Renewal Assessment Report

under Regulation (EC) 1107/2009

Zoxamide

Volume 3

Active substance
B.7 Residue data

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

Version history

Date	Subject
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B.7 RESIDUE DATA[1]

B.7.1 Storage stability of residues

A storage stability of residues in potatoes, grapes, grape juice and raisins was previously evaluated in DAR and considered to be acceptable. Although the grape and potato storage stability studies for parent zoxamide were previously accepted for Annex I inclusion, there were problems with the spiking solutions used in the studies. Therefore a new storage stability study was performed for parent zoxamide on grape (RAC, raisins, juice and wine) and potato (RAC), for the metabolites RH-141452 and RH-141455 in potato, and for the metabolite RH-150721 in grapes (RAC) and wine. A summary of the new study and studies evaluated for Annex I inclusion are presented below.

B.7.1.1 Matrices of plant origin

Study 1

Reference:	CA 6.1/01 Ross, J.R., Storage stability of RH-117281 Residues in Grapes, Grape Juice, Raisins and Potatoes under Condition of Frozen Storage. Report No. 34-98-161, December 15, 1998a
Guideline(s):	US EPA OPPTS 860.1380
Deviations:	Guideline Deviations – analysis of spike solutions aliquots sampled at each dosing interval were used for determining dosing level.
GLP:	Yes
Validity of the study:	Valid
Previous evaluation:	In DAR (2001)

Summary

Field residue trialswere conducted in the US on potaoes and grapes in 1996 and 1997, in which the crop was treated with RH-117281. Results of these trials are being reported separately (Refs. 1,2). Field residue trials were also conducted on potatoes and grapes in Europe in 1996 and 1997, and on Potatoes in Canada in 1998. In one of the 1996 US grape trials, both grape fruit (raw agricultural commodity, RAC) samples and samples of the processed commodities, grape juice and raisins, were generated from the study. Since the potato, grape, grape juice and raisin samples generated in these studies were stored for periods grater than thirty days prior to analysis, information on the stability of RH- 117281 in the 4 matrices of concern under conditions of frozen storage was generatedbeginning in 1997. Control samples of each matrix were fortified periodically with RH-117281 at a nominal concentration of 1ppm, and the fortified samples were stored frozen pending analysis. Dose verification vials were also prepared at each interval by injecting aliquots of dosing solution into sample vials. The grape samples were fortifiedand stored frozen fot up to 17 months prior to analysis, which is aproximately the maximum length of time that samples from the grape residue trials were stored.The potato, raisin, and grape juice were fortified and stored frozen for up to 11 months prior to analysis, which is less than the maximum length of time that these samples from corresponding studies were stored prior to analysis, so the stability studies will continue. All samples were analyzed at the end of the storage period. Data from freshly fortified samples analyzed concurrently with the stored samples were used to determinē the analytical method recovery.

[1] Uses applied for to support the setting of MRLs for uses beyond the representative uses(s) should be clearly identified.

As a supplement to this study, several treated samples from 1996 grape field trials were reanalyzed after approximately 15 months additional frozen storage, by the original analytical laboratory (per Amendment 2 to protocole 34P-97-14). These residues were compared with the values from the initial analysis.

Concentrations of RH-117281 in grape, grape juice and potato were determined by GC/ECD analysis. Concentrations of RH-117281 in raisins, and the grape samples from field residue trials, were determined by GC/MSD, a confirmatory detection technique described in the analytical method. Actual levels of fortification in the aged samples were determined by analysis of the dose verification vials. Recoveries of RH-117281 from stored samples were calculated by dividing the found concentration levels in stored samples by the amount fortified prior to storage. These recoveries were then corrected for the recovery of RH-117281 from freshly fortified samples analyzed cuncurrently with thestored samples. Study results can be found in Tables 1-4 and are graphically depicted in Figures 1-4. Results from analysis of the dose verification vials are shown. In Table 5.Results of both the original and of the grape residue study samples are presented in Table 6. Results of all the analyses showed the residues of RH-117281 are stable in frozen storage in grape for at least seventeen months, and in potato, grape juice and raisin for at least eleven months.

Results and discussion

The data show that residues of RH-117281 are stable in grape under conditions of frozen storage for at least 17 months and in grape juice, raisin and potato under conditions of frozen storage for at least 11 months. No residues above the LOQ of the method were found in any of the control samples.

Residues in the 6 samples from the grape residue trials, which were reanalyzed after an additional 15 months of frozen storage, were scattered above and below the residues determined in the original analyses. Each of the samples was analyzed two or three separate times in the original study, and was analyzed in duplicate in the reanalysis. Although there is a great deal of variability in the data, the average value of reanalysisversus initial analysis is 96.7%, as seen in table 6. We conclude that the residues in these reanalyzed grape samples are unchainged from their original values. There was certainly no pattern of decline in residues following an additional 15 months of frozen storage.

Conclusions

The results of these studies demonstrante that residues of RH-117281 remain relatively stable in grape juice, raisins and potato when stored at -20^{o}C for at least 11 month, and for at least 17 month in grape. Grape and grape juice show a very slow decline with freezer storage time, raisin shows a steady decline, and potato is essentially unchanged after frozen storage.

Study 2

Reference:	CA 6.1/02 Ross, J.R., Stability of RH-141455 and RH-141452 Residues in Potatoes, potato Chips and Potato Flakes under Conditions of Frozen Storage. Report No. 34-98-162, December 15, 1998
Guideline(s):	US EPA OPPTS 860.1380
Deviations:	Guideline Deviations – analysis of spike solutions aliquots sampled at each dosing interval were used for determining dosing level.
GLP:	Yes
Validity of the study:	Valid
Previous evaluation:	In DAR (2001)

Summary

Field residue trials were conducted on potatoes in 1996 and 1997 in the US, in which the crop was treated with RH-117,281. Results of these trials are being reported separately. A 14C-RH-117,281 potato metabolism study showed that the residues of concern in potato are the metabolites RH-141,455 and RH-141,452. In one of the 1997 US potato trials, both potato tuber (REC) samples and samples of processed commodities, potato chips and potato flakes, were generated from the study. Since the potato , potato chips and potato flake samples generated in these studies were stored for periods greater than thirty days prior to analysis, information on stability of RH-117,281, RH-141,455 and RH-141,452 residues in potatoes, and RH-141,455 and RH-141,452 residues in potato chips and flakes under condition of storage is required.

Storage stability studies were initiated on potato RAC, chips and flakes in late 1997. Because residue analytical methods were not developed yet, control samples of each matrix were fortified periodically with RH-141,455 and RH-141,452 at nominal concentration of 1 ppm, and the fortified samples were stored frozen pending analysis. Dose verification vials were also prepared at each interval by injecting aliquotsof dosing solution into empty vials. These studies were found to be irretrievably flawed at the time of analysis (approximately 10 months after study initiation), due to a solubility problem that resulted in vanishingly low spiking levels for the samples.

Since information on the stability of RH-141,455 and RH-141,452 residues in potatoes, potato chips and potato flakes under conditions of frozen storage is still required, an alternate method of assessing this stabiolity was empoyed. Ancillary studies, not originally designed as storage stability studies, provide strong evidence that residues of RH-141,455 and RH-141,452 in potatoes, potato chips and potato flakes are stable for period of up to 29 months.

Results and disussion

In late august 1998, a crystalline precipitate was noticed in the fortification stock solution from RH-117,281 soil storage stability study. Subsequent investigation showed that the test material has only limited solubility in n-heptane at low temperature. Analysis of dose verification vials for potato chips and flakes showed that the problem with the spiking solution was mor severe for RH-1452 and RH-1455 than for RH-7281. There was no detectable level of either analyte in any of the vials, which meant that virtually no analyte had been spiked into the study samples at any of the time intervals.

OPPTS Guideline 860.1380 allowes that analysis of metabolism study samples is acceptable for establishing storage stability as long as the method used is a valideted method for quantitation and is not based simply on counting the total radioactivity. In the potato metabolism study, RH-141,452 and RH-141,455 were isolated, purified, identified and quantitated by TLC using authentic standarts, and confirmed by HPLC and also by mass spec. A radiovalidation of the residue analytical method was performed 29 months later, using GC/ECD, and the results corresponded very closely to the values ariginally calculated in the metabolism study, indicating that there was no loss of the residues on frozen storage. The data are summarized in table B.7.1.1-1 below:

Table B.7.1.1-1

	Metabolism study April 1996 (ppm, parent equivalents)	Radiovalidation GC/ECD Analysis September 1998 (ppm, parent equivalents)
RH-141,452	0.037	0.038 ± 0.013
RH-141,455	0.069	0.065 ±0.015

The analysis of the potato processed fractions demonstartes that a loss of FH-1452 and RH-1455 residues in potato chips and flakes is unlikely over the 11-12 months the samples were stored prior to analysis. The

potatoes which were processed were harvested August 28, 1997. The potatoes were stored at 7oC, and were made into chips and flakes two weeks later. The samples were stored in freezer for 11-12 months between sampling and analysis.

The EPA theoretical maximum concentration factor for making potato flakes from potatoes is 4.7, and the theoretical maximum concentration factor for making potato chips from potatoes is 5. If the measured residues in potato flakes and chips after 12 months of frozen storage are close to the theoretical maximum level (based on the residues measured in the starting potatoes), it is unlikely that any significant degradation could have occurred. The measured residues of each analyte in the starting RAC, the flakes, and the chips are listed below, along with the calculated theoretical maximum levels.

Table B.7.1.1-2

	RH-141,452 Residues, ppm	RH-141,455 Residues, ppm
Residues in starting RAC	0.0208	0.0163
Theoretical in flakes (x4.7)	0.0978	0.0766
Actual level in flakes	0.121	0.056
Actual as % of theoretical, flakes	124%	73%
Theoretical in chips (x5)	0.104	0.0815
Actual level in chips	0.0259	0.0204
Actual as % of theoretical, chips	25%	25%

There is excellent agreement for both the analytes in flakes, especially considering that the residue levels in the RAC were near or below the LOQ of the analytical method (0.02ppm). These results indicate that residues of RH-141,455 and RH-141,452 are stable in potato flakes under conditions of frozen storage for at least 12 months. The lower-than-theoretical residue levels in potato chips do not necessarily indicate reduced stability for these analytes in potato chips in frozen storage. The residues in potato chips could be lower due to dilution from the weight of cooking oil in the chips, or due to loss duringfrying.

