment:

The study has been previously evaluated and considered acceptable for the Annex I inclusion by UK. The RMS believes the study is still acceptable.

B.8.2.1.2 Direct photochemical degradation

Studies from the original DAR (May 2001):

Reference:	Smalley J., Reynolds JL. (1998). Aqueous photolysis of [14C]-RH-117281, XenoBiotic Laboratories, Inc., Rohm and Haas Technical Report No. 34-96-215, May 12, 1998.
Guideline(s):	US EPA guidelines (Subdivision N, 161-2, 1982)
GLP:	Yes
Previous evaluation:	In DAR (May 2001)
Validity of the study:	Considered acceptable

A sterile sodium acetate solution was prepared containing [14C-phenyl-UL] RH-117281 (radiochemical purity ≥96.5, specific activity 20.95 mCi/g) at concentrations of 0.5 and 2.0 mg/l (1% acetonitrile & pH 4 to minimise hydrolysis). Samples were irradiated with artificial sunlight (Suntest Unit, xenon lamp, cut-off <290 nm) through alternating 12-hour light and dark cycles for up to 30 days at 25°C. The average light intensity (over the range 330-800 nm) and spectral distribution of the xenon lamp and of natural.

New Jersey summer sunlight (*ca* 42° N) were comparable (xenon lamp and natural sunlight average fluxes were 169 and 171.5 W/m^2 respectively).

Duplicate samples were taken at 0, 3, 7, 10, 14, 21 and 30 days post irradiation and neutral, acidic and basic volatiles were individually trapped. Radioactivity was quantified by LSC, extracted by SPE and characterised by radio-TLC, HPLC and electrospray (ESI) LC-MS.

Total recoveries were 94.5-99.8% (irradiated samples) and 97.1-102.1% (dark controls). Degradation of RH-117281 occurred in dark controls with an estimated first-order half-life of 22 days (r^2 1.0). RH-117281 levels decreased from 98% at day 0 to 38.9% at day 30. In the irradiated samples, RH-117281 degraded with a first-order half-life of 8 days (r^2 0.99). RH-117281 levels decreased from 98% at day 0 to 7% at day 30 (Table B.8.2.1.2-1).

Table B.8.2.1.2-1: Identification and mass balance of radioactivity during aqueous photolysis at pH 4 (% AR, mean of duplicates).

Day	parent RH-117281	RH-150721	RH-24549	RH-139432*	RH-129151	Unknown	Unknown	Total
0	97.98	0.65	ND	0.65	ND	ND	ND	99.27
3	80.97	3.89	5.03	8.67	ND	ND	ND	98.56
7	59.96	7.29	10.21	18.07	0.84	0.53	0.88	97.76
10	43.07	15.10	9.67	26.70	0.54	1.02	1.02	97.11
14	37.07	9.46	19.03	28.04	1.91	1.13	1.38	98.00
21	16.15	9.98	23.90	41.83	1.74	1.54	1.25	96.38
30	6.99	11.32	27.69	42.36	3.29	1.23	1.13	94.00
Dark 14	66.21	10.96	19.62	0.69	ND	0.39	ND	97.86
Dark 30	38.88	14.06	44.60	1.00	ND	0.60	ND	99.12

* Possibly contains low levels (<1%) of RH-141288, as indicated from the hydrolysis study of *Reynolds (1998b)*, see section B.8.2.1.1.

Major degradates, formed at greater than 10% of the applied radioactivity during the study, were RH-24549 (benzoic acid derivative), RH-139432 (amide) and RH-150721 (re-arranged product of RH-129151). RH-24549 and RH-150721 were not photo-products, however, they reached similar or greater concentrations in the dark control as in the irradiated samples. These compounds were also major hydrolysis degradates. RH-139432 was the only major photo-product in the study (only a very minor component in the dark control). The level of RH-139432 continued to increase throughout the study increasing to a maximum of 42% AR at day 30. The major metabolites did not degrade under the conditions of this study, therefore were not studied further.

Minor metabolites included RH-129151 (re-arranged cyclic product), and two other unidentified metabolites which individually did not account for >1.6% AR. Volatiles accounted for <0.2% AR (irradiated samples) and <0.3%AR (dark controls).

Mean quantum yield was calculated from degradation kinetics and absorption data to be 0.0225.

RMS comment:

The study has been previously evaluated and considered acceptable for the Annex I inclusion by UK. The RMS believes the study overall complies with the requirements of existing guidance document – OECD guideline 316 "Phototransformation of Chemicals in Water" (October 2008) and therefore is still considered acceptable.

B.8.2.1.3 Indirect photochemical degradation

There are no indications that the route and rate of degradation of zoxamide in the water phase can be significantly influenced by indirect photolysis. Data on indirect photochemical degradation are therefore not necessary.

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

B.8.2.2.1 "Ready biodegradability"

Studies from the original DAR (May 2001):

Reference:	Barnes S.P., Nave V. (1998). RH-117281 – Assessment of ready biodegradability: modified Sturm test, Huntingdon Life Sciences Limited, Rohm and Haas Report No. 98RC-1028, December 14, 1998.
Guideline(s):	Method C.4 Determination of "ready" biodegradability (Annex to Regulation (EC) No 440/2008).
GLP:	Yes
Previous evaluation:	In DAR (May 2001)
Validity of the study:	Considered acceptable

RH-117281 was added to two flasks containing a mineral salts medium inoculated with 30 mg/l domestic activated sludge, to give a nominal concentration of 10 mg/l. The following control samples were also established: inoculated medium unfortified control; inoculated medium/sodium benzoate reference (10 mg C/litre); inoculated medium /test compound/ sodium benzoate inhibition control. Samples were aerated for 29 days with CO_2-free air. Resulting CO_2 was trapped with barium hydroxide and quantified

by titration of residual $Ba(OH)_2$ with HCl. The pH of the samples remained within 7.4-7.6 throughout the study.

Sodium benzoate was biodegraded by 70% in 6 days and by 93% in 29 days. In the presence of RH-117281, sodium benzoate was biodegraded by 65% in 6 days, indicating that RH-117281 was not inhibitory to the microbial innoculum.

RH-117281 was biodegraded by 8% in 29 days, indicating that it is not readily biodegradable under these conditions.

RMS comment:

The study has been previously evaluated and considered acceptable for the Annex I inclusion by UK. The RMS believes the study is still acceptable.

B.8.2.2.2 Aerobic mineralisation in surface water

Studies submitted with the dossier for the renewal of the approval:

Reference:	Van den Bosch M.M.H. (2014). Aerobic mineralisation of zoxamide in surface water.
Guideline(s):	OECD guideline for the testing of chemicals 309. Aerobic Mineralisation in Surface water – Simulation Biodegradation Test. 13th April 2004
GLP:	Yes
Previous evaluation:	Submitted for the purpose of renewal
Validity of the study:	Considered acceptable

Executive Summary

The degradation of zoxamide was examined in surface water (pelagic test) according to OECD guideline 309. ^{14}C-zoxamide was incubated at two test concentrations (10 µg/L and 50 µg/L) in surface water at 20 ± 2 °C in the dark for 58 days. Two flasks were additionally treated with benzoic acid to act as a positive control. Samples were taken for analysis at 0, 1, 3, 7, 15, 28, 44 and 58 days after treatment. Analysis was performed by HPLC with selected samples analysed by LC-MS. Zoxamide degraded rapidly to non-detectable levels after 28 days with a DT_{50} of 7.6 to 8.4 days. A number of metabolites were detected above the relevant thresholds. RH-141455, RH-139432, RH-141288, RH-163353 and RH-24549 were detected at >5% on two consecutive occasions at respective maximums of 10.5% AR, 21.4% AR, 22.1% AR, 47.9% AR and 22.7% AR. M-7 was detected at a maximum of 9.1% AR but was multicomponent, consisting of 2-3 different substances which individually did not exceed 5% AR.

I. MATERIAL AND METHODS

[phenyl-UL-14C]-zoxamide

Batch:	2010041301
Radiochemical purity:	97.81%
Specific activity:	1.85 GBq/mmol (50 mCi/mmol)

16 flasks were prepared containing 300 ml of surface water sampled from Schoonrewoerdse Wiel, Leerdam, the Netherlands (properties given in Table B.8.2.2.2-1). Six of the flasks were treated with ^{14}C-zoxamide (stock solution) to give a nominal concentration of 10 µg/l and six further flasks were treated to give a higher concentration of 50 µg/l. For both test concentrations, three flasks were the test vessels, two were for measurement of the mass balance and one flask was the sterile control. Two flasks were treated with benzoic acid to act as a positive control and two flasks received no treatment and were used for monitoring oxygen/pH and temperature respectively.

Table B.8.2.2.2-1 Characteristics of surface water and stock solutions

Property	**Value**
Surface water	
pH (at sampling)	7.1
Temperature (at sampling)	7.5°C
Oxygen (at sampling)	6.8 mg/L (60%)
Total Organic Carbon Dissolved Organic Carbon (DOC)	10.8 mg/L 9.9 mg/L
Total Nitrogen as N	2.3 mg/L
Nitrate	< 2.2 mg/L
Nitrite	< 1.6 mg/L
Ammonium	1.0 mg/L
Total hardness	146 mg/L as $CaCO_3$
Phosphate/orthophosphates	0.6 mg/L
Total Phosphorus as P	0.2 mg/L
Stock solutions	
Dissolved Organic Carbon (DOC) content of water used to prepare stock solutions	-*

* for the preparation of the stock solutions only acetonitrile was used.

Flasks were incubated under aerobic conditions at 20 ± 2 °C in the dark for 58 days with constant agitation. During incubation aeration was continuous and air was trapped in polyurethane foam plugs, ethylene glycol (organic volatiles) and 2N NaOH (CO_2).

Samples were taken from the flasks immediately after treatment and at 1, 3, 7, 15, 28 and 58 days. Dissolved oxygen, pH and water temperature were determined at least once every two weeks. Radioactivity in water was directly determined by LSC, both before and after addition of HCl to remove evolved CO_2. Total radioactivity in volatile traps was determined by LSC and confirmation of the presence of CO_2 in the NaOH traps was performed by precipitation with barium hydroxide. Polyurathane foam plugs were extracted with acetonitrile and radioactivity determined by LSC. Radioactivity in the water samples was characterised by co-chromatography with reference standards using HPLC and LC-MS.

II. RESULTS AND DISCUSSION

Oxygen concentration measurements indicated aerobic conditions were maintained in the water throughout the test. The results of the reference control (benzoic acid) showed that the test system was sufficiently viable, see Table B.8.2.2.2-2.

Table B.8.2.2.2-2: Distribution of radioactivity in test system with reference control (mean values; % AR)

Time (days)	**Flask**	**PUF**	**EGEE**	**NaOH[1]**	**Total water layer**	**Total water layer stripped of CO_2**	**Mass balance**

0	9, 10	na	na	na	97.4	97.6	97.4
1	9, 10	na	0.1	3.4	75.5	43.6	79.0
3	9, 10	na	0.6	9.5	49.0	40.3	59.1
7	9, 10	na	0.0	46.3	24.6	14.9	71.0
15	9, 10	na	0.0	67.5	7.2	6.1	74.8
28	9, 10	na	0.0	73.7	5.1	4.7	78.8
58	9, 10	0.0	0.0	76.5	4.6	4.6	81.2

na: not applicable; [1] Values of flask 9 only. Flask 10 was not used in calculations for CO_2 trapping in the NaOH traps, since no CO_2 was trapped (possibly caused by a leak).

Mass balances in the mass balance flasks were 94.2 to 99.2% AR. The distribution of radioactivity in the high and low dose mass balance flasks is given in Tables B.8.2.2.2-3 and B.8.2.2.2-4.

Significant formation of CO_2 did not occur with <2% of activity recovered in the NaOH traps at both test concentrations and no organic volatiles (<1%) were detected.

Zoxamide degraded rapidly to non-detectable levels in both systems after 28 days of incubation. A number of metabolites were detected above the relevant thresholds (Tables B.8.2.2.2-5 and B.8.2.2.2-6). RH-141455, RH-139432, RH-141288, RH-163353 and RH-24549 were detected at >5% on two consecutive occasions at respective maximums of 10.5% AR, 21.4% AR, 22.1% AR, 47.9% AR and 22.7% AR. An additional fraction, M-7, was detected at a maximum of 9.1% AR. However, this was multicomponent, consisting of 2-3 different substances which individually did not exceed 5% AR.