The evidence from the radiovalidation study and the results of the potato processing study provide strong evidence that the residues of RH-141,455 and RH-141,452 are stable in potato RAC and potato flakes for at least the length of time that these samples were stored prior to analysis. The evidence on residue stability in potato chips is less conclusive, but consistent with physical principles involved with chip making. A conclusion of stability is also consistent with the chemical structure of these molecules (a simple hydroxymethylbenzoic acid and a benzenedicarboxylic acid).

Conclusion

The stability behavior of residues of RH-141,455 and RH-141,452 in potato matrices under conditions of frozen storage was assessed by analysis of the samples from the potato metabolism study. A comaprison of the results from the radiovalidation of the analytical method for RH-1452 and RH-1455 in potato, and the analytical results from the analysis of the potato processed fractions provide adequate proof of stability. Residues of RH-1452 and RH-1455 are stable in potato for at least 29 months and in potato chips and flakes for at least 12 months.

Study 3

Reference:	CA 6.1/03 Paul H. Reibach, Storage stability of RH-117,281 Residues in POtato Samples under Conditions of Frozen Storage: Supplement to TR 34-98-161 September 2, 2000
Guideline(s):	US EPA OPPTS 860.1380
Deviations:	Guideline Deviations – analysis of spike solutions aliquots sampled at each dosing interval were used for determining dosing level.
GLP:	Yes
Validity of the study:	Valid
Previous evaluation:	In DAR (2001)

Summary

Field residue trials in which the crop was treated with RH-117281 have been conducted at various locations around the world between 1996 and 2000. Results of these trials have being reported separately. Since potato samples generated in these studies were stored for periods grater than thirty days prior to analysis, information on the stability of RH-117281 in potato is required. A storage stability report, including results, for potato samples was previously issued (Reference 1), however additional analyses are required to completely cover the storage interval for potato RAC samples.This report provides additional analyses to cover these storage intervals.

The dosing portion of the study was performed at McKenzie Laboratories as detailed in Reference 1. Control samples of potato were fortified periodically with RH-117281 at anominal concentration of 1 ppm, and the fortified samples were stored frozen pending analysis. Dose verification vials were also prepared at each interval by pipeting aliquotsof dosing solution into empty vials. Dose verification vials were then analyzed at the time of dosing. Following analyses included in the previous report, the samples were shipped frozen to Rohm and Haas for additional analyses a later time points. The potato samples reported here were fortified at a actual concentration of 0.857 ppm and stored frozen for aproximately 24 months prior to analysis. Two year s is approximately the maximum length of time that samples from the residue trials were stored. Data from freshly fortified control samples, analyzed concurrently with the stored samples, were used to correct the results for analytical method recovery.

Objective

The objective of this study was to determine the stability of residues of RH-117281 in potato samples under conditions of frozen storage. Stability in potato was evaluated over a period of 708 days or about 24 months. This period was chosen to provide information over the length of time that samples from field residue trials were stored prior to analysis. The stability studies reported herein were conducted to comply with provisions of the US EPA OPPTS Section 860.1380 guidelines and related guidance documents.

The test material and reference material used in this study was RH-117281, an experimental fungicide which is also referred to as RH-7281.

Samples and dose verification vials were stored frozen for the duration of the study. Except for brief intervāls, to remove samples for spiking and then to return them to the boxes, all samples were stored in the freezer. The freezer temperature was maintained at approximately-20oC and monitored to ensure sample integrity. Except for brief periods when personnel were storing or removing samples, the interior of the freezer was dark.

Results

Results from the analysis of sample for the first year of the study and analysis of the dose verification vials are found in tabele B.7.1.1-3. Based on analyses at McKenzie the samples analyzed in this phase of the study were dosed at actual concentration of 0.857 ppm.

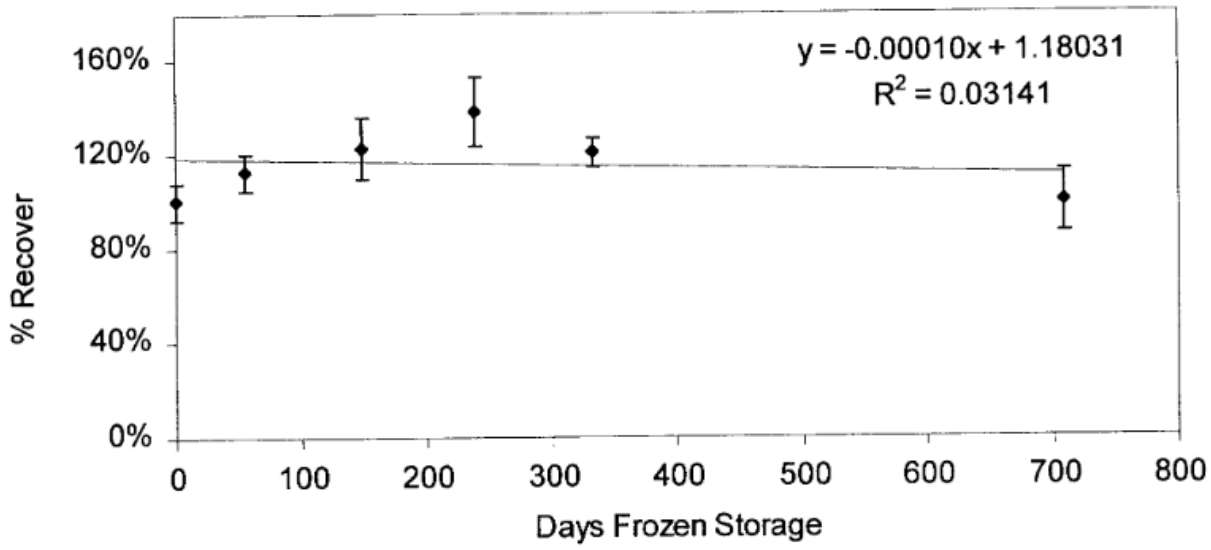
Table B.7.1.1-3

Fortification level (ppm)	Sample Number	Sample weight (g)	Peak Area	µg/ml	Final volume (ml)	Total µg	ppm
0.857	106	10	1126520	0.429	20	8.58	0.858
0.857	107	10	538292	0.201	20	4.02	0.402
0.857	108	10	939231	0.356	20	7.13	0.713
1.0	109	10	1070700	0.407	20	8.15	0.815
0.5	110	10	681195	0.256	20	5.12	0.512
0	111	10	ND	ND	20	ND	ND

Recovery data obtained from stored potato samples can be found in Table 1. The tabele shows the recovery found for three replicate samples from the 24 month storage interval (% theoretical dose), together with the recoveries obtained from freshly fortified samples analyzed cuncurrently with this set. The rightmost column lists the corrected recovery values for the aged forts. These values were used to prepare the decline curve. The fresh fortification values from each analytical run were averaged and the apparent recoveries of the aged and the fresh fortifications were corrected by dividing by the average recovery for that analysis day. The average corrected recoveries for potato are plotted versus freezer storage time in figurē 1, with the mean of fresh fortifications used as the Day 0 recovery.

This study was conducted over a period of 24 months and now has 4 time ppoints in addition of time 0. The data show that residues of RH-117,281 are relatively stable in potato under conditions of frozen storage for at least 24 months. Based on the slope of the decline curve from Figure 1, a loss of about 0.01% per day is expected. This extrapolates to a loss of about 3.6% per year or 7.3% after 2 years of storage. These results demonstrate that with respect to storage the analysis of actual residue samles are valid.

Figure 1: Corrected Recoveries for RH-117,281 in Potato vs. Days of frozen storage



Conclusions

The results of this study demonstrante that residues of RH-117281 remain relatively stable in potato when stored at -20°C for at least 24 months. These results demonstrante that storage stability is not a concern for actual field residue samples stored under similar conditions. The results also show that extrapolation to even longer time periods is appropriate.

Study 4

Reference:	CA 6.1/04 Weber, H., Kissmann, H. (2014) Storage Stability of residues of Zoxamide, RH-150721, RH-1452 and RH-1455 in Grape and Processed Products and Potato Report no.: S12-03952 Interim Report
Guideline(s):	Regulation (EC) no 1107/2009; Concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC EU guidance document 7032/VI/95, rev.5, Appendix H of EC document 1607/VI/95 rev.2 „Storage stability of residue samples", 22-Jul-1997. OECD guideline No. 506; Stability of pesticide residues in stored commodities (16/10/2007)
Deviations:	None
GLP:	Yes
Validity of the study:	Valid
Previous evaluation:	Submitted for the purpose of renewal.

Executive Summary

A storage stability study has been performed to investigate the frozen storage stability of zoxamide (RH-7281) in grapes (berries and juice), wine, raisins and potato (tubers), the storage stability of the metabolite RH-150721 in grapes (berries) and wine, and the storage stability of RH-141452 and RH-141455 in potato (tubers).

Residues of zoxamide are stable for at least 18 months in grape berries and at least 24 months in grape juice, raisins, wine and potato tubers when stored frozen at ≤-18°C.

RH-150721 is stable for at least 18 months in grape berries and at least 24 months in wine when stored frozen at ≤-18°C.