Table B.8.2.2.2-3: Distribution of radioactivity in test system at low test concentration (mean values; % AR)

Time (days)	Foam plug	Ethylene glycol	Total NaOH	Total water layer	Total water layer stripped of CO_2	Mass balance
0	na	na	na	95.4	96.1	95.4
1	na	0.1	0.2	97.2	97.7	97.5
3	na	0.0	0.0	98.3	101.3	98.3
7	na	0.1	0.0	96.1	97.0	96.2
15	na	0.0	0.1	94.6	94.8	94.6
28	na	0.0	0.4	93.8	96.0	94.2
58	0.5	0.0	1.6	93.9	93.4	96.0
58	0.5	0.0	1.1	93.8	94.3	95.3

na: not applicable

Table B.8.2.2.2-4: Distribution of radioactivity in test system at high test concentration (mean values; % AR)

Time (days)	Foam plug	Ethylene glycol	Total NaOH	Total water layer	Total water layer stripped of CO_2	Mass balance
0	na	na	na	98.3	99.3	98.3
1	na	0.0	0.0	98.5	99.1	98.5
3	na	0.1	0.0	97.1	97.9	97.2
7	na	0.0	0.0	96.2	97.0	96.3
15	na	0.0	0.1	95.8	96.2	96.0
28	na	0.0	0.2	95.4	95.9	95.6
44	na	0.0	0.4	96.3	97.4	96.7
58	0.4	0.0	0.7	96.2	96.5	97.3
58	0.5	0.1	0.7	98.0	98.1	99.2

na: not applicable

Table B.8.2.2.2-5: Parent and metabolites in test system at low concentration (% AR)

Time (days)	Replicate	Parent	RH-141455	RH-139432	RH-141288	RH-163353	RH-24549	RH-129151	M-7*
1	3	97.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	4	97.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	5	97.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	mean	97.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	3	96.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	4	97.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	5	101.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	mean	99.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7	3	94.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	4	96.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	5	96.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	mean	96.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
15	3	23.3	0.0	20.0	13.2	18.4	11.5	6.9	0.0
	4	17.3	0.0	0.0	24.6	37.7	15.9	0.0	0.0
	5	13.8	0.0	22.7	14.6	20.8	23.1	0.0	0.0
	mean	18.1	0.0	14.2	17.5	25.7	16.8	2.3	0.0
28	3	0.0	0.0	38.2	0.0	54.8	0.0	0.0	0.0
	4	0.0	0.0	0.0	19.0	52.4	21.6	0.0	0.0
	5	0.0	0.0	26.0	17.4	36.5	15.1	0.0	0.0
	mean	0.0	0.0	21.4	12.1	47.9	12.2	0.0	0.0
58	3	0.0	5.1	0.0	19.8	36.6	13.3	8.9	9.2
	4	0.0	11.1	0.0	17.1	52.2	13.9	0.0	0.0
	5	0.0	0.0	0.0	29.3	50.8	14.3	0.0	0.0
	mean	0.0	5.4	0.0	22.1	46.5	13.8	3.0	3.1

*multicomponent consisting of 2-3 substances

Table B.8.2.2.2-6: Parent and metabolites in test system at high concentration (% AR)

Time (days)	Replicate	Parent	RH-141455	RH-139432	RH-141288	RH-163353	RH-24549	RH-129151	M-7*
1	6	94.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	7	86.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	8	87.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	mean	89.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	6	88.9	3.2	0.0	3.5	0.0	0.0	0.0	0.0
	7	90.5	0.0	0.0	2.7	0.0	0.0	0.0	0.0
	8	81.3	3.0	0.0	5.1	3.9	1.8	0.0	0.0
	mean	86.9	2.1	0.0	3.8	1.3	0.6	0.0	0.0
7	6	64.0	3.8	7.1	7.0	4.3	3.1	0.0	0.0
	7	64.2	0.0	4.8	7.3	4.9	6.3	0.0	0.0
	8	73.1	2.8	2.6	5.9	3.5	5.8	0.0	0.0
	mean	67.1	2.2	4.8	6.7	4.2	5.1	0.0	0.0
15	6	11.6	0.0	18.2	16.7	23.6	16.2	4.5	0.0
	7	20.0	0.0	15.7	15.5	16.4	13.4	5.2	0.0
	8	30.8	0.0	18.5	17.5	14.2	13.5	3.1	0.0
	mean	20.8	0.0	17.5	16.6	18.1	14.4	4.3	0.0
28	6	0.0	6.6	13.9	17.5	32.2	20.0	4.4	0.0
	7	0.0	7.7	17.5	15.6	24.9	16.1	4.9	4.0
	8	0.0	10.4	22.5	19.5	24.2	16.0	5.3	0.0

	mean	0.0	8.2	18.0	17.5	27.1	17.4	4.9	1.3
44	6	0.0	9.5	9.7	13.7	23.5	21.1	3.1	4.0
	7	0.0	11.0	10.2	14.2	26.6	15.4	5.6	6.8
	8	0.0	11.1	12.8	14.1	20.0	21.1	4.6	5.0
	mean	0.0	10.5	10.9	14.0	23.4	19.2	4.4	5.3
58	6	0.0	7.8	5.8	13.4	29.6	24.1	5.5	7.9
	7	0.0	11.5	5.5	14.1	26.6	19.8	7.0	11.4
	8	0.0	8.3	0.0	18.8	26.8	24.1	6.0	7.9
	mean	0.0	9.2	3.8	15.4	27.7	22.7	6.2	9.1

*multicomponent consisting of 2-3 substances, individually not exceeding 5% AR

The kinetics of the decline of zoxamide and RH-139432 (high dose only) were determined according to FOCUS Kinetics Guidance Document (FOCUS 2006). For the metabolites RH-141455, RH-141288, RH-163353, RH-24549, RH-129151 and M-7, no meaningful calculations could be performed as the metabolites did not (significantly) degrade towards the end of the study.

SFO and FOMC kinetics were first applied to the data for zoxamide. SFO kinetics were considered the best fit to the data. A reliable fit for RH-139432 was not obtained (χ^2 % error = 82.6) therefore solely the results of the kinetic fits for zoxamide are given in Table B.8.2.2.2-7. Plots of the decline and the residuals are given in Figures B.8.2.2.2-1 and B.8.2.2.2-2.

Table B.8.2.2.2-7: Rates of degradation of zoxamide in the systems

System	Model	DT_{50}	DT_{90}	χ^2 % error	P/confidence interval acceptable?
High dose	SFO	7.6	25.4	12.1	Y
	FOMC	7.6	25.5	14.0	N
Low dose	SFO	8.4	28.0	21.9	Y
	FOMC	8.3	28.3	25.3	N

Figure B.8.2.2.2-1: Plots of the decline and the residuals for zoxamide in the high dose system

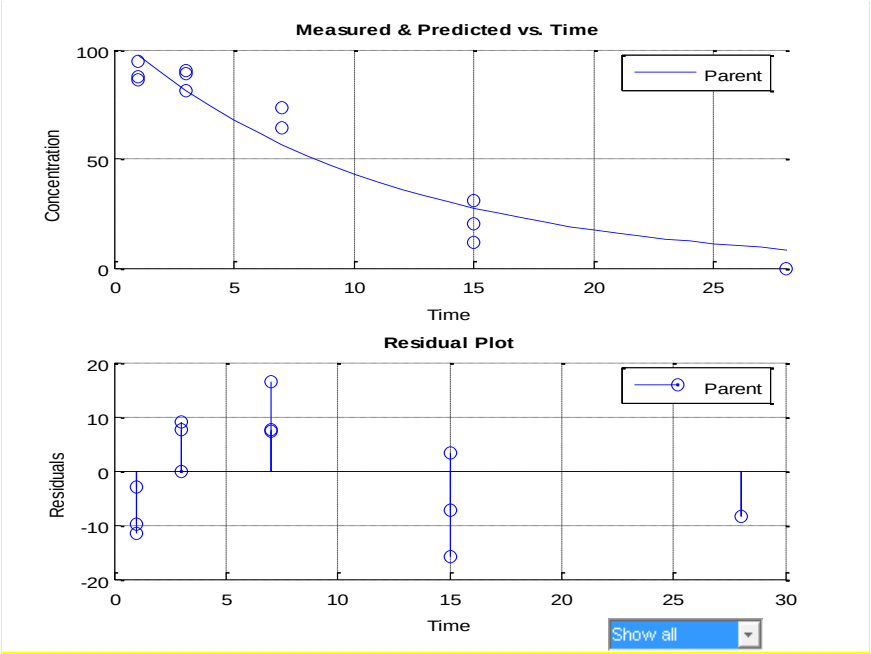
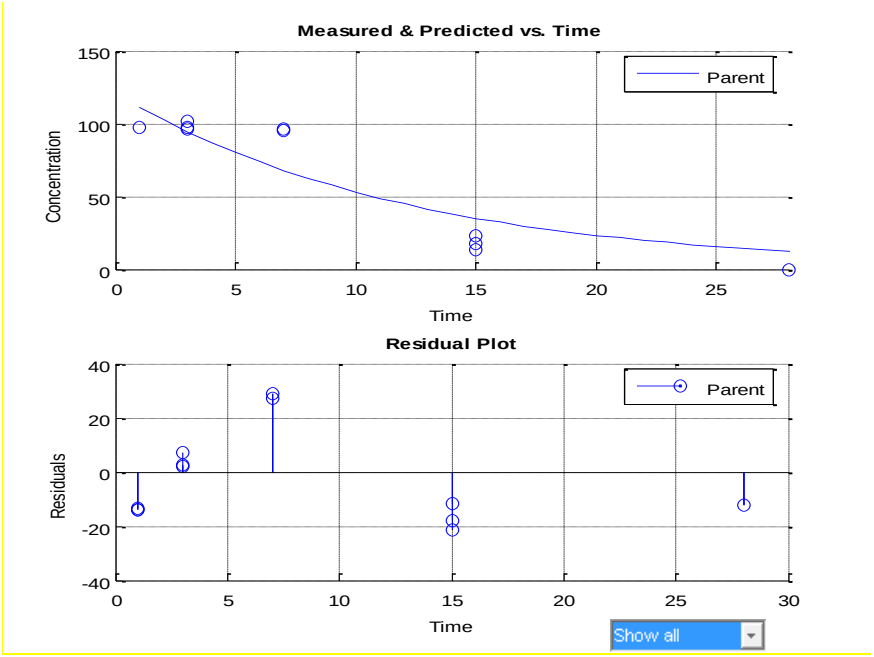


Figure B.8.2.2.2-2: Plots of the decline and the residuals for zoxamide in the low dose system



III. CONCLUSION

The degradation of zoxamide was examined in surface water according to OECD guideline 309. Zoxamide degraded rapidly to non-detectable levels after 28 days with a DT_{50} of 7.6 to 8.4 days. A number of metabolites were detected above the relevant thresholds. RH-141455, RH-139432, RH-141288, RH-163353 and RH-24549 were detected at >5% on two consecutive occasions at respective maximums of 10.5% AR, 21.4% AR, 22.1% AR, 47.9% AR and 22.7% AR. M-7 was detected at a maximum of 9.1% AR but was multicomponent, consisting of 2-3 different substances which individually did not exceed 5% AR.

RMS comment:

Study on aerobic mineralisation in surface water is relatively new data requirement which was not present at the time of Annex I inclusion of zoxamide.
Study was carried out strictly in line with requirements of existing guidance document – OECD guideline 309 "Aerobic Mineralisation in Surface Water – Simulation Biodegradation Test" (April 2004). The kinetics of the decline of zoxamide were determined according to FOCUS Kinetics Guidance Document (2006). Given the above mentioned the study is considered acceptable.

B.8.2.2.3 Water/sediment studies

Studies from the original DAR (May 2001):

Reference:	Morgenroth U. (1998). 14C-RH-117281: Degradation and metabolism in aquatic systems, RCC Umweltchemie Ag, Rohm and Haas Technical Report No. 34-98-47, September 15, 1998.
Guideline(s):	SETAC guidelines (Procedures for assessing the environmental fate and ecotoxicity of pesticides, 8.2. 1995)
GLP:	Yes
Previous evaluation:	In DAR (May 2001)
Validity of the study:	Considered acceptable for the first Annex I inclusion but partly acceptable for the renewal of the approval

Samples of untreated Rhine (river) and Judenweiher, Rheinfelden (pond) water from Switzerland (0.2 mm filtered, 550 ml) and associated 2mm sieved loamy sand and loam sediment (141g and 94g g dry weight, river and pond system respectively) were equilibrated in flasks for 1 month prior to treatment. Water and sediment were characterised at time of collection, prior to treatment and at the end of the study (see Table B.8.2.2.3-1). [Phenyl-14C-U]- RH-117281 (radiochemical purity 98.4-99.8%, specific activity 90.2 mCi/g) was added in acetone to the flasks at 0.072 mg a.s./l (equivalent to 216 g a.s./ha to a depth of 30 cm). Traps for volatiles (ethylene glycol: organics) and sodium hydroxide: CO_2) were fitted and flasks were incubated in the dark at 20°C $\pm$1°C or 10$\pm$1°C for up to 106 days. Aeration of the water was performed using moistened air. Gentle agitation of the surface was achieved without disturbing the sediment by use of a magnetic stirrer.

Table B.8.2.2.3-1: Physico-chemical characterisation of water and sediment before and following incubation with [Phenyl-14C-U]- RH-117281

Water/sediment source	Rhine, Mumpf, AG/Switzerland River		Judenweiher, Rheinfelden, AG/Switzerland Pond	
	Water (start, end) [2]	Sediment (start, end) [2]	Water (start, end) [2]	Sediment (start, end) [2]
Sediment textural class[1]		loamy sand		loam
% sand		78		45
% silt		17		32
% clay		6		23
Cation exchange capacity (mVal/100g dry sediment)		4.3, nd		13.8, nd
pH[3]	8.08-8.09, 8.35	7.35, nd	7.89-7.91, 8.09	6.55, nd
Redox potential[3] (mV)	216-230,	-76,	229-239,	-157,

	201	-213	180	-220
Oxygen content[3] (mg/l)	12.2-12.3, 6.8		12.0-12.2, 7.0	
Total organic carbon	3.2 mg/l, nd	0.48 %, nd	6.7 mg/l, nd	1.77%, nd

[1] US classification

[2] parameters determined before study (top line) and on day 106 of study in control flasks(bottom line).

[3] determined at surface & 5 cm above sediment initially.

nd Not determined.

The pH, DO and E_H of the system were also measured throughout the tests (Table B.8.2.2.3-2). There were no distinct trends in these characteristics. Throughout incubation the redox potential remained positive in the water and negative in the sediment. An aerobic/ anaerobic environment was maintained. Microbial viability was also maintained throughout the test period.