RH-141452 and RH-141455 are stable in potato for at least 24 months when stored frozen at ≤-18°C.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material-1:

Description:	Zoxamide (RH-7281)
Lot/batch No.:	10901
Purity:	98.5%
Expiry date:	27 December 2015
Development Code:	RH-7281
CAS No.:	156052-68-5
Spiking levels:	0.1 mg/kg

1. Test Material-2:

Description:	RH-150721 (as methane sulfonate)
Lot/batch No.:	F1132-136 and DB5000
Purity:	Lot F1132-136: 98.8% Lot DB5000: 98.5%
Expiry date:	Lot F1132-136: 24 April 2015 Lot DB5000: 07 July 2015
Development Code:	not applicable
CAS No.:	not available
Spiking levels:	0.1 mg/kg

1. Test Material-3:

Description:	RH-141452 (RH-1452)
Lot/batch No.:	021109
Purity:	99.82%
Expiry date:	01 March 2018
Development Code:	not applicable
CAS No.:	not available
Spiking levels:	0.1 mg/kg

1. Test Material-4:

Description:	RH-141455 (RH-1455)
Lot/batch No.:	ELM-1775
Purity:	98.8%
Expiry date:	07 February 2015, recertified expiry date 07 July 2017
Development Code:	not applicable
CAS No.:	not available
Spiking levels:	0.1 mg/kg

2. Test Commodity

Crop:	Grape, Potato
Type:	Not applicable
Variety:	Not reported
Botanical Name:	*Vitis vinifera*, *Solanum tuberosum*
Crop Part or Processed Commodity:	Grape berries, raisins, grape juice, wine, potato tubers
Sample Size:	Grape berries, grape juice, wine, potato tubers: 10 g Raisin: 5 g

B. STUDY DESIGN

1. Test Procedure

Samples of grapes, grape juice, wine and potato (10 g samples) or raisins (5 g samples) were fortified with zoxamide, or RH-150721, RH-141452 or RH-141455 as appropriate at 0.1 mg/kg (10 x LOQ) and placed in a freezer at ≤-18°C for up to 18 months for grapes or up to 24 months for raisins, grape juice, wine and potato. Samples were analysed on the day of storage initiation (day 0), and after storage for 6, 12, 18 and 24 months at ≤-18°C (except grapes, where samples were analysed after 6, 12 and 18 months).

On day 0, three freshly fortified samples and one untreated control sample were analysed for each analyte and matrix separately. At the subsequent time points, for each analyte and matrix, one untreated control sample, one untreated sample freshly fortified just before analysis at 0.1 mg/kg, and two stored samples were analysed.

2. Description of analytical procedures

Analysis for zoxamide

Grape berries, grape juice, wine, raisin and potato samples were analysed for zoxamide using the QuEChERS LC-MS/MS method, validated in EAS study S12-03949. The method validation data are presented in Point B.5.1.2., study CA 4.1.2/01.

Samples were extracted by shaking with acetonitrile. After addition of a salt mixture of magnesium sulfate, sodium chloride, trisodium citrate dihydrate and disodium hydrogen citrate sesquihydrate, the samples were shaken again and then centrifuged.

An aliquot of the supernatant was cleaned up by dispersive solid phase extraction using primary secondary amine (PSA). After centrifugation, the extract was diluted in methanol/0.05% acetic acid and analysed for residues of zoxamide by LC-MS/MS. The limit of quantification (LOQ) was 0.01 mg/kg for zoxamide in all matrices.

Analysis for RH-150721

Grape berry and wine samples were analysed for the metabolite RH-150721 using an LC-MS/MS method, validated in EAS study S12-03950. The method validation data are presented in Point B.5.1.2., study CA 4.1.2/02.

Samples of grape (berries) were extracted and homogenised with acetone and water. After filtration, the organic solvent was evaporated and 4% aqueous potassium hydrogen carbonate solution added.

Samples of grape juice and wine were mixed with 1% aqueous potassium hydrogen carbonate solution.

In each case, the resulting extract was cleaned up by solid phase extraction using a reversed phase column. The analyte was eluted with methanol containing 1% glacial acetic acid, and the extract diluted with water and analysed for residues of RH-150721 by LC-MS/MS. The limit of quantification (LOQ) for RH-150721 in grape berries, grape juice and wine was 0.01 mg/kg.

Analysis for RH-141452 and RH-141455

Potato samples were analysed for the metabolites RH-141452 and RH-141455 using an LC-MS/MS method, validated in EAS study S12-03951. The method validation data are presented in Point B.5.1.2., study CA 4.1.2/03.

Samples of potato tubers were extracted twice by homogenising with methanol/0.01 N sodium hydroxide solution (7:3, v/v). After filtration, an aliquot of the combined extract was diluted with 0.01 N sodium hydroxide solution and cleaned up by solid phase extraction using a mixed-mode polymeric phase

(OASIS MAX by Waters). The analytes were eluted with methanol/37% hydrochloric acid (100:1, v/v). The extract was evaporated to approximately 1 mL using a nitrogen stream and diluted with methanol/0.2% formic acid (2:8, v/v) to 10 mL. The final extract was analysed for residues of RH-141452 and RH-141455 using LC-MS/MS. The limit of quantification (LOQ) for RH-141452 and RH-141455 in potatoes was 0.01 mg/kg.

II. RESULTS AND DISCUSSION

The storage stability results are summarized in Tables 6.1/1 to 6.1/4.

No residues of zoxamide, RH-150721, RH-141452 or RH-141455 above the LOD were found in any of the corresponding control samples.

Table 6.1/1 Summary of the frozen storage stability of zoxamide in grapes (berries, juice, wine, raisins) and potato (tubers) (fortification level 0.1 mg/kg)

Matrix	Storage period (months)	Residue level in stored sample (mean in parenthesis) (mg/kg)	Average Recovery (%)		Corrected Average Stored Recovery (%)[1]
			Procedural	Stored (uncorrected)	
Grape berries	0	0.104, 0.099, 0.108 (0.104)	-	104	100*
	6	0.099, 0.098 (0.099)	102	99	97
	12	0.099, 0.097 (0.098)	98	98	100
	18	0.082, 0.081 (0.082)	98	82	84
Grape juice	0	0.100, 0.108, 0.108 (0.105)	-	105	100*
	6	0.092, 0.091 (0.092)	97	92	95
	12	0.093, 0.093 (0.093)	91	93	102
	18	0.101, 0.105 (0.103)	105	103	98
	24	0.091, 0.093 (0.092)	99	92	93
Wine	0	0.090, 0.083, 0.093 (0.089)	-	89	100*
	6	0.080, 0.081 (0.081)	78	81	104
	12	0.080, 0.076 (0.078)	80	78	98
	18	0.090, 0.091 (0.091)	89	91	102
	24	0.081, 0.080 (0.081)	92	81	88
Raisins	0	0.103, 0.099, 0.102 (0.101)	-	101	100*
	6	0.104, 0.104 (0.104)	104	104	100
	12	0.095, 0.094 (0.095)	101	95	94
	18	0.103, 0.101 (0.102)	105	102	97
	24	0.088, 0.093 (0.091)	90	91	101
Potato tubers	0	0.105, 0.103, 0.100 (0.103)	-	103	100*
	6	0.088, 0.090 (0.089)	95	89	94
	12	0.087, 0.083 (0.085)	90	85	94
	18	0.094, 0.104 (0.099)	102	99	97
	24	0.085, 0.084 (0.085)	94	85	90

* Zero time recovery set to 100% by default

[1] Corrected for the procedural recovery of the freshly fortified sample in the same analytical set

Table 6.1/2 Summary of the frozen storage stability of RH-150721 in grapes berries and wine (fortification level 0.1 mg/kg)

Matrix	Storage period (months)	Residue level in stored sample (mean in parenthesis) (mg/kg)	Average Recovery (%)		Corrected Average Stored Recovery (%)[1]
			Procedural	Stored (uncorrected)	
Grape berries	0	0.076, 0.075, 0.075 (0.075)	-	75	100*
	6	0.077, 0.070 (0.074)	75	74	99
	12	0.081, 0.084 (0.083)	83	83	100
	18	0.071, 0.084 (0.078)	85	78	92
Wine	0	0.089, 0.092, 0.097 (0.093)	-	93	100*
	6	0.107, 0.107 (0.107)	98	107	109
	12	0.092, 0.096 (0.094)	79	94	119
	18	0.101, 0.089 (0.095)	93	95	102
	24	0.077, 0.074 (0.076)	91	76	84

* Zero time recovery set to 100% by default

[1] Corrected for the procedural recovery of the freshly fortified sample in the same analytical set

Table 6.1/3 Summary of the frozen storage stability of RH-141452 in potato (tubers) (fortification level 0.1 mg/kg)

Matrix	Storage period (months)	Residue level in stored sample (mean in parenthesis) (mg/kg)	Average Recovery (%)		Corrected Average Stored Recovery (%)[1]
			Procedural	Stored (uncorrected)	
Potato tubers	0	0.088, 0.089, 0.087 (0.088)	-	88	100*
	6	0.095, 0.089 (0.092)	89	92	103
	12	0.081, 0.086 (0.084)	85	84	99
	18	0.092, 0.094 (0.093)	93	93	100
	24	0.099, 0.096 (0.098)	84	98	117

* Zero time recovery set to 100% by default

[1] Corrected for the procedural recovery of the freshly fortified sample in the same analytical set

Table 6.1/4 Summary of the frozen storage stability of RH-141455 in potato (tubers) (fortification level 0.1 mg/kg)

Matrix	Storage period (months)	Residue level in stored sample (mean in parenthesis) (mg/kg)	Average Recovery (%)		Corrected Average Stored Recovery (%)[1]
			Procedural	Stored (uncorrected)	
Potato tubers	0	0.086, 0.089, 0.085 (0.087)	-	87	100*
	6	0.086, 0.086 (0.086)	94	86	91
	12	0.082, 0.076 (0.079)	90	79	88
	18	0.081, 0.085 (0.083)	86	83	97

	24	0.096, 0.089 (0.093)	96	93	97

* Zero time recovery set to 100% by default

[1] Corrected for the procedural recovery of the freshly fortified sample in the same analytical set

III. CONCLUSION

Residues of zoxamide were shown to be stable for at least 18 months in grape berries, and at least 24 months in grape juice, raisins, wine and potato when stored frozen at ≤-18°C.

Residues of RH-150721 were shown to be stable for at least 18 months in grape berries and at least 24 months in wine when stored frozen at ≤-18°C.

Residues of RH-141452 and RH-141455 were shown to be stable for at least 24 months in potato tubers when stored frozen at ≤-18°C.

Samples from residue trials in potato presented under point 7.3.1 were stored for up to 19 months prior to analysis for CGA zoxamide and RH-141452 and RH-141455, with the exception of 4 trials in northern Europe and 4 trials in Southern Europe that were stored for up to 32 months.

Samples from the residue trials in grapes presented under point 7.3.2 were stored for up to 10 months prior to analysis for zoxamide.