Table B.8.2.2.3-2: Physico-chemical characterisation of water and sediment during incubation with [Phenyl-14C-U]- RH-117281

Water/sediment source		Rhine, Mumpf, AG/Switzerland River		Judenweiher, Rheinfelden, AG/Switzerland Pond	
		Water	Sediment	Water	Sediment
pH	(10°C)	8.20-8.55	nd	7.93-8.31	nd
	(20°C)	8.29-8.51		7.86-8.27	
DO (mg/l)	(10°C)	7.0-8.9	nd	5.8-8.9	nd
	(20°C)	6.0-7.0		6.0-7.9	
Redox potential (mV)					
	(10°C)	100-212	-76 - -174	133-231	0 - -227
	(20°C)	143-209	-99 - -171	152-212	-89 - -274

Duplicate flasks were analysed initially post-treatment and subsequently at 8 time points. Radioactivity in water was quantified by LSC and RH-117281 and metabolites were extracted with ethyl acetate and analysed by reverse phase HPLC and TLC with radiometric and UV (254 nm) detection. Sediment was sequentially extracted with acidified acetonitrile, including a Soxhlet extraction, and the resulting combined extracts concentrated and analysed by HPLC and TLC as above. Non-extracted radioactivity was quantified by combustion LSC and subjected to alkaline extraction, pH partitioning and centrifugation to separate humic (including clay minerals and aluminium oxides) and fulvic fractions. Compound identification was achieved by comparison to reference standards. Radioactivity in the volatile traps was directly quantified by LSC. Identification of CO_2 was confirmed by precipitation as barium carbonate.

Total recoveries were 92.3-103% AR for both systems. Distribution of radioactivity in both systems and at both temperatures was similar (Table B.8.2.2.3-3). A slightly higher proportion of applied radioactivity

partitioned into the pond sediment (maximum 80.6% AR) compared to the river sediment (maximum 64.5% AR. Maximum levels of radioactivity extracted from sediment were 44-56% AR (pond system, day 7-56) and 36-44% AR (river system, day 14), whilst levels of non-extractable radioactivity increased throughout the studies, exceeding that of extracted radioactivity on day 106 except in the pond system at 10°C. Maximum levels of non-extracted radioactivity were 33-43% AR and were higher in the pond than in the river system. Organic volatiles were not detected at any time. Maximum levels of CO_2 evolved by day 106 were similar in both systems (19.7-21.9% AR maximum, 20°C). The extent of mineralisation was higher in the systems incubated at 20°C than at 10°C (4-6.5% AR maximum). Results for the Rhine river/sediment system, incubated at 10°C, are shown in Figure B.8.2.2.3-1.

Table B.8.2.2.3-3: Distribution of radioactivity in Rhine river and Judenweiher pond water and associated sediments during incubation with [Phenyl-14C-U]- RH-117281

	Day 0	Day 0.25	Day 1	Day 2	Day 7	Day 14	Day 28	Day 56	Day 106
River, 20 °C									
Water	96.0	94.7	90.1	86.8	66.1	31.7	30.0	25.3	16.7
Total Sediment	3.1	2.8	10.2	11.0	30.0	59.2	60.2	60.8	64.4
Extractables from sediment	2.6	2.4	8.3	9.3	22.3	36.4	36.1	33.1	27.8
Non-extractables from sediment	0.5	0.4	1.9	1.8	7.7	22.9	24.1	27.7	36.6
CO_2	---	< 0.1	< 0.1	< 0.1	0.4	3.4	6.3	11.1	21.9
River, 10 °C									
Water	94.9		84.1	67.7	50.5	33.1	28.6	22.1	22.5
Total Sediment	3.6		15.1	31.6	48.4	61.0	64.4	71.7	64.5
Extractables from sediment	3.1		12.3	25.7	38.7	43.9	40.1	43.0	31.0
Non-extractables from sediment	0.5		2.9	5.9	9.7	17.1	24.2	28.7	33.5
CO_2	---		< 0.1	0.1	0.1	0.4	1.7	1.9	6.5
Pond, 20 °C									
Water	98.0	95.7	93.5	85.5	31.9	22.5	18.9	7.8	6.9
Total Sediment	3.6	3.6	4.1	13.6	63.5	68.6	72.3	79.3	71.9
Extractables from sediment	3.0	3.1	3.3	11.0	44.3	41.7	42.6	39.4	33.0
Non-extractables from sediment	0.6	0.5	0.7	2.6	19.2	26.8	29.7	39.9	39.0
CO_2	---	< 0.1	< 0.1	< 0.1	1.0	1.2	5.0	9.4	19.7
Pond, 10 °C									
Water	93.1		82.6	69.7	53.2	37.2	31.7	12.6	10.7

Total Sediment	5.1		15.4	27.8	44.3	55.2	63.5	83.2	80.6
Extractables from sediment	4.4		12.4	23.3	36.9	41.4	45.1	55.8	43.3
Non-extractables from sediment	0.7		3.0	4.6	7.4	13.7	18.4	27.4	37.3
CO_2	---		< 0.1	< 0.1	< 0.1	< 0.1	0.3	0.7	4.0

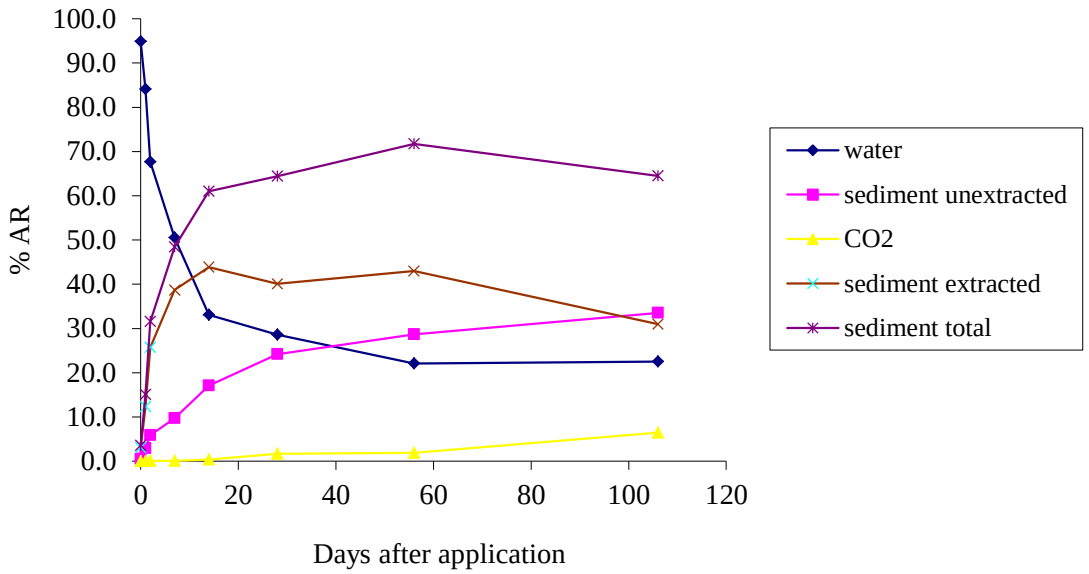


Figure B.8.2.2.3-1: Distribution of radioactivity in Rhine river/sediment system, 10°C

Distribution of RH-117281 and the formation of metabolites was similar in both river and pond systems. RH-117281 was rapidly lost from the water phase, with maximum levels occurring in sediment of 13-26% AR (river system, day 7-14) and 23-30 % AR (pond system, day 7) and declining thereafter. Major metabolites formed were identified as RH-127450 (derivative of parent, dechlorinated in alkyl chain) and RH-163353 (carboxylic acid derivative). Metabolite RH-127450 increased from zero to maximum levels of 7.5-17% AR (day 14-28) and 19.8-23% AR (day 28-56) in water and sediment respectively, and then declined. Metabolite RH-163353 increased from zero to maximum levels of 6-16% AR (day 28-106) and 6-14% AR (day 106) in water and sediment respectively, and then declined in water. These metabolites were first detected on days 2-14. Levels of major metabolites in sediment were slightly higher in systems incubated at 10°C than at 20°C.

Six minor metabolites were tentatively identified as RH-24549, RH-139432, RH-129151, RH-141288, RH-141455, RH-141643 and eighteen unidentified degradates were also found. The total levels of these compounds in any system, at any time point, were individually <8% AR.

Of the non-extractable radioactivity in sediment, 16-20% AR was associated with the fulvic acid fraction, 8-13% AR was associated with the humic acid fraction and 9-10% AR was found in the insoluble humin fraction.

Results for the Rhine (river) and Judenweiher (pond) system are shown in Table B.8.2.2.3-4 to Table B.8.2.2.3-7 and Figure B.8.2.2.3-2.

Table B.8.2.2.3-4: Pattern of ^{14}C-RH-117281 and metabolites in the river aquatic system (incubation at 20°C). Mean values of two independent samples given in percent of the radioactivity applied.

River / 20 °C		INCUBATION TIME IN DAYS								
Radioactive fraction		0	0.25	1	2	7	14	28	56	106
PARENT	Water	94.8	93.4	86.6	72.3	39.6	2.5	*	*	*
RH-117281	Sediment	2.6	2.4	8.3	9.0	10.1	12.9	0.4	*	*
	Total	**97.4**	**95.9**	**94.9**	**81.3**	**49.6**	**15.3**	**0.4**	*	*
M 1	Water	*	*	0.8	2.9	0.5	0.6	*	*	0.1
RH-139432	Sediment	*	*	*	*	*	2.9	1.7	1.0	1.4
	Total	*	*	**0.8**	**2.9**	**0.5**	**3.5**	**1.7**	**1.0**	**1.5**
M 2	Water	*	**0.3**	**0.6**	*	**0.3**	*	*	*	*
RH-129151	Sediment	*	*	*	*	*	*	*	*	*
	Total	*	**0.3**	**0.6**	*	**0.3**	*	*	*	*
M 3	Water	*	*	*	1.5	4.0	12.8	10.3	10.2	2.4
RH-127450	Sediment	*	*	*	0.2	5.8	10.9	19.8	19.2	14.2
	Total	*	*	*	**1.7**	**9.8**	**23.7**	**30.0**	**29.3**	**16.5**
M 4	Water	*	*	*	2.5	3.2	0.8	*	*	*
RH-24549	Sediment	*	*	*	*	1.8	1.2	*	*	*
	Total	*	*	*	**2.5**	**5.0**	**2.0**	*	*	*
M 7	Water	*	*	*	0.3	*	*	*	*	*
unknown	Sediment	*	*	*	*	*	1.0	2.9	3.6	0.3
	Total	*	*	*	**0.3**	*	**1.0**	**2.9**	**3.6**	**0.3**
M 8	Water	*	*	*	*	*	*	*	*	*
unknown	Sediment	*	*	*	*	*	*	0.6	1.2	0.9
	Total	*	*	*	*	*	*	**0.6**	**1.2**	**0.9**
M 9	Water	*	*	*	1.2	*	0.4	*	*	0.4
unknown	Sediment	*	*	*	*	*	*	0.4	*	0.8
	Total	*	*	*	**1.2**	*	**0.4**	**0.4**	*	**1.2**
M 18	Water	*	*	*	*	1.7	1.7	*	*	*
unknown	Sediment	*	*	*	*	*	*	0.4	*	*
	Total	*	*	*	*	**1.7**	**1.7**	**0.4**	*	*
M 14	Water	*	*	*	1.7	7.5	5.7	15.8	14.7	8.8
RH-163353	Sediment	*	*	*	*	0.4	2.9	4.1	5.9	7.4
	Total	*	*	*	**1.7**	**7.9**	**8.6**	**20.0**	**20.6**	**16.1**
M 23	Water	*	*	0.3	4.3	3.0	3.3	1.5	*	*
RH-141288	Sediment	*	*	*	*	0.5	1.2	2.9	1.2	0.5
	Total	*	*	**0.3**	**4.3**	**3.4**	**4.5**	**4.5**	**1.2**	**0.5**
M 24	Water	*	*	*	*	1.3	*	*	*	*
unknown	Sediment	*	*	*	*	1.1	0.9	0.6	*	*
	Total	*	*	*	*	**2.4**	**0.9**	**0.6**	*	*
M 25	Water	*	*	*	*	4.4	0.6	*	*	*
unknown	Sediment	*	*	*	*	2.2	2.1	2.2	*	*
	Total	*	*	*	*	**6.6**	**2.7**	**2.2**	*	*
M 26	Water	*	*	*	*	*	1.3	*	*	*
RH-141643	Sediment	*	*	*	*	*	*	*	*	1.8
	Total	*	*	*	*	*	**1.3**	*	*	**1.8**

*: Not detected or below the limit of quantification

Additionally, the following metabolites were detected: M5 (RH-141454): 0.2% (day 106); M6 (RH-141455): 1.8% (day 106); M 10: 0.2% (day 106); M11: 0.4%, 1.1% (day 14, 106); M13: 1.1% (day 56); M15: 0.2% (day 7); M17: 0.2% (day 106); M20 0.4% (day 106); M21: 0.3% (day 106); M22 (RH-141453): 0.2% (day 106); M29: 0.3% (day 7)

Table B.8.2.2.3-5: Pattern of ^{14}C-RH-117281 and metabolites in the river aquatic system (incubation at 10°C). Mean values of two independent samples given in percent of the radioactivity applied.