B.7.1.2 Summary of storage stability

Storage stability of zoxamide (RH-7281) residues was shown to be acceptable for at least 24 months in potatoes and for at least 18 months in grapes. Stability of zoxamide was demonstrated over 24 months in grape juice, raisins and wine when stored frozen at ≤-18°C.

Residues of RH-141452 and RH-141455 were shown to be stable for at least 24 months in potato tubers when stored frozen at ≤-18°C.

Residues of RH-150721 were shown to be stable for at least 18 months in grape berries and at least 24 months in wine when stored frozen at ≤-18°C.

B.7.2 Metabolism, distribution and expression of residues

B.7.2.1 Plants

The metabolism of zoxamid in fruits (grapes) and root and tuber vegetables (potatoes) was evaluated during the first review of the active substance under Directive 91/414/EEC (DAR, 2002). As the use pattern in previously evaluated grape metabolism study is significantly different from the use pattern for Zoxamide 240 SC, a new grape metabolism study has been performed and is summarised below. In addition, metabolism studies have been performed in tomato, cucumber and peas. All studies are summarised below.

B.7.2.1.1 Fruits and fruiting vegetables

Grapes 1

Reference:	CA 6.2.1/01 Reibach, PH and Spencer, WO, ^{14}C-RH-117,281: Nature of Residue in

	fruiting grape plants ER ref. No. 14.5
Guideline(s):	US EPA Pesticide Assessment Guidelines
Deviations:	-
GLP:	Yes
Validity of the study:	Valid
Previous evaluations:	In DAR (2002)

In a 1995 study, Concord grapes grown in Pennsylvania USA were treated with 14C phenyl labelled RH 7281 (radiochemical purity 97.6%) formulated as an emulsifiable concentrate (EC) containing 5% w/w active substance. The study was conducted according to US EPA Pesticide Assessment Guidelines. One grape vine was treated with three applications, each at a rate of 1867 g/ha (≈ 12N). Applications were made at 30 day (± 4 day) intervals. The Applicant stated that the slight drizzle about two hours following the first application did not wash off the test material. The Applicant also stated that although the formulation caused transient and insignificant grape leaf injury, the yield of the grapes was not affected.

Aliquots of spray solutions collected at the time of each application were analyzed by LSC for total radioactivity and by TLC for radiopurity. The results confirmed that the intended amount of test material was applied, and that the material was stable in spray solution.

The harvest of mature grapes occurred 1 day after the last spray. The samples were taken to the analysing laboratory where they were frozen until processed for analysis. Sample analyses were conducted 5 to 6 months after harvest.

Grape samples were ground using a Waring food processor with dry ice. All grape samples from the control plot were processed prior to any samples from the treated plot. The dry ice was allowed to sublime and the samples were stored frozen until required for analysis

Total ^{14}C activity was determined by combustion radio-assay. Based on the specific activity of the RH-7281 used for treatment, the total terminal residue (TRR) for grapes at harvest was determined to be 0.74 mg/kg.

Extraction of samples with methanol extracted 94.0 % TRR. The remaining post extracted solids (PES) were dried at room temperature and the remaining radioactivity was determined by combustion analysis to be 5.1 % of the TRR.

The methanol solution was concentrated to remove the methanol and partitioned between water and ethyl acetate. 72.1% of TRR was found in the ethyl acetate fraction (EAl). Following acidification, the aqueous fraction was again partitioned with ethyl acetate, this time removing only 2.2% of the TRR (EA2). The resulting aqueous fraction (AQ1) contained 16.2% of the TRR. The aqueous fraction was fractionated by means of a C-18 solid phase extraction cartridge. This procedure resulted in an organic fraction which was eluted with methanol, containing 15.4% of the TRR, and a second aqueous fraction containing 0.4% of the TRR. The total recovery of each analytical procedure and the distribution of TRR in each fraction were determined via liquid scintillation counting or combustion.

The ethyl acetate fractions (EA1 and EA2), and the C-18 methanol fraction (MeOH) were compared to authentic standards of several potential metabolites using normal phase TLC (silica gel plates). Extracts were analyzed by streaking or spotting the samples ≈1.5 cm from the bottom of the plate (standards were co-spotted along the origin). The plates were developed in various solvent systems in a solvent saturated chamber.

Non radiolabeled standards were viewed under ultraviolet light and marked with a soft lead pencil. Radioactivity on the TLC plates was imaged using a PhosphorImager® SI optical imager. Radioactivity

from the plates was quantified using the PhosphorImager ImageQuant® software. Quantitation was based on the % radiolabel in the peak of interest following background subtraction.

The fractions were first analyzed in a non-polar solvent system consisting of hexane/ethyl acetate/acetic acid, designed primarily to analyze for parent and several of the more non polar standards. This analysis demonstrated that the EA1 fraction was composed primarily of parent RH-7281. There was significant activity remaining at the origin, demonstrating the formation of some polar metabolites.

Fractions EA2 and MeOH showed little or no parent. In these two fractions the majority of the radiolabel remained at the origin (78.4 and 94.7% respectively). Due to the more polar nature of the origin material, chloroform/methanol/acetic acid or chloroform/methanol/methanoic acid were used as solvent systems to resolve these metabolites.

The ethyl acetate fraction EA1 was further analyzed by two dimensional TLC. An aliquot of EA1 was mixed with standards of RH-129151, RH-7281, RH-127450, RH-139432, RH-24549, and RH-141288 and spotted in the lower corner of a silica plate. The plate was developed in the first dimension with hexane/ ethyl acetate / formic acid, and in the second dimension with chloroform/methanol/ammonium hydroxide. After drying, comparison of the UV visible spots from the standards with the radioactive image, confirmed that RH-7281 was the major component and that RH-141288, RH-139432, RH-129151 were also present together with several other low level metabolites.

The EA1 fraction was found to contain RH-7281 as the only significant component (greater than 10%). RH-7281 accounted for 58.3% of the TRR. Further confirmation of RH-7281 was obtained by reversed phase HPLC analysis of the ethyl acetate fraction EA1 using a C-18 column, UV detection and an acidic mobile phase composed of ACN/H_2O/phosphoric acid.

The fraction with the highest percentage of unidentified components was the MeOH fraction where 4.2% of the TRR remained at the origin. All other unknown degradates were present at less than 5% of the TRR. The total accountability for the study is summarised in table B.7.2.1.1.-1

Table B.7.2.1.1-1 Total accountability for the metabolism of zoxamide in grapes

Metabolite	% TRR	mg/kg[1]
RH-7281	58.3	0.429
RH-129151	3.0	0.022
RH-141288	1.7	0.013
RH-139432	1.9	0.014
RH-150721	2.8	0.021
RH-149736	1.1	0.008
RH-149737	1.6	0.012
Post extraction solids	5.1	0.036
Total MeOH unknowns	15.3[2]	0.113
Total EA 1 unknowns	2.8	0.021
Total EA 2 unknowns	1.1	0.008
C-18 AQ	0.4	0.003
Total accounted for	95.1	0.699

(1) Expressed as parent equivalents
(2) In the methanol fraction 4.2% of TRR remained at the origin. Three other bands were observed but not characterised further.

Grape samples retained from supervised field trials were analysed for the metabolite RH-150721. Trials were conducted in accordance with the appropriate GAP unless otherwise stated. Samples had been stored at –20 °C prior to analysis. Samples were dispersed in acetonitrile/ 0.01M hydrochloric acid and homogenised. The pH was adjusted to 2.0 by adding 1 M hydrochloric acid. After filtering through Celite, the filtrate was evaporated to dryness and the residue taken up again in acetonitrile/ 0.01 M hydrochloric acid. After the pH was adjusted to 7.0 with 5% sodium bicarbonate solution the residue was extracted with ethylacetate. The organic extract was concentrated under reduced pressure. RH-150721 was determined using GC with Electron Capture detection. The LOQ for the method was 0.03 mg/kg.

The results of the analyses are presented in table B.7.2.1.1-2

Table B.7.2.1.1-2 Determination of RH-150721 in samples from selected supervised field trials

Country/ Year	PHI (Days)	RH-150721 (mg/kg)	RH-7281 (mg/kg)	RH-7281/ RH-150721	Trial Reference	Dossier ref (DP No.)
France N 1999	28	0.074	0.19	2.6	EA990175 FR 01	6.3.2/19 (97899)
France S 1999	28	0.086	0.33	3.8	EA990176 FR01	6.3.2/20 (97900)
France 1999	28	0.062	0.19	3.1	EA990177 FR01	
France 1999	28	0.185	0.27	1.6	EA990177 FR02	
Italy 1999	28	0.090	1.32	14.6	A/IT/F/99/66	
Germany 1999	56	0.055	0.49	8.9	A/GE/F/99/6 5	
Germany 1997	57	<0.03	0.84	28	A/GE/F/97/1	6.3.2/06 (82140)
Germany* 1997	57	<0.03	0.6	20	A/GE/F/97/2	6.3.2/06 (82140)
Germany 1997	57	<0.03	0.66	22	A/GE/F/97/3	6.3.2/06 (82140)
Germany* 1997	57	0.03	0.35	11.66	A/GE/F/97/4	6.3.2/06 (82140)

(*) Crops were treated with a spray concentration of 45 kg a.s/ hl instead of 15 kg/hl as stated in the proposed GAP

Grapes 2

Reference:	CA 6.2.1/05 Staffa, C., Möndel, M. (2014) ^{14}C-phenyl UL Zoxamide: Plant Metabolism in Grape Report no.: AS209 Amendment No. 1
Guideline(s):	OECD guideline No. 501; Metabolism in Crops (08/01/2007)
Deviations:	None
GLP:	Yes
Validity of the study:	Valid
Previous evaluations:	No; Submitted for the purpose of renewal of a.s. approval

Executive Summary

After foliar application of [^{14}C]-zoxamide to grape vines at a 28 day PHI at a nominal rate of 3 x 500 g a.s./ha, the total radioactive residue (TRR) in grapes (RAC) was 3.975 mg/kg.

The majority of the residue was removed in the surface rinses and comprised mainly parent zoxamide.