River / 10 °C		INCUBATION TIME IN DAYS							
Radioactive fraction		0	1	3	7	14	28	56	106
PARENT	Water	94.1	82.0	59.4	37.4	21.7	4.2	0.2	*
RH-117281	Sediment	3.1	12.3	24.0	26.4	15.4	11.2	2.7	*
	Total	**97.2**	**94.2**	**83.4**	**63.8**	**37.1**	**15.4**	**2.9**	*
M 1	Water	*	0.6	1.8	0.8	*	*	*	0.1
RH-139432	Sediment	*	*	0.2	1.4	3.0	2.6	2.1	2.2
	Total	*	**0.6**	**2.0**	**2.2**	**3.0**	**2.6**	**2.1**	**2.3**
M 2	Water	*	*	**0.4**	*	*	*	*	*
RH-129151	Sediment	*	*	*	**0.4**	*	**0.9**	*	*
	Total	*	*	**0.4**	**0.4**	*	**0.9**	*	*
M 3	Water	*	*	0.9	1.8	6.2	17.1	16.3	3.4
RH-127450	Sediment	*	*	1.2	3.7	14.1	11.8	23.1	10.5
	Total	*	*	**2.1**	**5.4**	**20.3**	**28.9**	**39.3**	**13.9**
M 4	Water	*	*	*	*	0.2	*	*	*
RH-24549	Sediment	*	*	0.3	4.1	2.2	4.0	0.9	2.2
	Total	*	*	**0.3**	**4.1**	**2.4**	**4.0**	**0.9**	**2.2**
M 6	Water	*	*	*	*	*	*	*	0.5
RH-141455	Sediment	*	*	*	0.2	*	0.2	*	*
	Total	*	*	*	**0.2**	*	**0.2**	*	**0.5**
M 7	Water	*	*	*	0.7	1.1	*	*	*
unknown	Sediment	*	*	*	1.5	2.3	2.0	3.0	*
	Total	*	*	*	**2.1**	**3.4**	**2.0**	**3.0**	*
M 9	Water	*	*	*	*	0.3	0.9	1.9	0.8
unknown	Sediment	*	*	*	*	*	0.6	*	1.7
	Total	*	*	*	*	**0.3**	**1.5**	**1.9**	**2.5**
M 14	Water	*	*	2.7	8.3	*	0.6	0.7	15.3
RH-163353	Sediment	*	*	*	0.4	3.8	3.4	6.1	12.7
	Total	*	*	**2.7**	**8.7**	**3.8**	**4.0**	**6.8**	**28.0**
M 23	Water	*	*	0.6	0.6	1.5	3.9	*	*
RH-141288	Sediment	*	*	*	*	0.7	1.1	1.9	0.4
	Total	*	*	**0.6**	**0.6**	**2.2**	**5.0**	**1.9**	**0.4**
M 25	Water	*	*	*	0.6	0.2	*	*	*
unknown	Sediment	*	*	*	0.6	2.3	1.3	1.1	*
	Total	*	*	*	**1.3**	**2.4**	**1.3**	**1.1**	*
M 26	Water	*	*	*	*	*	*	0.3	0.2
unknown	Sediment	*	*	*	*	*	*	1.4	1.4
	Total	*	*	*	*	*	*	**1.7**	**1.6**

*: Not detected or below the limit of quantification
Additionally, the following metabolites were detected: M 5 (RH-141454): 0.3% & 0.2% (days 56 & 106); M 8: 0.7% (day 28); M 11: 0.6% (day 106); M 15: 0.5% (day 56); M18: 0.4% (day 28); M20: 0.1% (day 106); M 24: 0.3% & 0.5% (days 14 & 56)

Table B.8.2.2.3-6: Pattern of ^{14}C-RH-117281 and metabolites in the pond aquatic system (incubation at 20°C). Mean values of two independent samples given in percent of the radioactivity applied.

Pond / 20 °C		INCUBATION TIME IN DAYS								
Radioactive fraction		0	0.25	1	2	7	14	28	56	106
PARENT	Water	97.0	94.6	90.6	79.9	22.3	6.6	*	*	*
RH-117281	Sediment	3.0	3.1	3.3	11.0	23.1	11.6	3.3	0.8	*
	Total	**99.9**	**97.7**	**93.9**	**90.9**	**45.4**	**18.1**	**3.3**	**0.8**	*
M 1	Water	*	0.3	0.9	1.7	0.8	*	*	*	*
RH-139432	Sediment	*	*	*	*	1.1	0.4	2.1	1.1	0.8
	Total	*	**0.3**	**0.9**	**1.7**	**1.8**	**0.4**	**2.1**	**1.1**	**0.8**
M 2	Water	*	*	**0.9**	**1.1**	*	*	*	*	*
RH-129151	Sediment	*	*	*	*	**1.3**	**0.2**	*	**0.2**	*
	Total	*	*	**0.9**	**1.1**	**1.3**	**0.2**	*	**0.2**	*
M 3	Water	*	*	*	*	3.0	7.5	4.8	2.1	1.8
RH-127450	Sediment	*	*	*	*	6.8	13.8	18.0	22.1	17.9
	Total	*	*	*	*	**9.8**	**21.3**	**22.8**	**24.1**	**19.7**
M 4	Water	*	*	*	*	1.3	1.5	1.5	*	*
RH-24549	Sediment	*	*	*	*	1.9	2.8	1.5	*	*
	Total	*	*	*	*	**3.2**	**4.3**	**2.9**	*	*
M 6	Water	*	*	*	*	0.7	*	*	*	2.1
RH-141455	Sediment	*	*	*	*	*	*	*	*	*
	Total	*	*	*	*	**0.7**	*	*	*	**2.1**
M 7	Water	*	*	*	*	*	*	*	*	*
unknown	Sediment	*	*	*	*	1.8	2.3	5.2	4.7	1.3
	Total	*	*	*	*	**1.8**	**2.3**	**5.2**	**4.7**	**1.3**
M 8	Water	*	*	*	*	*	*	*	*	*
unknown	Sediment	*	*	*	*	*	0.3	0.5	*	0.6
	Total	*	*	*	*	*	**0.3**	**0.5**	*	**0.6**
M 9	Water	*	*	*	*	*	0.4	*	*	*
unknown	Sediment	*	*	*	*	*	*	0.4	0.5	1.8
	Total	*	*	*	*	*	**0.4**	**0.4**	**0.5**	**1.8**
M 14	Water	*	*	*	*	1.3	1.2	9.3	4.3	2.5
RH-163353	Sediment	*	*	*	*	2.0	1.9	2.3	4.4	6.0
	Total	*	*	*	*	**3.3**	**3.1**	**11.6**	**8.7**	**8.5**
M 18	Water	*	*	*	*	*	*	*	*	*
unknown	Sediment	*	*	*	*	*	0.3	1.5	*	0.8
	Total	*	*	*	*	*	**0.3**	**1.5**	*	**0.8**
M 23	Water	*	*	*	1.0	0.9	3.3	2.0	0.3	*
RH-141288	Sediment	*	*	*	*	0.5	1.8	4.2	3.0	1.9
	Total	*	*	*	**1.0**	**1.3**	**5.2**	**6.2**	**3.3**	**1.9**
M 24	Water	*	*	*	*	*	*	*	*	*
unknown	Sediment	*	*	*	*	2.2	1.4	0.6	*	*
	Total	*	*	*	*	**2.2**	**1.4**	**0.6**	*	*
M 25	Water	*	*	*	*	0.6	0.3	*	*	*
unknown	Sediment	*	*	*	*	3.5	4.6	2.3	2.0	*
	Total	*	*	*	*	**4.2**	**4.9**	**2.3**	**2.0**	*
M 26	Water	*	*	*	*	0.4	0.3	*	*	*
RH-141643	Sediment	*	*	*	*	*	*	*	*	1.4
	Total	*	*	*	*	**0.4**	**0.3**	*	*	**1.4**

*: Not detected or below the limit of quantification
Additionally, the following metabolites were detected: M10: 0.6% (day 56); M15: 0.8% (day 28); M20: 0.4% (day 14); M22 (RH-141453): 0.4%,0.3% (days 7,106)

Table B.8.2.2.3-7: Pattern of ^{14}C-RH-117281 and metabolites in the pond aquatic system (incubation at 10°C). Mean values of two independent samples given in percent of the radioactivity applied.

Pond / 10 °C		INCUBATION TIME IN DAYS							
Radioactive fraction		0	1	3	7	14	28	56	106
PARENT	Water	92.4	81.2	68.0	46.0	29.4	23.6	1.8	*
RH-117281	Sediment	4.4	12.4	22.6	30.2	27.6	16.3	8.0	0.9
	Total	**96.7**	**93.5**	**90.6**	**76.2**	**57.0**	**39.9**	**9.8**	**0.9**
M 1	Water	*	0.5	0.3	0.7	*	*	*	*
RH-139432	Sediment	*	*	0.1	0.2	0.3	0.7	1.6	0.9
	Total	*	**0.5**	**0.4**	**0.9**	**0.3**	**0.7**	**1.6**	**0.9**
M 2	Water	*	*	*	*	*	*	*	*
RH-129151	Sediment	*	*	*	**1.3**	**0.4**	*	*	*
	Total	*	*	*	**1.3**	**0.4**	*	*	*
M 3	Water	*	*	*	1.0	2.7	4.7	5.9	2.6
RH-127450	Sediment	*	*	0.6	1.7	7.3	12.7	22.6	14.7
	Total	*	*	**0.6**	**2.7**	**10.0**	**17.4**	**28.5**	**17.3**
M 4	Water	*	*	*	*	*	*	0.8	0.2
RH-24549	Sediment	*	*	*	0.5	0.1	0.5	*	1.2
	Total	*	*	*	**0.5**	**0.1**	**0.5**	**0.8**	**1.4**
M 6	Water	*	*	*	*	*	*	*	0.5
RH-141455	Sediment	*	*	*	*	*	*	*	*
	Total	*	*	*	*	*	*	*	**0.5**
M 7	Water	*	*	*	*	2.4	0.4	*	*
unknown	Sediment	*	*	*	0.9	3.5	6.4	8.0	5.3
	Total	*	*	*	**0.9**	**5.8**	**6.8**	**8.0**	**5.3**
M 8	Water	*	*	*	*	*	*	*	*
unknown	Sediment	*	*	*	*	*	0.3	3.7	0.2
	Total	*	*	*	*	*	**0.3**	**3.7**	**0.2**
M 14	Water	*	*	*	1.4	*	1.2	2.4	6.0
RH-163353	Sediment	*	*	*	*	0.4	3.6	3.8	13.8
	Total	*	*	*	**1.4**	**0.4**	**4.8**	**6.3**	**19.9**
M 23	Water	*	*	*	*	*	*	0.5	*
RH-141288	Sediment	*	*	*	*	*	1.2	1.5	2.1
	Total	*	*	*	*	*	**1.2**	**2.0**	**2.1**
M 24	Water	*	*	*	0.7	*	*	0.1	*
unknown	Sediment	*	*	*	*	*	*	1.4	0.8
	Total	*	*	*	**0.7**	*	*	**1.5**	**0.8**
M 25	Water	*	*	*	0.3	0.3	*	*	*
unknown	Sediment	*	*	*	0.5	1.2	2.8	3.8	2.1
	Total	*	*	*	**0.9**	**1.4**	**2.8**	**3.8**	**2.1**
M 26	Water	*	*	*	*	*	*	*	0.1
RH-141643	Sediment	*	*	*	*	*	*	1.2	0.8
	Total	*	*	*	*	*	*	**1.2**	**0.9**

*: Not detected or below the limit of quantification

Additionally, the following metabolites were detected: M6: 0.5% (day 106); M9: 0.4% (days 28 & 106); M10: 0.8% (day 56); M13: 0.7% (day 14); M 15: 0.4% (day 7); M 20: 0.2% (day 106); M 28: 0.2% (day 106)

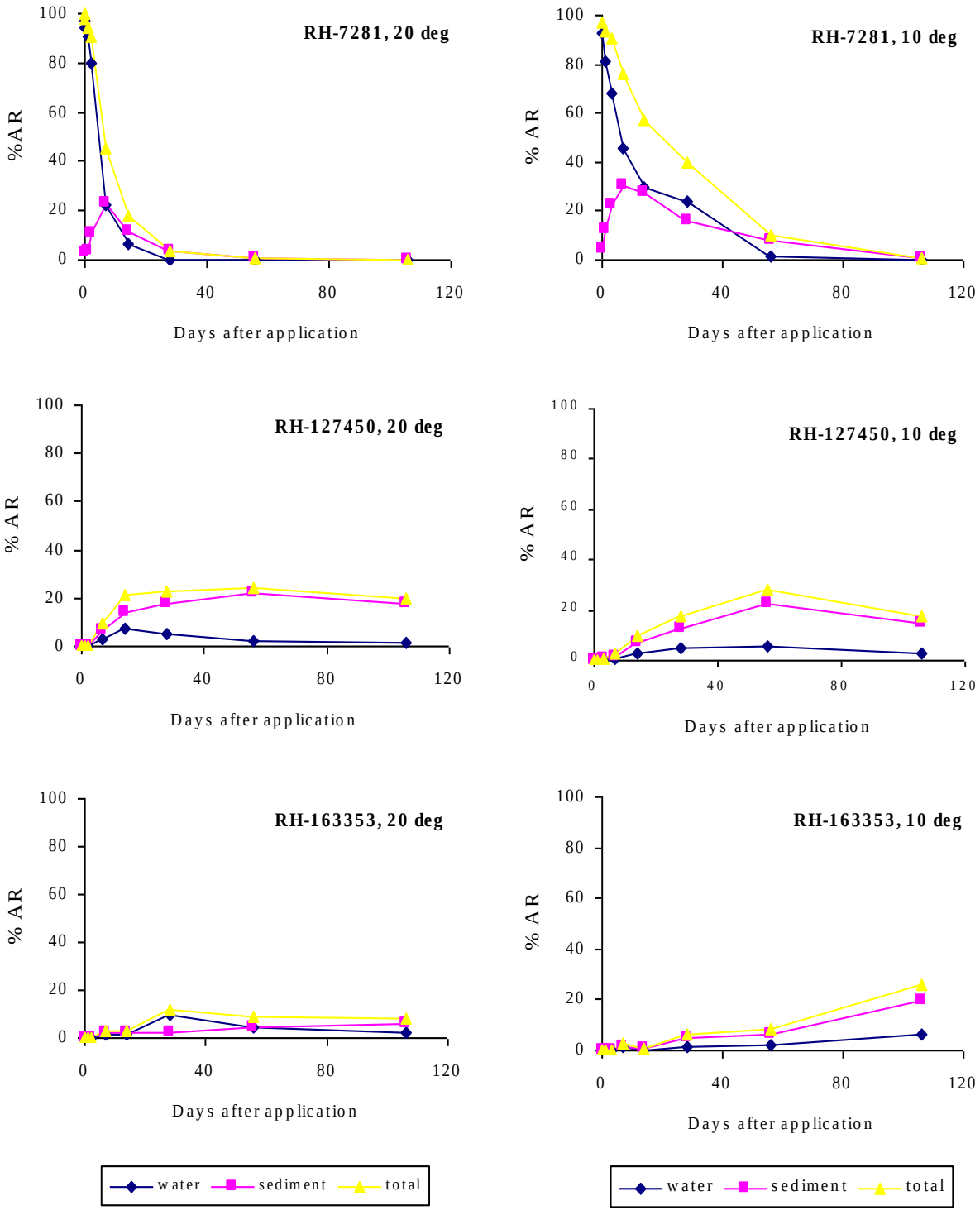


Figure B.8.2.2.3-2: Distribution of RH-117281 and the formation of metabolites in the Judenweiher pond system

The proposed metabolic pathway of RH-117281 in sediment/water systems is shown in Figure B.8.2.2.3-3. First order DT50s and DT90s, calculated by the Rapporteur, are shown in Table B.8.2.2.3-8.

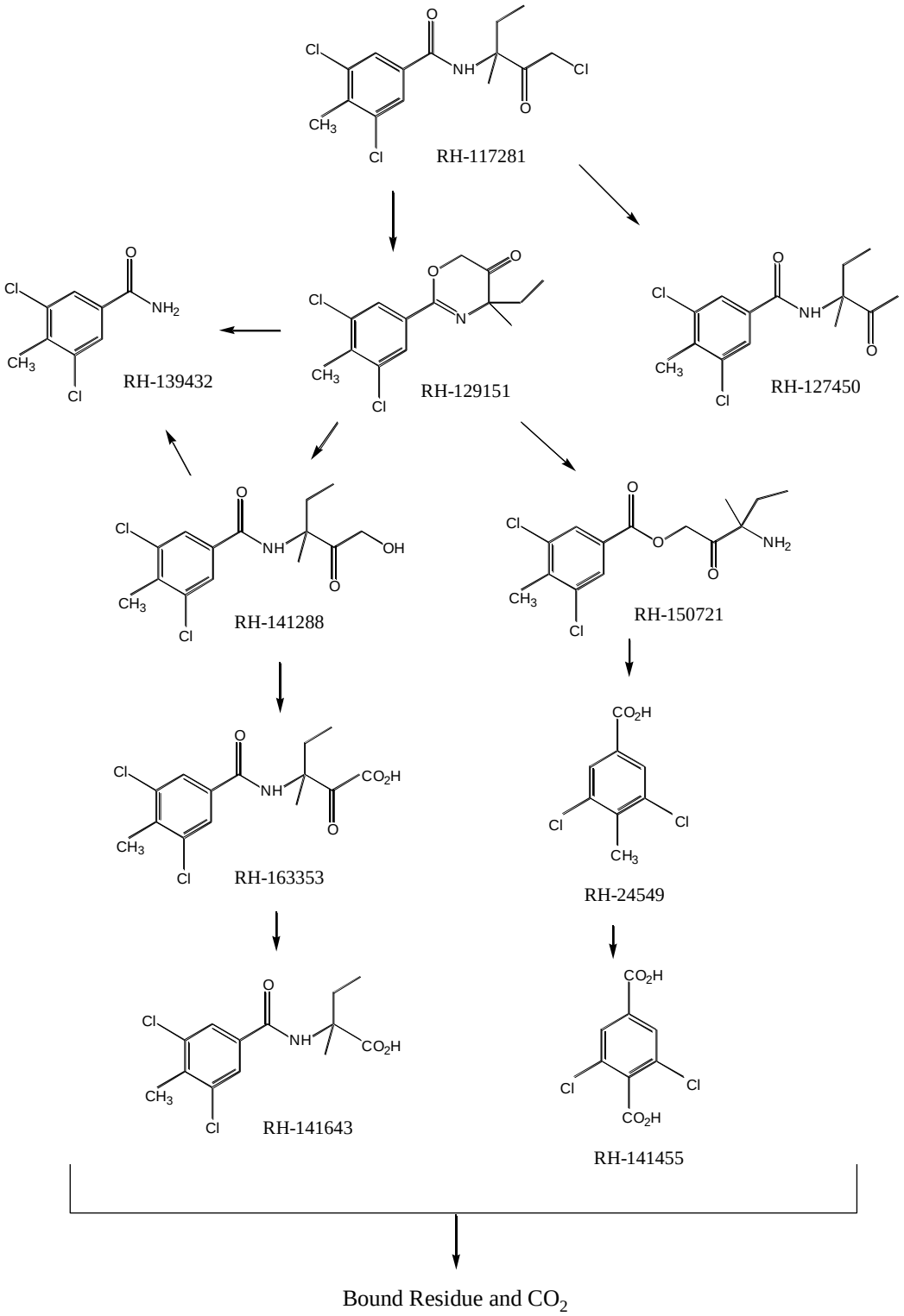


Figure B.8.2.2.3-3: Proposed metabolic pathway of RH-117281 in sediment/water systems

Table B.8.2.2.3-8: DT_{50}s and DT_{90}s for RH-117281 and major metabolites in water/sediment systems

RH-117281 *	River 20 °C Days	Order r^2	River 10 °C Days	Order r^2	Pond 20 °C Days	Order r^2	Pond 10 °C Days	Order r^2
DT_{50} – surface water	~~3.0~~	~~1st r² 0.94~~	~~6[2]~~	~~1st r² 1.0~~	~~3.0~~	~~1st r² 0.99~~	~~11~~	~~1st r² 0.96~~
DT_{50} – sediment	~~0.8[1]~~	~~√1st r² 0.98~~	~~16[2]~~	~~1st r² 0.98~~	~~10[2]~~	~~1st r² 0.97~~	~~19~~	~~1st r² 0.99~~
DT_{50} - total system	~~3.6[2]~~	~~1st r² 0.97~~	~~11[2]~~	~~1st r² 1.0~~	~~8~~	~~1st r² 0.96~~	~~16~~	~~1st r² 0.99~~
DT_{90} – surface water	~~9.0~~	~~1st r² 0.94~~	~~21[2]~~	~~1st r² 1.0~~	~~12~~	~~1st r² 0.99~~	~~35~~	~~1st r² 0.96~~
DT_{90} – sediment	~~9.0[1]~~	~~√1st r² 0.98~~	~~52[2]~~	~~1st r² 0.98~~	~~34[2]~~	~~1st r² 0.97~~	~~65~~	~~1st r² 0.99~~
DT_{90} - total system	~~12[2]~~	~~1st r² 0.97~~	~~36[2]~~	~~1st r² 1.0~~	~~25~~	~~1st r² 0.96~~	~~52~~	~~1st r² 0.99~~
RH-127450	River 20 °C		River 10 °C		Pond 20 °C		Pond 10 °C	
DT_{50} – surface water	~~39~~	~~1st r² 0.88~~	~~32[1]~~	~~1st r² 0.89~~	~~19~~	~~√1st r² 0.93~~	-	-
DT_{50} – sediment	~~160~~	~~1st r² 0.92~~	-	-	-	-	-	-
DT_{50} - total system	~~85~~	~~1st r² 0.90~~	-	-	-	-	-	-
DT_{90} – surface water	~~130~~	~~1st r² 0.88~~	~~100[1]~~	~~1st r² 0.89~~	~~200~~	~~√1st r² 0.93~~	-	-
DT_{90} – sediment	~~>500~~	~~1st r² 0.92~~	-	-	-	-	-	-
DT_{90} - total system	~~280~~	~~1st r² 0.90~~	-	-	-	-	-	-
RH-163353	River 20 °C		River 10 °C		Pond 20 °C		Pond 10 °C	
DT_{50} – surface water	~~89[1]~~	~~1st r² 0.94~~	-	-	~~22[1]~~	~~√1st r² 1.0~~	-	-
DT_{50} – sediment	-	-	-	-	-	-	-	-
DT_{50} - total system	-	-	-	-	-	-	-	-
DT_{90} – surface water	~~290[1]~~	~~1st r² 0.94~~	-	-	~~240[1]~~	~~√1st r² 1.0~~	-	-
DT_{90} – sediment	-	-	-	-	-	-	-	-
DT_{90} - total system	-	-	-	-	-	-	-	-

* All DT50s and DT90s generated with four or more data points, unless indicated.

1 Three data points only; 2 Where residues <0.1% AR, values not used for estimation.

RMS comment:

The study has been previously evaluated and considered acceptable for the Annex I inclusion by UK. The RMS believes the study is still overall acceptable except for the kinetic evaluation which is outdated. However a new kinetic evaluation of the study is provided by applicant, please see *Callow & Hilton (2013b).*

Studies submitted with the dossier for the renewal of the approval:

Reference:	Callow B., Hilton M. (2013b). Determination of rates of decline for zoxamide and its metabolites, in sediment-water studies according to the guidance within the FOCUS Kinetics Guidance Document.
Guideline(s):	FOCUS Kinetics Guidance Document (2006)
GLP:	No (calculation - GLP is not relevant)
Previous evaluation:	Submitted for the purpose of renewal
Validity of the study:	Considered acceptable

Executive Summary

The rates of degradation in the water/sediment study of Morgenroth (1998) have been re-evaluated according to the recommendations of the FOCUS Kinetics Guidance Document (FOCUS 2006).

The SFO model satisfactorily described the decline of zoxamide in all four systems, giving both an acceptable visual and statistical fit. Zoxamide degraded with DT_{50}s of 6.3 to 6.4 days at 20°C and 10.4 to 19.4 days at 10°C. For the metabolite RH-127450 it was concluded that the fit was acceptable in three of the systems. RH-127450 degraded with DT_{50}s of 88.9 to 326.1 days at 20°C and 123 days at 10°C. An acceptable fit to the data for RH-163353 could not be obtained.

I. MATERIAL AND METHODS

Rates of degradation were calculated according to the guidance of the FOCUS Degradation Kinetics Workgroup, using KinGui Version 2.0 (Bayer CropScience 2011).

The approach used followed that given in Chapter 10 of the FOCUS Kinetics Guidance Document. The suitability of the fit of the models was evaluated both visually and statistically by calculating the minimum % error required to pass the χ^2 test at a probability of 0.05 (acceptability criteria χ^2 error < 15%). A t-test was also performed to evaluate whether the determined rate constants were significantly different to 0 (acceptability criteria $P \leq 0.1$ for sediment water studies).

Prior to running the kinetic evaluation the appropriate metabolism scheme was investigated. For the purposes of simplifying the kinetic assessment, and due to the low formation and often transient nature of intermediate metabolites, direct formation from the parent was assumed for RH-163353 as well as RH-127450.

Two levels of kinetics are proposed for the determination of rates of degradation from sediment/water studies. P-I is for single compartment approaches and P-II is proposed for multi-compartment approaches where degradation in both the water and sediment is considered. In this exercise only the single compartment (P-I) approach was used for the kinetic evaluation of degradation of zoxamide in the whole

system. For the determination of metabolite degradation kinetics the term M-I is used to describe single compartment approaches. The M-I approach was used for the determination of metabolite degradation in the whole system.

II. RESULTS AND DISCUSSION

The detections of zoxamide and its metabolites in Morgenroth (1998) are given in Tables B.8.2.2.3-5 to B.8.2.2.3-8.

The results of the determinations are summarised in Table B.8.2.2.3-9 and the plots of the decline and the residuals are given in Figures B.8.2.2.3-4 to B.8.2.2.3-6.

For all systems first order kinetics provided an acceptable visual and statistical fit to the data for zoxamide. The fitting of FOMC kinetics, as advised in the FOCUS guidance for the determination of the persistence endpoint, was not performed as there were no indications of any biphasic pattern in the data. For both systems first order kinetics for zoxamide is appropriate for use as both the persistence and modelling end-points.

For RH-127450 an acceptable visual and statistical fit was obtained for the Pond 20°C system. For the River 20°C and the Pond 10°C, although the statistical fit was not within the FOCUS thresholds the visual fit was considered acceptable. In the River 10°C system the fit was considered both visually and statistically unacceptable.

For RH-163353 the visual and statistical fit in all systems was unacceptable. This was largely due to the lack of predicted degradation.

Table B.8.2.2.3-5: Detections of zoxamide and its metabolites in the river system, 20°C (% AR; mean of two samples, whole system).