Zoxamide was the only significant component of the residue, accounting for 3.665 mg/kg, 92.2% TRR. The metabolites RH-149736, RH-149737, RH-139432 and RH-129151 were identified as minor components of the residue (up to 2.4% TRR). Two unidentified metabolites were also detected at up to 1.5% TRR.

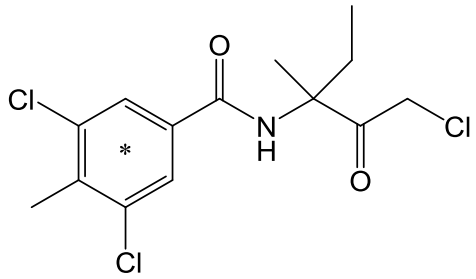
The degradation pathway in grapes proceeds via hydrolysis/cyclisation to form RH-129151. RH-139432 is probably formed by photolysis, and then oxidised to form RH-149737 and RH-149736.

I. MATERIAL AND METHODS

A. MATERIALS

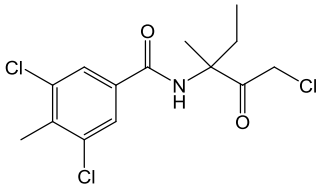
1. Test Material

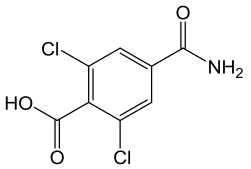
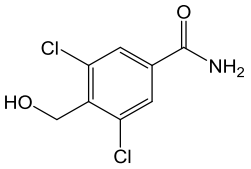
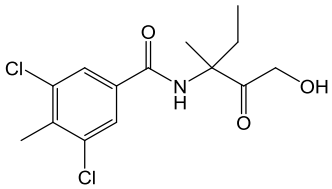
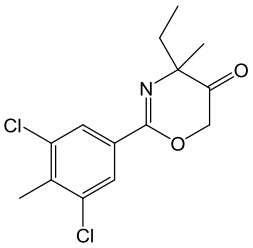
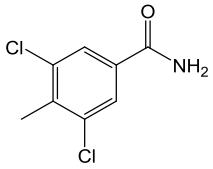
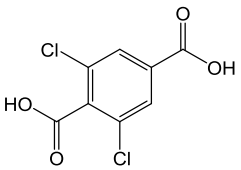
Description:	Zoxamide (unlabelled) [Ring-UL-^{14}C]-zoxamide (phenyl label)
Lot/batch No.:	90924 (unlabelled) 10-135-36-30 (phenyl label)
Purity:	Chemical purity: 95.0% (unlabelled) Radiochemical purity: ≥ 98.0% (phenyl label)
Specific activity:	0.0487 mCi/mg (1801.9 kBq/mg) (phenyl label as received)
CAS No.:	156052-68-5
Development Code:	RH-7281
Position of Radiolabel:	



The purity and structures of the key reference materials used in this study are given in Table B.7.2.1.1-3 below.

Table B.7.2.1.1-3 Key reference materials used in the identification of metabolites

Reference Standard Name/code	Structure	Batch Number Purity Molecular Weight (MW) Conversion Factor (CF)[1]
Zoxamide		90924 95.0% MW 336.6

Reference Standard Name/code	Structure	Batch Number Purity Molecular Weight (MW) Conversion Factor (CF)[1]
RH-149736		ELM-1774 94.99% MW 234.04 CF 0.6953
RH-149737		ELM-1772 96.26% MW 220.05 CF 0.6537
RH-141288		KY-00-25-37 100% MW 318.20 CF 0.9453
RH-129151		KY-00-25-17 >99% MW 300.19 CF 0.8918
RH-139432		ELM-1740 98.3% MW 204.06 CF 0.6062
RH-141455		ELM-1775 98.8% MW 235.02 CF 0.6982

[1] Conversion Factor (CF) is the molecular weight ratio of the metabolite/zoxamide. This value is used to convert residue values expressed as mg analyte/kg to mg parent equivalents/kg.

2. Test Commodity

Crop: Grape vine
Type: Not applicable
Variety: Muskat delekta
Botanical Name: *Vitis vinifera*
Crop Part or Processed Commodity: Grape bunches, leaves
Sample Size: 4.1-4.6 kg (grape bunches), 0.21-0.54 kg (leaves)

3. Soil

Type: Not reported

B. STUDY DESIGN

1. Test Procedure

^{14}C-Phenyl ring-labelled zoxamide was isotopically diluted with unlabelled zoxamide to a final specific activity of 938 kBq/mg. This was formulated as an 1000 mg/L WG formulation (Electis WG) and applied to a grape vine at a target application rate of 3 x 500 g as/ha, corresponding to a total application rate of 1500 g as/ha. The three foliar spray applications were made at BBCH 81 (1st and 2nd applications) and BBCH 83 (3rd application), with 9-11 days between each application. The water volume was 500 L/ha. A second control grape vine was not treated. Plants were maintained outdoors, and cultivation and harvesting was carried out according to common agricultural practice.

The field phase was carried out in 2011 at RLP AgroScience GmbH, Breitenweg 71, 67435 Neustadt, Rhineland-Palatinate, Germany.

2. Sampling

Grapes and leaves were harvested 28 days after the last application, at BBCH 89. Any immature and diseased berries were removed from the stem and discarded. The harvested leaves and grapes were weighed.

Due to the large sample size, the treated grape bunches were separated into five fractions for surface rinsing. The freshly sampled grapes were rinsed three times with acetonitrile/water (1:1 v/v) to remove surface residues. Aliquots of the surface rinse were analysed by LSC and profiled by HPLC.

The rinsed grapes were then frozen, homogenised and lyophilised. Aliquots of the homogenised samples were combusted and analysed by LSC for the determination of the total radioactive residue (TRR).

The leaf samples were frozen after harvesting, but not analysed.

3. Extraction and analysis

Two sub-samples of homogenised, lyophilised rinsed grapes were extracted five times with methanol. After each extraction, the extract was centrifuged, and the supernatant decanted and filtered. Water was added to the combined methanol extracts, and the extraction mixture evaporated to an aqueous remainder. The resulting aqueous extract was partitioned against ethyl acetate, and the extracts combined to give one water extract and one ethyl acetate extract. The water extract was acidified with 6N HCl and partitioned again against ethyl acetate. The ethyl acetate extracts from the two partitioning steps were combined to a single sample. Aliquots of each extract were analysed by LSC. The post-extraction solids (PES) were dried and analysed for radioactivity by combustion and LSC.

The surface rinse samples, water extracts and ethyl acetate extracts were analysed by reverse-phase (C18) HPLC to determine the metabolite profile, using UV and liquid scintillation cell radiochemical detection. Metabolite identification was performed by co-chromatography with authentic reference standards, and the results were confirmed using an alternative HPLC gradient.

HPLC analysis of the water extracts showed a single broad peak, therefore the water extracts were acidified with 6M HCl and heated under reflux for 1 hour. The hydrolysed water extracts were analysed by HPLC.

To determine the storage stability of zoxamide in the extracts, selected extracts of control grapes were spiked with known concentrations of zoxamide and stored under the same conditions

(≤ -18°C) as the treated sample extracts. The purity of the zoxamide in the extracts before and after storage was determined by HPLC.

All mg/kg values are expressed as parent equivalents in the tables below.

II. RESULTS AND DISCUSSION

A. TOTAL RADIOACTIVE RESIDUES (TRR)

The total radioactive residue (TRR) in grapes (RAC) was 3.975 mg/kg. The majority of the residue was removed in the surface rinses (93.0% TRR), as shown in Table B.7.2.1.1-4 below.

Table B.7.2.1.1-4 Summary of TRR in grapes

Crop part	Location of residue	Total radioactive residue (TRR)	
		mg/kg	% TRR
Grapes	Surface rinse	3.698	93.0
	Washed grapes	0.278	7.0
	Total TRR	3.975	100

B. EXTRACTION AND CHARACTERISATION OF RESIDUES

1. Extraction and characterisation of residues

The washed grapes were extracted with methanol, releasing a further 6.5-6.7% TRR (0.26-0.27 mg/kg). Partitioning of the methanol extracts showed that 5.2-5.4% TRR (0.21 mg/kg) was organo-soluble (in the ethyl acetate fraction) and 1.2% TRR (0.047 mg/kg) was water-soluble. Only 0.4% TRR (0.016 mg/kg) remained unextracted.

2. Identification of metabolites

The acetonitrile/water surface rinses, and ethyl acetate and water extracts were profiled by radio-HPLC (see Table B.7.2.1.1-5).

The majority of the radioactivity in the surface rinses was parent zoxamide (3.504 mg/kg, 88.1% TRR). Four minor metabolites were also detected in the surface rinses. Two were identified by co-chromatography with reference items as RH-139432 (0.088 mg/kg, 2.2% TRR) and RH-129151 (0.30 mg/kg, 0.7% TRR). The two unidentified metabolite fractions did not exceed 0.058 mg/kg, 1.4% TRR.

The radioactivity in the ethyl acetate extracts also comprised mainly parent zoxamide (0.162 mg/kg, 4.1% TRR). Small amounts of the metabolites RH-149736 (0.005 mg/kg, 0.1% TRR), RH-149737 (0.036 mg/kg, 0.9% TRR) and RH-139432 (0.006 mg/kg, 0.2% TRR) were identified in the extracts, and one unidentified metabolite (0.001 mg/kg, <0.1% TRR) was also detected.

All of the radioactivity in the aqueous extracts chromatographed as a broad peak at the retention time of RH-149737. Based on the HPLC retention time, the radioactivity in this fraction was characterised as conjugated RH-149737, however acid hydrolysis of the aqueous fraction did not release any conjugates.