Days after treatment	Zoxamide	RH-127450	RH-163353
0	97.4	nd	nd
0.25	95.9	nd	nd
1	94.9	nd	nd
2	81.3	1.7	1.7
7	49.6	9.8	7.9
14	15.3	23.7	8.6
28	0.4	30.0	20.0
56	nd	29.3	20.6
106	nd	16.5	16.1

nd – not detected

Table B.8.2.2.3-6: Detections of zoxamide and its metabolites in the river system, 10°C (% AR; mean of two samples, whole system).

Days after treatment	Zoxamide	RH-127450	RH-163353
0	97.2	nd	nd
1	94.2	nd	nd
3	83.4	2.1	2.7
7	63.8	5.4	8.7
14	37.1	20.3	3.8
28	15.4	28.9	4.0
56	2.9	39.3	6.8
106	nd	13.9	28.0

nd – not detected

Table B.8.2.2.3-7: Detections of zoxamide and its metabolites in the pond system, 20°C (% AR; mean of two samples, whole system).

Days after treatment	Zoxamide	RH-127450	RH-163353
0	99.9	nd	nd
0.25	97.7	nd	nd
1	93.9	nd	nd
2	90.9	nd	nd
7	45.4	9.8	3.3
14	18.1	21.3	3.1
28	3.3	22.8	11.6
56	0.8	24.1	8.7
106	nd	19.7	8.5

nd – not detected

Table B.8.2.2.3-8: Detections of zoxamide and its metabolites in the pond system, 10°C (% AR; mean of two samples, whole system).

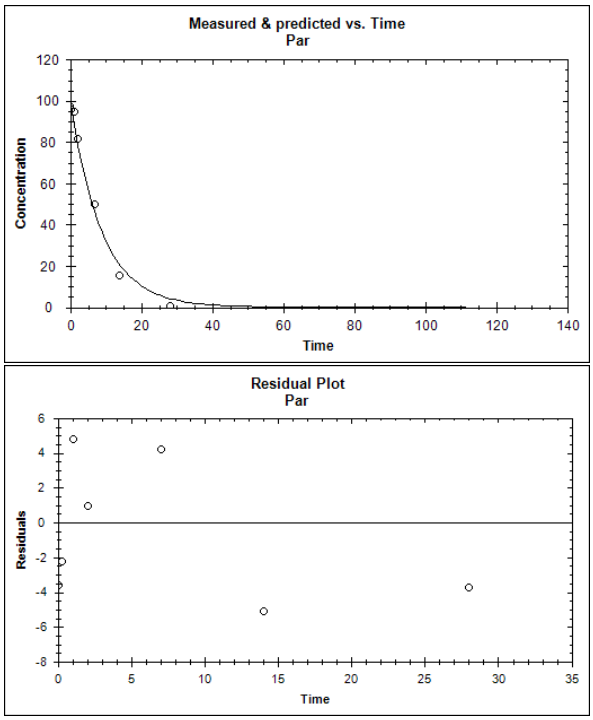
Days after treatment	Zoxamide	RH-127450
0	96.7	nd
1	93.5	nd
3	90.6	0.6
7	76.2	2.7
14	57.0	10.0
28	39.9	17.4
56	9.8	28.5
106	0.9	17.3

nd – not detected; RH-163353 not tabulated as detections were not high enough to allow kinetic evaluation

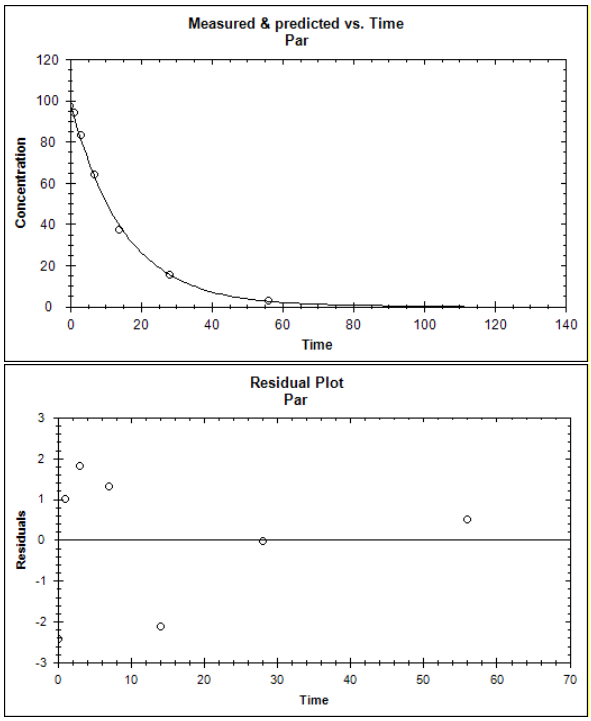
Table B.8.2.2.3-9: Calculated DT_{50}s for zoxamide and its metabolites in sediment water systems

Parameter	River system		Pond system	
	20°C	10°C	20°C	10°C
	SFO	SFO	SFO	SFO
	Zoxamide			
DT_{50} (days)	6.4	10.4	6.3	19.4
DT_{90} (days)	21.1	34.7	20.9	64.6
χ^2 error (%)	5.921	2.59	6.044	3.424
	RH-127450			
DT_{50} (days)	148.4	101.3	326.1	123
DT_{90} (days)	493.1	336.4	1083.3	408.7
χ^2 error (%)	16.271	26.89	7.265	20.12
FF	0.31	0.38	0.24	0.33

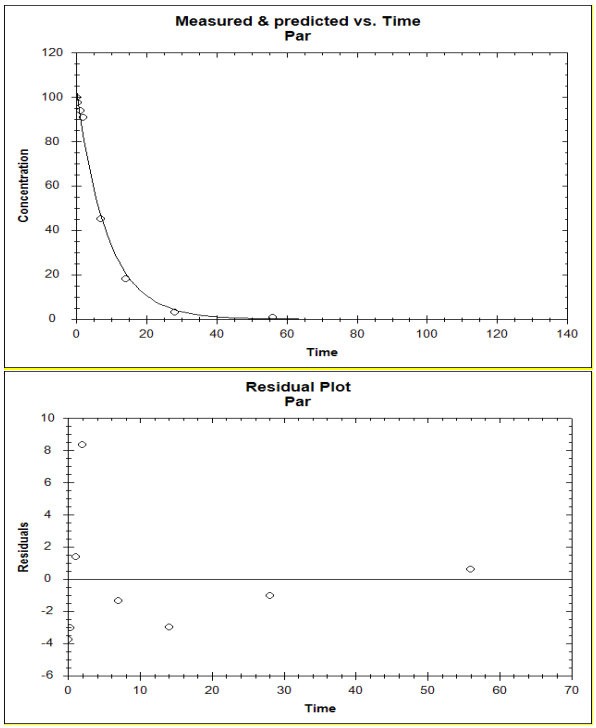
Figure B.8.2.2.3-4: Plot of the decline and the residuals –zoxamide



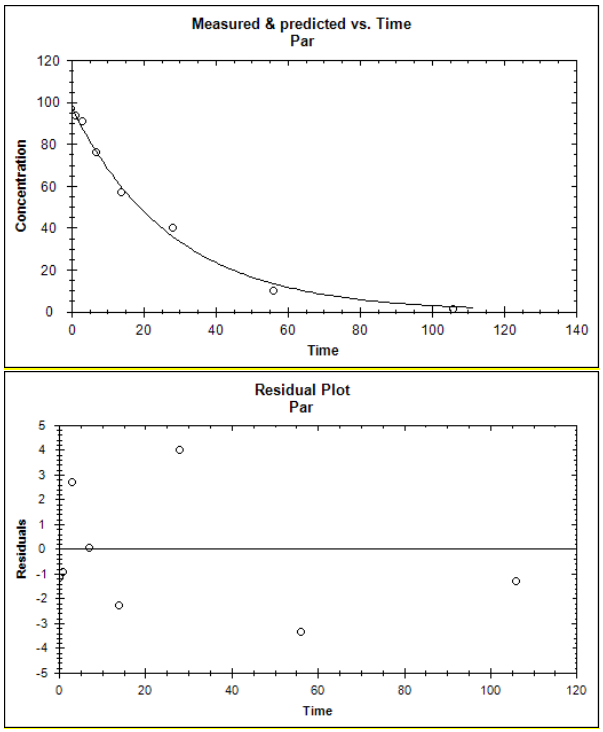
River system 20°C



River system 10°C

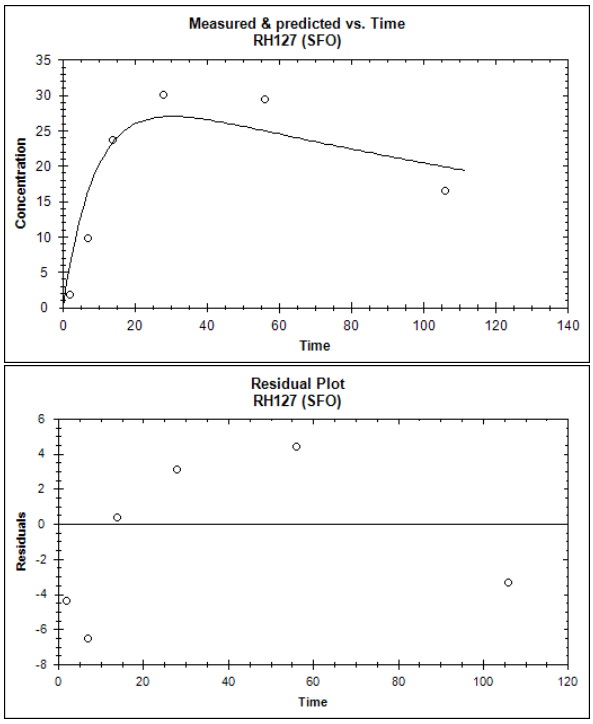


Pond system 20°C

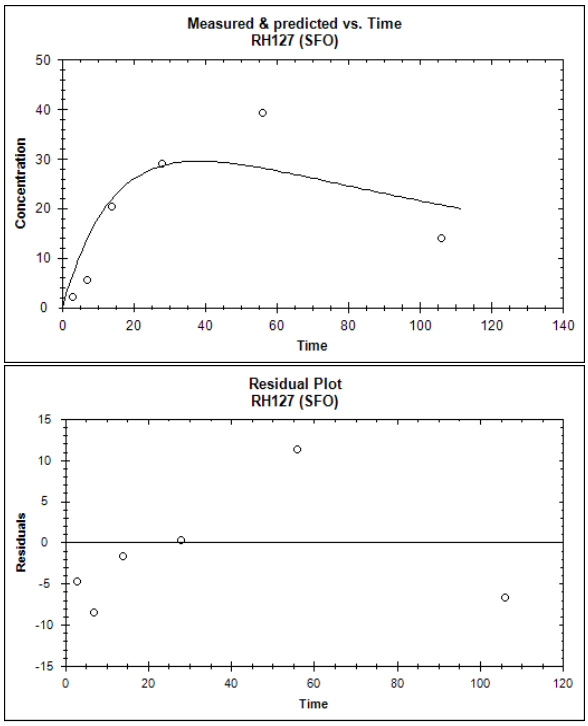


Pond system 10°C

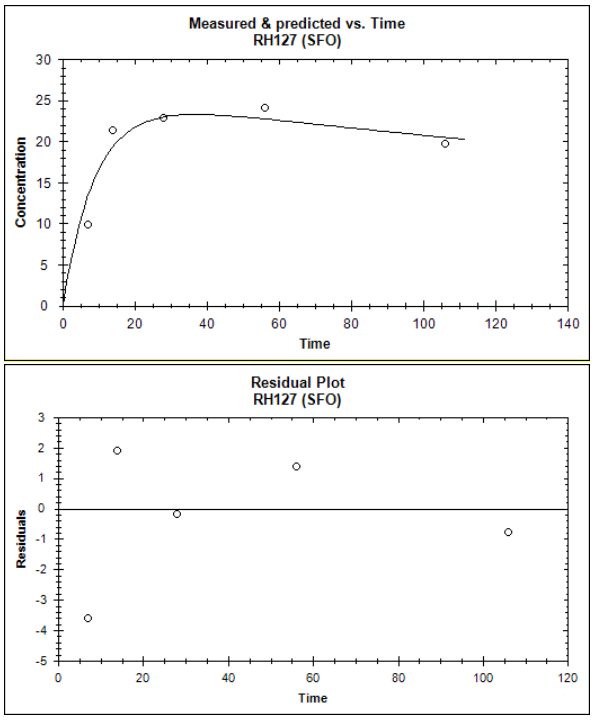
Figure B.8.2.2.3-5: Plot of the decline and the residuals –RH-127450



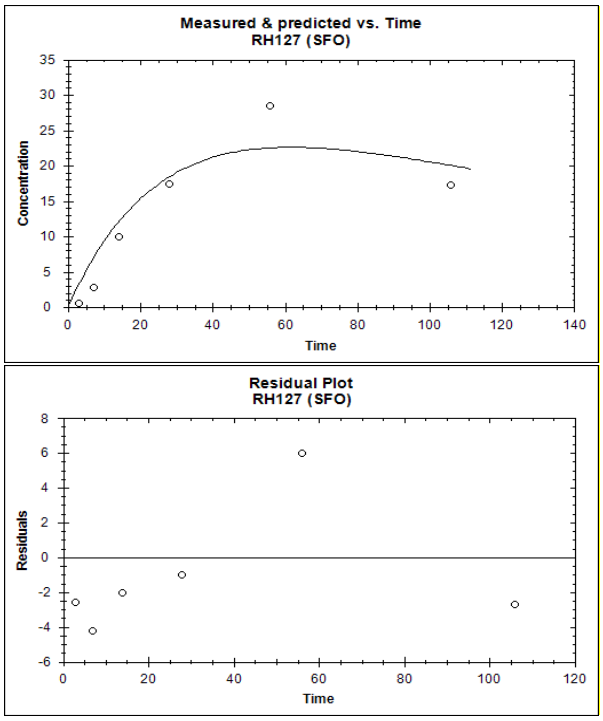
River system 20°C



River system 10°C

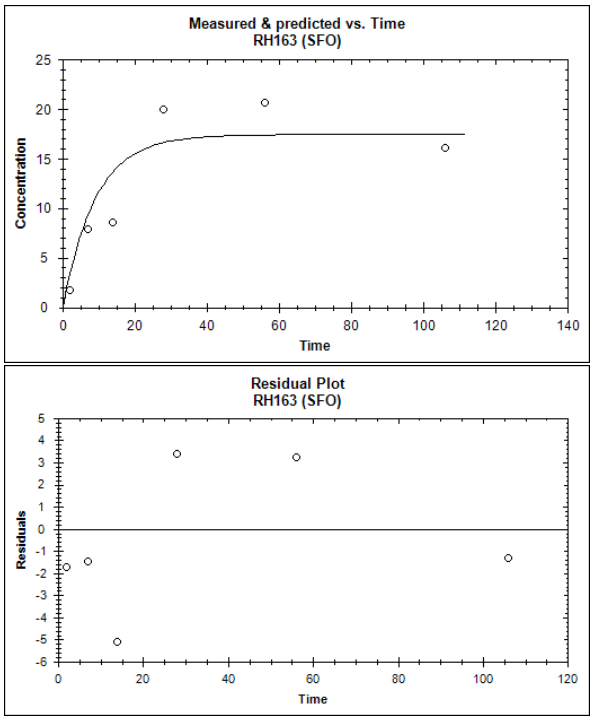


Pond system 20°C

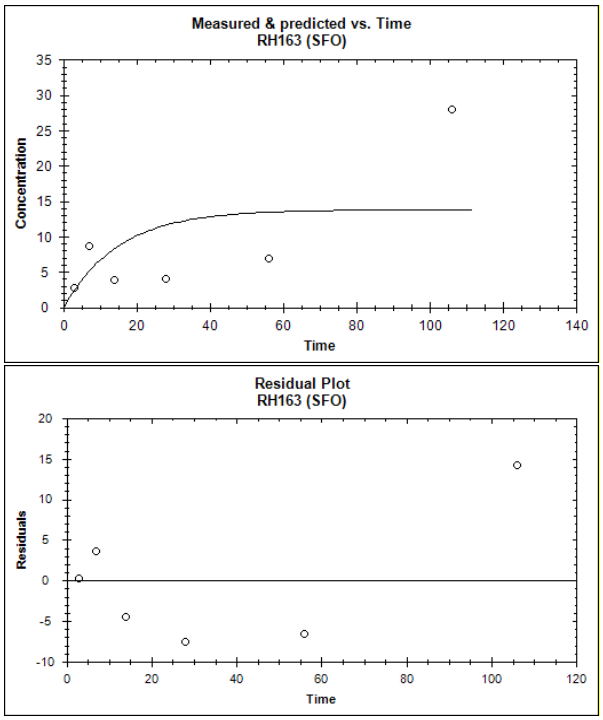


Pond system 10°C

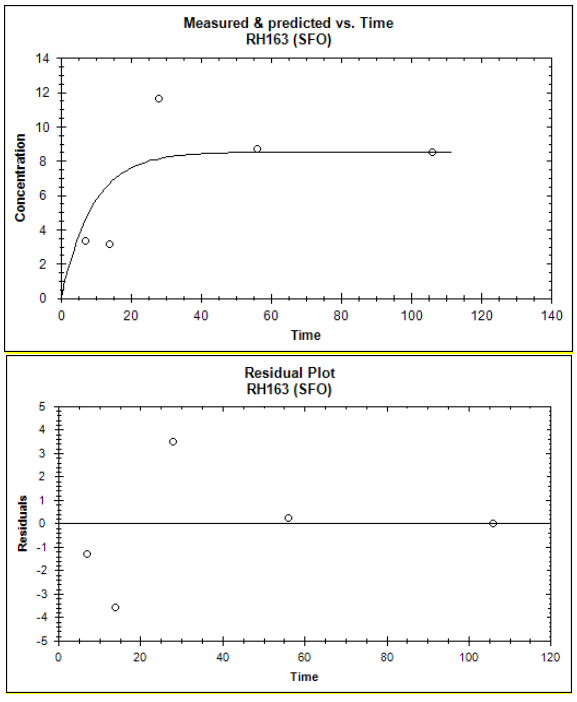
Figure B.8.2.2.3-6: Plot of the decline and the residuals –RH-163353



River system 20°C



River system 10°C



Pond system 20°C

III. CONCLUSION

The decline of zoxamide and its metabolites, RH-127450 and RH-163353, in two sediment/water systems incubated at 10 and 20°C was modelled according to the recommendations of the FOCUS Kinetics Guidance Document. The SFO model satisfactorily described the decline in all four systems, giving both an acceptable visual and statistical fit. Zoxamide degraded with DT_{50}s of 6.3 to 6.4 days at 20°C and 10.4 to 19.4 days at 10°C. For the metabolite RH-127450 it was concluded that the fit was acceptable in three of the systems. RH-127450 degraded with DT_{50}s of 88.9 to 326.1 days at 20°C and 123 days at 10°C. An acceptable fit to the data for RH-163353 could not be obtained.

RMS comment:

The degradation rates of zoxamide and its metabolites in water/sediment systems from study of *Morgenroth (1998)* have been re-evaluated according to the recommendations of the FOCUS Kinetics Guidance Document (FOCUS 2006). The study is acceptable and the results can be used for risk assessment purposes.

B.8.2.2.4 Irradiated water/sediment study

Photochemical degradation of zoxamide is not considered to be of importance, therefore data on the degradation in irradiated water/sediment systems are not necessary.

B.8.2.3 Degradation in the saturated zone

Based on the simulation modelling presented in Vol 3 CP – B8 of RAR zoxamide and its metabolites are predicted to have a low risk to groundwater. Studies on its behaviour in the saturated zone are therefore not necessary.

Summary of fate and behaviour in water and sediment

Under the original review it was concluded that zoxamide hydrolyses in the pH range 4-9 in the absence of light under sterile conditions, with first-order DT_{50}s of 8 (pH 9) and 16 (pHs 4,7) days. Major metabolites exceeding 10% AR were RH-129151 (re-arranged cyclic product), RH-150721 (amine), RH-24549 (benzoic acid derivative) and RH-141288 (alcohol). The hydrolytic stability of zoxamide decreases as temperature and pH increase. Hydrolysis of zoxamide proceeds by de-chlorination and cyclisation to RH-129151, followed by oxazine ring-opening, amination, hydroxylation and subsequent cleavage at the epoxyimino- and epidioxy- bridges. Degradation rate constants were calculated for the major metabolites at each pH, assuming first order kinetics using linear and non-linear compartmental regression analysis. DT_{50}s were estimated to be 2 and 9 days (RH-129151, pH 9, 7) and 18 days (RH-150721, pH 4). RH-24549 and RH-141288 were stable (concentrations were still increasing during the study).

The aqueous photolysis of zoxamide was studied at pH 4 to minimise any effects of hydrolysis. When irradiated with a xenon lamp (equivalent light intensity of New Jersey summer sunlight, 42°N) the half life corresponding to natural irradiation with a 12 hour photoperiod was 8 days. However degradation of zoxamide also occurred in dark controls with a calculated half life of 22 days. The major degradates occurring at >10% AR, RH-24549 and RH-150721, were not photo-products, however, as they reached similar or greater concentrations in the dark control as in the irradiated samples and were also major hydrolysis degradates. RH-139432 (amide, occurring at a maximum of 42.4%AR) was the only major

photo-product in the study and was photolytically stable. The other major metabolites did not degrade under the conditions of this study.

The ready biodegradability of zoxamide was assessed using a method suitable for poorly soluble compounds. Zoxamide was not inhibitory to the microbial inoculum and was only biodegraded by 8% in 29 days, indicating that it is not readily biodegradable under these conditions.

In Rhine river and Rheinfelden pond water/sediment studies conducted at 20 and 10°C, zoxamide degraded in the whole system with first order DT_{50}s calculated under the first review of 3.6-11 days (river) and 7.6-16 days (pond) respectively. Zoxamide dissipated from water with 1st order DT_{50}s of 3-6 days (river) and 3-11 days (pond), at 20 and 10°C respectively. A slightly higher proportion of applied radioactivity partitioned into the pond sediment (maximum 80.6% AR) compared to the river sediment (maximum 64.5% AR, however the pond sediment had a higher %oc content than the river sediment. In sediment, zoxamide increased to account for a maximum of 13-26% AR (river, day 7-14) and 23-30 % AR (pond, day 7), then declined with DT_{50}s of 0.8-16 days (river) and 10-19 days (pond).

Preliminary metabolites were identical to those generated during hydrolysis studies; however the extent of metabolism was greater in natural systems. Metabolites were similar to those found in soil and levels were all <10% AR except for RH-127450 (max. 17% AR (day 28) and 23% AR (day 56) in water and sediment respectively) and RH-163353 (max 16% AR (day 28) and 13.8% AR (day 106) in water and sediment respectively). RH-127450 and RH-163353 were first detected on days 2-14 and dissipated from water with DT_{50}s calculated under the first review of $\leq$39 days and $\leq$89 days respectively. Levels of major metabolites in sediment were slightly higher in systems incubated at 10°C than at 20°C. Of the non-extractable radioactivity in sediment, 16-20% AR was fulvic acid-associated (soluble at low pH), 8-13% AR was humic acid-associated (soluble at high pH) and 9-10% AR was found in the insoluble humin fraction. Therefore a higher proportion was incorporated into the less mobile sediment organic matter. The extent of mineralisation was similar in river and pond systems and was higher at 20°C (20-22% AR) than at 10°C (4-6.5% AR).

Under the first review it was concluded that in natural waters, the major dissipation routes of zoxamide will be hydrolysis, microbial degradation and partitioning to sediment, although photolysis may play a minor role. In sediment, zoxamide dissipated moderately rapidly. Significant levels of non-extracted residues are formed, a high proportion being associated with the less mobile humin/humic acid fraction. RH-127450 and RH-163353 were major sediment/water metabolites, dissipating from water, although appearing to be more stable in sediment (levels continued to increase throughout study, or peaked on day 56 and declined slowly). Lifetimes of these metabolites in natural aquatic systems are likely to be controlled by biological and chemical degradation rather than by photochemical degradation.

Since the original review a new data requirement under Regulation 1107/2009 is the aquatic mineralisation study to OECD guideline 309. This study is provided and zoxamide degraded rapidly to non-detectable levels after 28 days with SFO DT_{50}s of 7.6 to 8.4 days. A number of metabolites were detected above the relevant thresholds. RH 141455, RH 139432, RH 141288, RH 163353, RH 24549 and M-7 were detected at >5% on two consecutive occasions at respective maximums of 10.5% AR, 21.4% AR, 22.1% AR, 47.9% AR, 22.7% AR and 9.1% AR.

The rates of degradation in the sediment/water study of Morgenroth (1998) have been re-evaluated according to the recommendations of the FOCUS Kinetics Group (FOCUS 2006). Zoxamide degraded with DT_{50}s of 6.3 to 6.4 days at 20°C and 10.4 to 19.4 days at 10°C. RH-127450 degraded with DT_{50}s of 88.9 to 326.1 days at 20°C and 123 days at 10°C. DT_{50}s for RH-163353 could not be calculated. The calculated DT_{50}s are given in Table B.8.2-1.

Table B.8.2-1: Calculated DT_{50}s for zoxamide and its metabolites in sediment water systems

Parameter	River system		Pond system	
	20°C	10°C	20°C	10°C
	SFO	SFO	SFO	SFO
	Zoxamide			
DT_{50} (days)	6.4	10.4	6.3	19.4
DT_{90} (days)	21.1	34.7	20.9	64.6
	RH-127450			
DT_{50} (days)	148.4	-	326.1	123
DT_{90} (days)	493.1	-	1083.3	408.7

B.8.3 Fate and behaviour in air

B.8.3.1 Route and rate of degradation in air

Adequate data to assess the route and rate of degradation of zoxamide in air were evaluated during the first EU review and no further data are considered necessary.

The vapour pressure of zoxamide is 1.33 x 10^{-5} Pa at 25°C and the water solubility at 20°C is 0.68 mg/l (pH 4-9). Using these values a Henry's Law constant of <6.59 x 10^{-3} Pa/mol.m^3 was derived. These figures suggest that zoxamide is only very slightly volatile. Volatilisation of zoxamide from soil and leaf surfaces under standardised climatic conditions was investigated. Losses were very low with losses of 5.1% AR from leaf surfaces and 3.9% AR from soil after 24 hours. Concentrations of zoxamide in air will therefore be negligible.

A theoretical calculation of the photo-oxidation of zoxamide in the atmosphere, using the method of Atkinson (1988), gave a DT_{50} of 7.5 hours. Therefore in the unlikely event of residues entering air, they will be rapidly degraded.