Table B.7.2.1.1-5 Identification of metabolites in grapes

Compound	Residues of [^{14}C]-zoxamide in Grapes							
	Surface rinse		Ethyl acetate extract		Water extract		Total	
	mg/kg[1]	% TRR	mg/kg[1]	% TRR	mg/kg[1]	% TRR	mg/kg[1]	% TRR
Zoxamide	3.504	88.1	0.162	4.1	-	-	3.665	92.2
RH-149736	-	-	0.005	0.1	-	-	0.005	0.1
RH-149737 [2]	-	-	0.036	0.9	0.047	1.2	0.083	2.1
RH-139432	0.088	2.2	0.006	0.2	-	-	0.094	2.4
RH-129151	0.030	0.7	-	-	-	-	0.030	0.7
Unknown 1	0.058	1.4	0.001	<0.1	-	-	0.059	1.5
Unknown 2	0.018	0.5	-	-	-	-	0.018	0.5
Unextracted							0.016	0.4
Total							3.970	99.9

- not detected

[1] Residue expressed as mg parent equivalents/kg

[2] Sum of RH-149737 and its conjugates, expressed as RH-149737

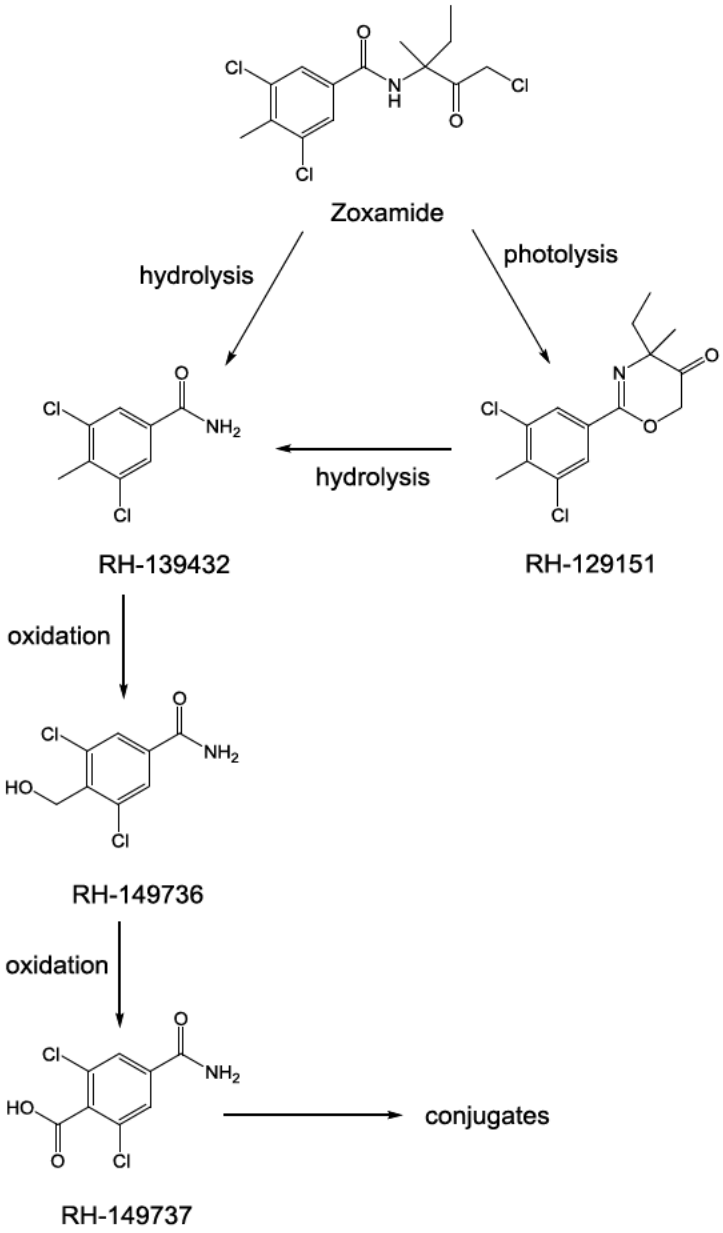
3. Storage stability of residues

The storage stability investigations demonstrated that parent zoxamide is stable in the extracts over the 6-month storage period under frozen storage conditions (≤ -18°C).

4. Proposed metabolic pathway

The degradation pathway in grapes proceeds via photochemical degradation on the surface of the grapes to form RH-129151. Hydrolysis of zoxamide or RH-129151 forms RH-139432, and this is then further oxidised to form RH-149737 and RH-149736. A metabolic pathway is proposed as follows:

Figure B.7.2.1.1-6 Proposed metabolic pathway for zoxamide in grapes



III. CONCLUSION

After foliar application of [^{14}C]-zoxamide to grape vines at a 28 day PHI at a nominal rate of 3 x 500 g a.s./ha, the total radioactive residue (TRR) in grapes (RAC) was 3.975 mg/kg.

The majority of the residue was removed in the surface rinses and comprised mainly parent zoxamide. Zoxamide was the only significant component of the residue, accounting for 3.665 mg/kg, 92.2% TRR.

RH-149736, RH-149737, RH-139432 and RH-129151 were identified as minor components of the residue, with two additional unidentified metabolites.

Tomatoes

Reference:	CA 6.2.1/06 Sharma, A.K. (1999a) RH-117,281: Nature of Residue in fruiting Tomato Plants Report no.: 34-99-159
Guideline(s):	US EPA Guideline OPPTS 860.1300
Deviations:	None
GLP:	Yes
Validity of the study:	Valid
Previous evaluations:	No; Submitted for the purpose of renewal of a.s. approval

Executive Summary

After foliar application of [^{14}C]-RH-7281 to tomato plants at a 1 day PHI at a nominal rate of 3 x 860 g a.s./ha, the total radioactive residue (TRR) was 0.290 mg/kg in green tomatoes and 0.497 mg/kg in red tomatoes.

The extractability of radioactive residues was very similar between red and green tomatoes, with 92-94 %TRR being readily solvent extractable and 6-8% TRR remaining in the post-extraction solids.

Parent RH-7281 was the only significant component of the residue, accounting for 0.139 mg/kg, 48% TRR in green tomatoes and 0.219 mg/kg, 44% TRR in red tomatoes. The metabolites RH-141452, RH-141288, RH-24549 and RH-127450 were identified as minor components of the residue (up to 3% TRR) in the ethyl acetate soluble fraction. The n-butanol soluble fraction was comprised numerous polar metabolites which included three components that were identified as glucose and aspartic acid conjugates.

The degradation pathway in tomatoes proceeds via photolysis, followed by oxidation/hydrolysis and conjugation with sugars and amino acids.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material Zoxamide (RH-7281)

Description: [^{14}C]-RH-7281
[^{13}C]-RH-7281

Lot/batch No.: 912.0103 (^{14}C-label)
CLM 3762/P6909 (^{13}C-label)

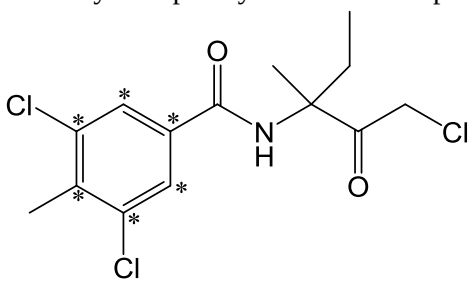
Purity: Radiochemical purity: 98.5% (^{14}C-label)
Chemical purity: 96.8% (^{13}C-label)

Specific activity: 40.36 µCi/mg (1493 kBq/mg) (^{14}C-label)

CAS No.: 156052-68-5

Development Code: RH-7281

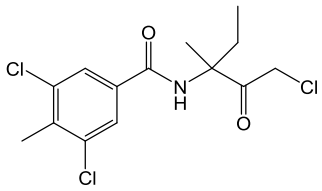
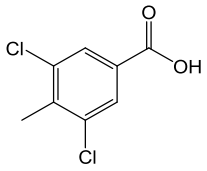
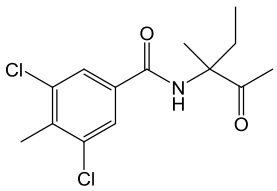
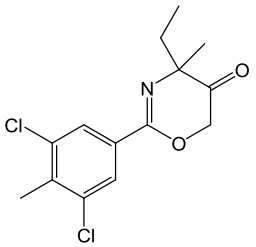
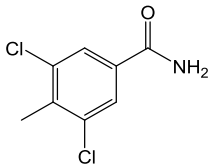
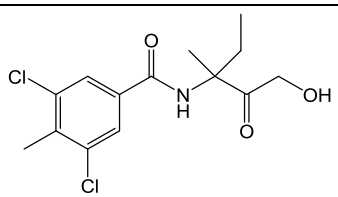
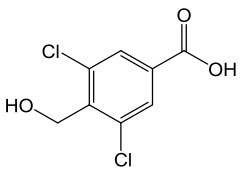
Position of Radiolabel: Uniformly isotopically labelled in the phenyl ring

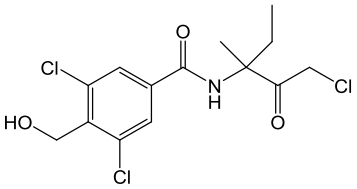
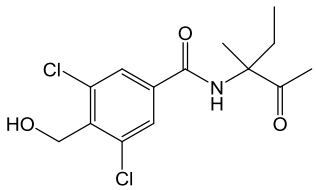
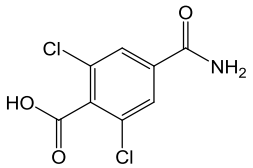
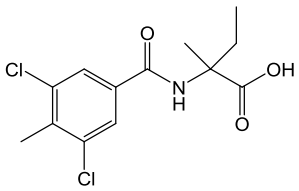
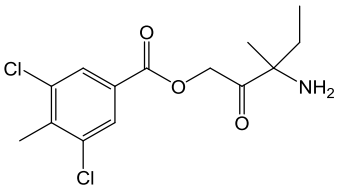
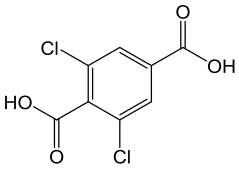


*position of ^{14}C and ^{13}C labels

The purity and structures of the key reference materials used in this study are given in Table B.7.2.1.1-7 below.