Studies from the original DAR (May 2001):

Reference:	Burgener A. (1998b). Investigation of the volatilization of 14C-RH-117281 from soil and dwarf runner bean, RCC Ltd, Rohm and Haas Technical Report No. 34-98-132, August 24, 1998.
Guideline(s):	BBA, Richtlinien Fur die Prufung von Phlanzenschutzmitteln im Zulassungsverfahren, 6.1, Teil IV, Juli 1990
GLP:	Yes
Previous evaluation:	In DAR (May 2001)
Validity of the study:	Considered acceptable

Volatilisation of RH-117281 from soil and leaf surfaces under standardised climatic conditions was investigated. Dwarf runner bean leaves (minimised to 2 leaves per plant) and soil surfaces (100 g 2mm sieved, German standard Speyer 2.1, soil moisture 60% MWC) were treated with [14C-phenyl-U]-RH-117281 (radiochemical purity 97.8%, specific activity 3.34 MBq/mg), formulated as RH-117281 2F, was applied to the experimental surfaces at a concentration corresponding to 0.15 kg a.s./ha. Samples were incubated in a climatic chamber for 24 hours and samples taken at 0, 1, 3, 6 and 24 hours. Mean climatic

values for leaf and soil chambers were as follows: Wind speed (m/s) 1.55, 1.46; Air humidity (%rH) 66.2, 76.8; Air temperature (°C) 19.7, 20.9; Soil temperature (°C) 19.0, 20.1.

Samples were extracted in acidified acetonitrile and analysed by LSC. Non-extracted radioactivity was then quantified by combustion LSC. Concurrent recoveries were performed. Analysis was also performed by TLC with radiometric and UV (254 nm) detection.

Losses of 5.1% AR occurred from leaf surfaces and 3.9% AR from soil after 24 hours.

RMS comment:

The study has been previously evaluated and considered acceptable for the Annex I inclusion by UK. The RMS believes the study is still acceptable.

B.8.3.2 Transport via air

Based on the available information on fate and behaviour in air the potential for transport via air is negligible.

B.8.3.3 Local and global effects

Based on the available information on fate and behaviour in air the potential for local/global effects is negligible.

B.8.4 Monitoring data concerning fate and behaviour of the active substance, metabolites, degradation and reaction products

No data are submitted and are not considered to be necessary.

B.8.5 Definition of the residue

Soil

The metabolites exceeding 10% AR in the aerobic soil metabolism studies were RH-127450 (maximum 15.1% AR), RH-24549 (maximum 33.8% AR) and RH-163353 (maximum 15% AR). In addition the metabolite RH-141455 was detected at >5% AR on 2 or more consecutive occasions. Anaerobic conditions are unlikely to be encountered and similar metabolites were identified in the soil photolysis study. The definition of the residue for risk assessment in soil is therefore:

Zoxamide, RH-127450, RH-24549, RH-163353 and RH-141455.

Groundwater

The metabolites exceeding the thresholds in Sanco/221/2000 in the aerobic soil metabolism studies were RH-127450 (maximum 15.1% AR), RH-24549 (maximum 33.8% AR), RH-163353 (maximum 15% AR) and RH-141455 (Maximum 8% AR). Anaerobic conditions are unlikely to be encountered and similar

metabolites were identified in the soil photolysis study. The definition of the residue for risk assessment in groundwater is therefore:

Zoxamide, RH-127450, RH-24549, RH-163353 and RH-141455.

Surface water

The metabolites RH-127450 (maximum 15.1% AR in soil and a maximum of 39.3% AR in the total water/sediment system), RH-141288 (22.1% AR in the aquatic mineralisation study) and RH-163353 (maximum 15% AR in soil, 28% AR in the total water/sediment system and 47.9% AR in the aquatic mineralisation study) were detected at >5% AR in soil and/or individually in the sediment or water phases of water/sediment systems or in the aquatic mineralisation study. RH-24549 (maximum 33.8% AR in soil) was detected at >10% in soil, therefore exposure of surface waters via run-off/drainage needs to be assessed. In addition the metabolites RH-141288 and RH-141455 were detected at >5% AR on 2 or more consecutive occasions in soil and water/sediment system, respectively. In the aqueous photolysis study photodegradation proceeded with a DT_{50} of 8 days (in comparison with a DT_{50} of 22 days in the dark controls) and one photodegradate was formed from the parent at >10% (RH-139432 at a maximum of 42.4% AR). The definition of the residue for risk assessment in surface water is therefore:

Zoxamide, RH-127450 (>10% in soil & water), RH-163353 (>10% in soil & water), RH-141288 (>5% on 2 consecutive occasions in water), RH-24549 (>10% in soil only), RH-141455 (>5% of 2 or more consecutive occasions in soil) and photodegradate RH-139432 (>10% in water).

Air

Zoxamide

B.8.6 References relied on

Literature review

Open literature search has been carried out according to requirements of guidance document of EFSA (Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009. EFSA Journal 2011; 9(2):209). The notifier performed a literature search in 34 databases, in total 124 records were retrieved after removing duplicates from all database searches. The majority of the articles (89 titles) did not meet the relevance criteria (i.e. risk assessment parameters relating to the environment).
Articles of potential relevance to the regulatory data package for the active substance were investigated in further detail by examining the abstract and/or the full article text. The reliability of articles considered to meet the criteria for relevance was assessed using the approach described in Klimisch *et al*., (1997).

No publications were found that showed new/unknown effects or information potentially contradictory to the regulatory data package for the active substance, relevant metabolites and/or plant protection product with respect to the environment, which could impact the regulatory endpoints or the risk assessment parameters.

References relied on

Data point	**Annex point (old)**	**Author(s)**	**Year**	**Title Source (where different from company) Company, Report No, GLP or GEP status (where relevant), Published or not**	**Data Protection Claimed Y/N**	**Owner**	**Previous Evaluation Y/N**
CA, 7.1.1.1	IIA, 7.1.1.1.1	Smalley, J., Reynolds, J.L.	1997	Aerobic soil metabolism of [14C]-RH-117281 Fungicide, XenoBiotic Laboratories, Inc., Rohm and Haas Technical Report No. 34-96-07, June 26, 1997, GLP, unpublished. ER ref. no. 6.13	N	Gowan	Y
CA, 7.1.1.1	IIA, 7.1.1.1.1	Burgener, A.	1998a	14C-RH-117281: Rate of degradation and metabolism in four soils incubated under aerobic conditions, RCC Umweltchemie Ag, Rohm and Haas Technical Report No. 34-98-45, September 17, 1998, GLP, unpublished. ER ref. no. 18.2	N	Gowan	Y
KCA, 7.1.2.1.1	-	Callow, B. and Hilton, H.	2013a	Determination of rates of decline for zoxamide and its metabolites in soil according to the guidance within the FOCUS Kinetics Guidance Document Exponent International Ltd, Harrogate, HG2 8E, UK Report No. 0907598.UK0 EWC 0021 Not GLP, Not published	Y	Gowan	N

Data point	Annex point (old)	Author(s)	Year	Title Source (where different from company) Company, Report No, GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N	Owner	Previous Evaluation Y/N
KCA, 7.1.2.1.2	-	Van den Bosch, M.M.H.	2013a	Determination of the aerobic degradation rate of RH-141,455 in soil. WIL Research Europe B.V., Hambakenwetering 7 5231 DD 's-Hertogenbosch, The Netherlands Project 500850 GLP, Not published	Y	Gowan	N
CA, 7.1.1.2	IIA, 7.1.1.1.2.1	Volkel, W.	1998a	14C-RH-117281: degradation in one soil incubated under anaerobic conditions, RCC Umweltchemie Ag, Rohm and Haas Technical Report No. 34-98-46, September 3, 1998, GLP, unpublished. ER ref. no. 4.5	N	Gowan	Y
CA, 7.1.1.2	IIA, 7.1.1.1.2.1	Kim-Kang, H.	1997	Anaerobic soil metabolism of [14C]RH-117281, XenoBiotic Laboratories, Inc., Rohm and Haas Technical Report No. 34-97-43, April 9, 1997, GLP, unpublished. ER ref. no. 8.16	N	Gowan	Y
CA, 7.1.1.3.	IIA, 7.1.1.1.2.2	Reynolds, J.L.	1997	Soil photolysis of [14C]RH-117281, XenoBiotic Laboratories, Inc., Rohm and Haas Technical Report No. 34-96-214, July 31, 1997, GLP, unpublished. ER ref. no. 10.2	N	Gowan	Y
CA, 7.1.3.1.1	IIA, 7.1.2.1	Shelby, D.J.	1996	Adsorption and desorption of RH-117281 to soil, Ricerca, Inc., Rohm and Haas Technical Report No. 34-96-01, February 9, 1996, GLP, unpublished. ER ref. no. 7.2	N	Gowan	Y
CA, 7.1.3.1.2	IIA, 7.1.2.2	Reynolds, J.L.	1998a	Adsorption and desorption of 14C-RH-24549 in three soils, XenoBiotic Laboratories, Inc., Rohm and Haas Technical Report No. 34-98-53, October 14, 1998, GLP, unpublished. ER ref. no. 18.1	N	Gowan	Y
CA, 7.1.3.1.2	IIA, 7.1.2.2	Volkel, W.	1998b	Adsorption/Desorption of RH-127450 on Three Soils, RCC Ltd., Rohm and Haas Technical Report No. 34-98-54, December 15, 1998, GLP, unpublished. ER ref. no. 25.4	N	Gowan	Y
CA, 7.1.3.1.2	IIA, 7.1.2.2	Volkel, W.	2000	Adsorption/Desorption of RH-163,353 In Three Soils, RCC Ltd, Rohm and Haas Technical Report No. 34-00-06, January 31, 2000, GLP, unpublished. ER ref. no. 40.7	N	Gowan	Y

Data point	Annex point (old)	Author(s)	Year	Title Source (where different from company) Company, Report No, GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N	Owner	Previous Evaluation Y/N
CA, 7.1.3.1.2	IIA, 7.1.2.2	Volkel, W.	1998c	Determination of the Adsorption Coefficient of 14C-RH-163353 on Soil and its Octanol/Water Partition Coefficient Using High Performance Liquid Chromatography (HPLC), RCC Ltd., Rohm and Haas Technical Report No. 34-98-55, November 9, 1998, GLP, unpublished. ER ref. no. 31.4	N	Gowan	Y
KCA, 7.1.3.1.2	-	Van den Bosch, M.M.H.	2013b	Adsorption / desorption of RH-141,455 on three soils WIL Research Europe B.V., Hambakenwetering 7 5231 DD 's-Hertogenbosch, The Netherlands Project 500851 GLP, not published	Y	Gowan	N
CA, 7.1.4.1.2	IIA, 7.1.3.2	Volkel, W.	1998d	14C-RH-117281: Leaching characteristics of aged residues in one soil, RCC Umweltchemie Ag, Rohm and Haas Technical Report No. 34-98-48, September 15, 1998, GLP, unpublished. ER ref. No. 4.4	N	Gowan	Y
CA, 7.2.1.1	IIA, 7.2.1.1	Reynolds, J.L.	1998b	Hydrolysis of [14C]RH-117281 in Water at pH 4, 7, and 9, XenoBiotic Laboratories, Inc., Rohm and Haas Technical Report Number 34-98-39, September 29, 1998, GLP, unpublished. ER ref. No. 15.2	N	Gowan	Y
CA, 7.2.1.1	IIA, 7.2.1.1	Chong, B.P.	1998	RH-117281 Fungicide: Hydrolysis rates of relevant degradation products, Rohm and Haas Technical Report No. 34-98-26, September 30, 1998, GLP not relevant, unpublished. ER ref. No. 30.16	N	Gowan	Y
CA, 7.2.1.2	IIA, 7.2.1.2	Smalley, J. and Reynolds, J.L.	1998	Aqueous photolysis of [14C]-RH-117281, XenoBiotic Laboratories, Inc., Rohm and Haas Technical Report No. 34-96-215, May 12, 1998, GLP, unpublished. ER ref. No. 12.5	N	Gowan	Y
CA, 7.2.2.1	IIA, 7.2.1.3.1	Barnes, S.P, Nave, V.	1998	RH-117281 – Assessment of ready biodegradability: modified Sturm test, Huntingdon Life Sciences Limited, Rohm and Haas Report No. 98RC-1028, December 14, 1998, GLP, unpublished. ER ref. No. 29.1	N	Gowan	Y

Data point	**Annex point (old)**	**Author(s)**	**Year**	**Title Source (where different from company) Company, Report No, GLP or GEP status (where relevant), Published or not**	**Data Protection Claimed Y/N**	**Owner**	**Previous Evaluation Y/N**
KCA, 7.2.2.2	-	Van den Bosch, M.M.H.	2014	Aerobic mineralisation of zoamide in surface water WIL Research Europe B.V., Hambakenwetering 7 5231 DD 's-Hertogenbosch, The Netherlands Project 503495 (DRAFT Interim Report) GLP, not published	Y	Gowan	N
CA, 7.2.2.3	IIA, 7.2.1.3.2	Morgenroth, U.	1998	14C-RH-117281: Degradation and metabolism in aquatic systems, RCC Umweltchemie Ag, Rohm and Haas Technical Report No. 34-98-47, September 15, 1998, GLP, unpublished. ER ref. no. 4.3	N	Gowan	Y
KCA, 7.2.2.3	-	Callow, B. and Hilton, H.	2013b	Determination of rates of decline for zoxamide and its metabolites, in sediment-water studies according to the guidance within the FOCUS Kinetics Guidance Document Exponent International Ltd, Harrogate, HG2 8E, UK Report No: 0907598.UK0/EWC0020 Not GLP, Not published.	Y	Gowan	N
CA, 7.3.2	IIA, 7.2.2	Burgener, A.	1998b	Investigation of the volatilization of 14C-RH-117281 from soil and dwarf runner bean, RCC Ltd, Rohm and Haas Technical Report No. 34-98-132, August 24, 1998, GLP, unpublished. ER ref. no. 14.2	N	Gowan	Y