Table B.7.2.1.1-7 Key reference materials used in the identification of metabolites

Reference Standard Name/code(s)	Structure	Batch Number Purity
RH-117281 RH-7281 Zoxamide		T66246D 97.35%
RH-24549 RH-4549		HLR2-18 97.06%
RH-127450 RH-7450		RWS6-8 98.9%
RH-129151 RH-9151		RWS7-6 99.14%
RH-139432		LJG7-39A 99.69%
RH-141288 RH-1288		TAM76:34 98.41%
RH-141452 RH-1452		ELM-1771 99.7%

Reference Standard Name/code(s)	Structure	Batch Number Purity
RH-141453 RH-1453		TAM76:42 94.03%
RH-141454 RH-1454		TAM76:45 90.96%
RH-149736		ELM-1774 94.99%
RH-141643 RH-1643		RPO102:69B 98.41%
RH-150721		LMB-4422 95.53%
RH-141455 RH-1455		ELM-1775 94.5%

2. Test Commodity

Crop: Tomato
Type: Not applicable
Variety: Celebrity
Botanical Name: *Solanum lycopersicum*
Crop Part or Processed Commodity: Ripe fruit, green fruit, foliage
Sample Size: 99-660 g (ripe fruit), 1080-1312 g (green fruit), 3360-3484 g (foliage)

3. Soil

Type: Loamy sand

Table B.7.2.1.1-8 Soil physicochemical properties

Texture	Loamy sand

Sand	86 %
Silt	10 %
Clay	4 %
CEC	2.7 meq/100g
pH	6.2
Organic matter	0.7 %
1/3 Bar moisture	6.1 %
Disturbed bulk density	1.58 g/cm^3

B. STUDY DESIGN

1. Test Procedure

The field phase was carried out in 1998 at Grayson Research in North Carolina, USA.

The metabolism of zoxamide (RH-7281) was studied in tomato plants. Tomato plants were grown from seed, and transplanted into the test plots. The control and treated plots each consisted of 4 tomato plants in a plastic lined aluminium container (2 feet wide, 8 feet long, 2 feet deep) of loamy sand soil, each located in a separate greenhouse cell. Tomato plants were staked and tied, and pollinated with a hand pollinator. Plants were fertilized with Osmocote^{TM} timed release fertiliser and watered as needed. Calcium chloride was applied three times to try to control blossom end rot of tomato fruit.

^{14}C-RH-7281 was isotopically diluted with ^{13}C-RH-7281 to a final specific activity of 20.18 µCi/mg (747 kBq/mg). This was formulated as a 6% EC formulation and applied as a foliar spray application to tomato plants at an application rate of 3 x 860 g as/ha (3 x 0.77 lb/Acre), corresponding to a total application rate of 2580 g as/ha (2.3 lb/Acre). The first application was made shortly after the appearance of first fruit, with 18-19 days between each application. The water volume was 470 L/ha (50 gallons per acre). A second control tomato plot was not treated. Plants were maintained in a greenhouse.

The stability and purity of the formulated radiolabelled test substance was confirmed at the time of each application.

2. Sampling

Tomatoes that were ripe after the 2nd application were harvested to prevent them rotting on the plant. These ripe tomato samples were retained but not analysed.

Green (unripe) tomato fruit, red (ripe) tomato fruit and foliage were harvested 1 day after the final application, at BBCH 89. Yields of ripe tomatoes were reduced by blossom end rot, and ripe tomatoes with blossom end rot were retained as a separate sample but not analysed. Green and red tomatoes were sampled by hand. As the quantity of disease-free ripe red tomatoes was low at harvest (5-6 fruits, 331g), both red and green tomatoes were retained as separate samples, thus ensuring sufficient material was available for analysis. Foliage was sampled by cutting the tomato plant stems above the soil line. Samples were weighed and frozen within 20 minutes of sampling.

The green tomatoes and ripe tomatoes were quartered and then homogenised with dry ice. The frozen foliage was broken into small pieces and then homogenised with dry ice. Aliquots of the homogenised samples were combusted and analysed by LSC for the determination of the total radioactive residue (TRR).

3. Extraction and analysis

Sub-samples of homogenised green (unripe) and red (ripe) tomato fruit were extracted by homogenisation with acetonitrile containing 1% formic acid, and then twice with acetonitrile/water (80:20 v/v) containing 1% formic acid. After each extraction, the extract was centrifuged, and the supernatant collected. The combined acetonitrile/water extract was rotary evaporated to an aqueous remainder and then partitioned three times against ethyl acetate. The ethyl acetate fractions were combined and concentrated to a small volume. The aqueous fraction was acidified with concentrated hydrochloric acid, filtered and then partitioned three times against n-butanol. The n-butanol fractions were combined and concentrated to a small volume.

The residual aqueous fraction from green tomato was filtered through a filter paper, and then passed through an Amicon ultrafiltration disc with 1000 molecular weight cut-off. No significant residue was retained by the ultrafiltration disc. The aqueous filtrate was applied to a C18 filter disc and the retained activity eluted with acetonitrile. Analysis of the acetonitrile fractions showed that no significant radioactivity activity was retained by the C18 disc, and the aqueous eluent was not analysed further. The residual aqueous fraction from red tomato was not analysed further as it was assumed to behave similarly to the green tomato aqueous fraction.

A sub-sample of homogenised tomato foliage was extracted by blending with methanol/water (9:1 v/v). The extract was centrifuged and the supernatant rotary-evaporated to an aqueous remainder. The aqueous remainder was acidified with concentrated hydrochloric acid and partitioned three times with ethyl acetate and then three times with n-butanol. The combined ethyl acetate extract and combined n-butanol extract were each concentrated to a small volume. The n-butanol extract was used for comparison with the polar metabolites found in the red tomato n-butanol extract.

The tomato and foliage n-butanol extracts were subjected to acid, base and enzyme hydrolysis experiments as follows.

An aliquot of the n-butanol extract was mixed with methanol/water (9:1 v/v) and concentrated hydrochloric acid and heated at 60°C for 3-4 hours. After cooling, the solvent was evaporated and the residue redissolved in acetonitrile/water (9:1 v/v) for LSC, TLC and/or HPLC analysis.

An aliquot of the n-butanol extract was mixed with methanol/water (9:1 v/v) and 1N KOH and heated at 60°C for 3-4 hours. After cooling, the extract was acidified with concentrated hydrochloric acid, the solvent was evaporated and the residue redissolved in acetonitrile/water (9:1 v/v) for LSC, TLC and/or HPLC analysis.

An aliquot of the n-butanol extract in methanol/water (9:1 v/v) was mixed with milli-Q water and Cellulase in KH_2PO_4 buffer (pH 5) added. The resulting solution was incubated in a shaking water bath at 37°C for 12-36 hours. The reaction mixture was then acidifed to pH 1 with concentrated hydrochloric acid, and partitioned sequentially twice with ethyl acetate and twice with n-butanol. The organic extracts were concentrated to a small volume for analysis.

Aliquots of each extract were analysed by LSC. The post-extraction solids (PES) were analysed for radioactivity by combustion and LSC.

The red and green tomato ethyl acetate and n-butanol extracts were analysed by reverse-phase HPLC (LC-18DB) with UV and radiochemical detection, or fraction collection and LSC for radiochemical detection. Ethyl acetate extracts were also analysed by normal phase TLC, however TLC analysis did not resolve the components in the n-butanol extracts. Metabolite identification was performed by co-chromatography against reference standards and by GC-MS and LC-MS/MS analysis.

4. Radiovalidation of the residue analytical method

A sub-sample of the treated red tomato sample was extracted following the procedure described in 'Residue Analytical Method for RH-7281 in Tomatoes'. This method uses an acetonitrile extraction followed by analysis by GC-ECD.

II. RESULTS AND DISCUSSION

A. TOTAL RADIOACTIVE RESIDUES (TRR)

One day after the first application, the treated plants showed symptoms of phytotoxicity, namely leaf burning mostly to the veins of older leaves and severe burning of the top leaves. Phytotoxicity was most evident at 3 days after the first application. Some additional burning of the top leaves occurred after the second application. There was no obvious phytotoxicity one day after the last application.

The total radioactive residues (TRR) determined by combustion of red and green tomatoes were 0.474 mg/kg and 0.263 mg/kg, respectively. The standard deviations in the TRRs obtained by combustion were high, and this variability is attributed to the small sample size and high water content of the crop. Larger sample sizes were therefore used for the determination of the total radioactive residue (TRR) by extraction and combustion of the debris, and using this method the TRRs were 0.497 mg/kg and 0.290 mg/kg, for red and green tomatoes respectively. This is summarised in Table B.7.2.1.1-9 below.

Table B.7.2.1.1-9 Summary of TRR in tomato fruit

Crop part	Total radioactive residue (TRR) by combustion (mg/kg)	Total radioactive residue (TRR) by extraction (mg/kg)
Red (ripe) tomatoes	0.474	0.497
Green (unripe) tomatoes	0.263	0.290

B. EXTRACTION AND CHARACTERISATION OF RESIDUES

1. Extraction and characterisation of residues

The distribution of the radioactive residues in the solvent extracts and post-extraction solids of the tomato fruit is shown in Table B.7.2.1.1-10. The extractability of radioactive residues was very similar between red and green tomatoes.

Extraction of the fruit with acidified acetonitrile and acidified acetonitrile/water released 92-94% TRR from red and green tomatoes, with 6-8% TRR remaining in the post-extraction solids. Partitioning of the acetonitrile/water extracts showed that 68-75% TRR was organo-soluble in the ethyl acetate fraction, 15-22% TRR was organo-soluble in the n-butanol fraction, and 2-4% was water soluble.

The tomato fruit ethyl acetate and n-butanol fractions were subjected to chromatographic analysis. The water-soluble fraction was characterised by ultrafiltration and solid phase extraction (SPE). The water soluble residue was shown to comprise small (molecular weight <1000), very polar molecules that were not retained by C-18 SPE. No further analysis was performed on the post-extraction solids due to the small amount of sample remaining.

The foliage was extracted with methanol/water. The TRR value for foliage was not determined, but the residue extracted with methanol/water is calculated to be 28.08 mg/kg. Partitioning of the foliage methanol/water extract showed that 96.5% of the radioactivity in this extract was organo-soluble in the ethyl acetate fraction, 7.8% was organo-soluble in the n-butanol fraction, and 0.3% was water soluble.

Table B.7.2.1.1-10 Distribution of radioactive residues in tomato fruit

Compound	^{14}C-Residues in Green Tomatoes		^{14}C-Residues in Red Tomatoes	
	mg/kg	% TRR	mg/kg	% TRR
Extractable ($MeCN/H_2O$)[1]	0.273	94.04	0.457	91.95
Liquid-liquid partition of primary $MeCN/H_2O$ extract				
Volatile residues	*0.000*	*0.0*	*0.000*	*0.0*
EtOAc fraction	*0.218*	*75.20*	*0.339*	*68.13*
n-BuOH fraction	*0.044*	*15.15*	*0.107*	*21.57*
Aqueous fraction	*0.011*	*3.70*	*0.011*	*2.25*
Unextracted residue	0.017	5.96	0.040	8.05
Total[2]	0.290	100	0.497	100

[1] Combined acetonitrile (with 1% formic acid) and acetonitrile/water (with 1% formic acid) extracts
[2]TRR values from extraction and combustion of the debris

2. Identification of metabolites

The ethyl acetate and n-butanol extracts of red and green tomatoes were profiled by radio-HPLC and TLC (see Table B.7.2.1.1-11).

In both red and green tomatoes, parent RH-7281 was the largest component of the residue, comprising 0.139 mg/kg, 48% TRR in green tomatoes and 0.219 mg/kg, 44% TRR in red tomatoes. The remainder of the residue was made up of small amounts of a large number of highly degraded or polar metabolites. No individual metabolite exceeded 3% TRR. The metabolites RH-24549, RH141288 and RH-127450 were identified in the ethyl acetate extracts by co-chromatography with authentic reference standards.

Nearly 15-22% TRR was polar compounds in the n-butanol extracts, and an additional 12-15% TRR was polar material in the ethyl acetate extracts. The polar material in the ethyl acetate extracts contained some free RH-141452 (approximately 3% TRR) and several other components. Chromatographic analysis of the n-butanol extract showed that it contained numerous low level metabolites, the largest of which represented only 1-3% TRR. The most abundant polar metabolite was glucose conjugate-1 (0.02 mg/kg, 2.5% TRR) in red tomato. Two additional conjugates (glucose conjugate-2 and aspartic acid conjugate-3) were also found, each accounting for approximately 2% TRR.

The n-butanol extracts were subjected to acid, base and enzyme hydrolysis to characterise the residue. In red tomato, hydrolysis of the n-butanol extract with strong base released only one major identifiable aglycone, RH-141452 (0.03 mg/kg, 6% TRR). In green tomato, hydrolysis of the n-butanol extract with strong base released RH-141452, RH-24549 and another material more polar than RH-141452. Acid and enzyme hydrolysis did not provide any further information on the nature of the conjugates or aglycones. Almost every fraction listed in the table below was found to be multi-component.

Table B.7.2.1.1-11 Identification of metabolites in tomato fruit

Chromatography fraction	Compound	^{14}C-Residues in Green Tomatoes		^{14}C-Residues in Red Tomatoes	
		mg/kg	% TRR	mg/kg	% TRR
Quantitation data for EtOAc fraction					
Fraction 2	RH-141452[1]	0.044	15.05[1]	0.056	11.19[1]
Fraction 3	RH-141288[1]	0.020	6.82[1]	0.014	2.74[1]
Fraction 4	RH-24549	0.007	2.49	0.004	0.84
Fraction 5	RH-127450	0.003	1.10	0.007	1.36
Fraction 6	RH-7281 (zoxamide)	0.139	48.03	0.219	43.96
All other fractions		0.005	1.71	0.039	8.05
Quantitation data for n-BuOH fraction					
Fraction 3		0.003	1.15	0.028	5.61
Fraction 4	Glucose conjugate-2[2]	0.011	3.63	0.050	10.01[4]
Fraction 5	Glucose conjugate-1[3]	0.008	2.86		
Fraction 6	Aspartic acid conjugate-3[5]	0.003	1.07	0.009	1.79
Fraction 7		0.005	1.81	0.009	1.89
Fraction 8		0.004	1.46	0.005	0.94
Fraction 9		0.009	3.16	0.003	0.66

[1] Compound identified represented only a portion (~50%) of the activity in these fractions
[2] The glucose conjugate of RH-141452 accounted for a part of the residue in this fraction
[3] Glucose conjugate-1 accounted for a part of the residue in this fraction
[4] For red tomato, fractions 4 and 5 are shown together as 10% TRR, and they contained both conjugates-1 and -2.
[5] Aspartic acid conjugate-3 accounted for a portion of this fraction

3. Storage stability of residues

Samples were initially extracted and profiled by TLC within 3 months of harvest, and showed that the major component of the residue was parent RH-7281, with some polar radioactivity near the origin. RH-7281 can degrade to polar components, however the nature of the RH-7281 degradates is different to the polar components found in the tomato extracts, and therefore the polar residue formed in tomato fruits by metabolism of RH-7281 is not due to decomposition of RH-7281 during storage. The duration of storage of samples during the study therefore did not impact on the conclusions drawn in the study.

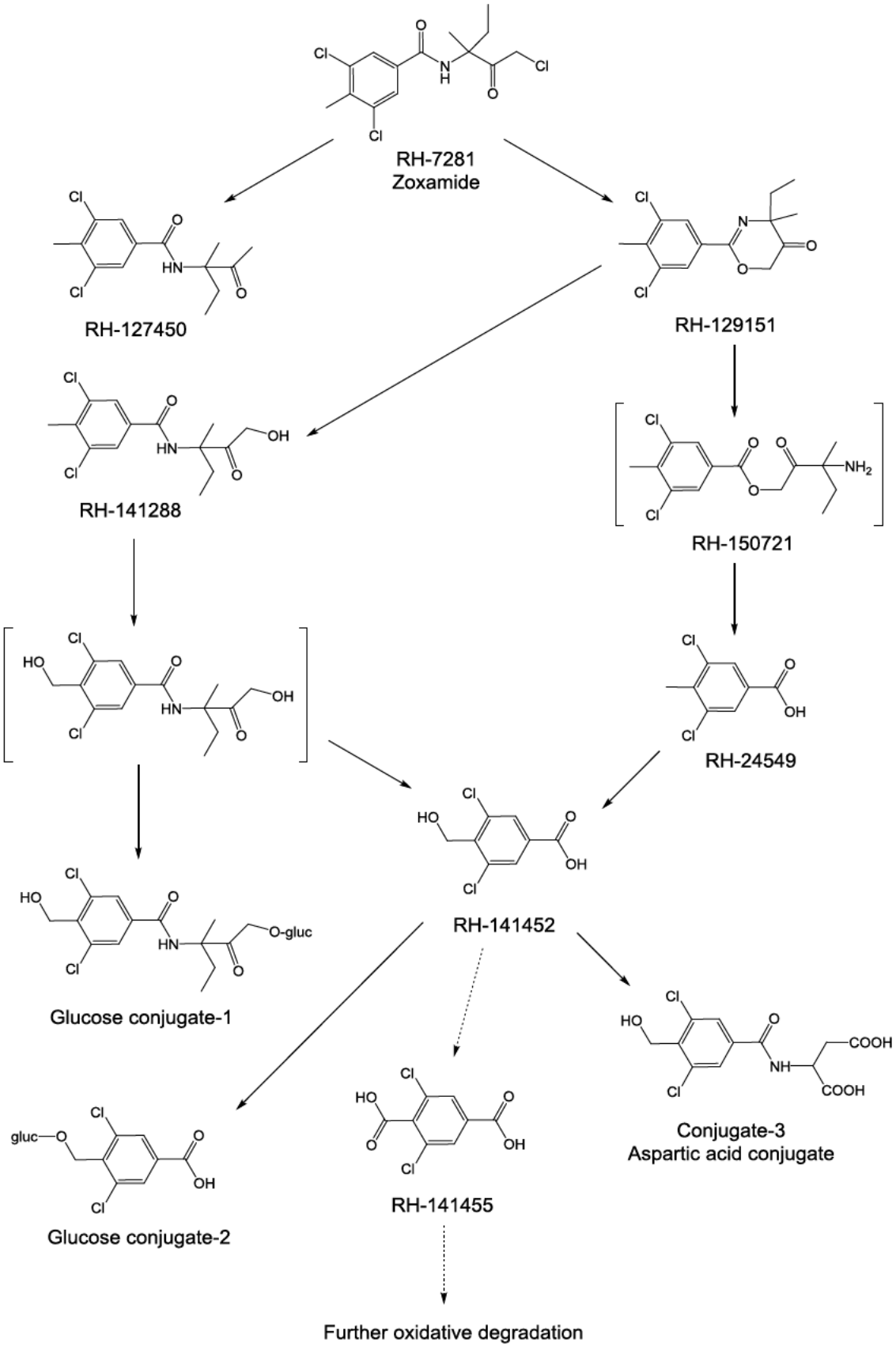
4. Radiovalidation

There is a residue analytical method for tomatoes that employs an acetonitrile extraction and analysis by GC-ECD. Analysis of a tomato red fruit sample using this method gave a recovery of RH-7281 of ~90% of the value obtained in the metabolism study, based on uncorrected recovery values.

5. Proposed metabolic pathway

The degradation pathway of RH-7281 in tomatoes proceeds via hydrolysis and photolysis. Photolytic dechlorination resulted in the formation of RH-127450, and cyclisation to form RH-129151. Subsequent hydrolysis and/or oxidisation formed RH-141288, RH-141452 and RH-24549. The resulting carboxylic acid metabolites were conjugated with sugars or amino acids. A metabolic pathway is proposed as follows:

Figure B.7.2.1.1-12 Proposed metabolic pathway for zoxamide in tomato



III. CONCLUSION

After foliar application of [^{14}C]-RH-7281 to tomato plants at a 1 day PHI at a nominal rate of 3 x 860 g a.s./ha, the total radioactive residue (TRR) was 0.290 mg/kg in green tomatoes and 0.497 mg/kg in red tomatoes.

The majority of the residue in tomatoes was readily solvent extractable and comprised mainly parent RH-7281, accounting for 0.139 mg/kg, 48% TRR in green tomatoes and 0.219 mg/kg, 44% TRR in red tomatoes.

The metabolites RH-141452, RH-141288, RH-24549 and RH-127450 were identified as minor components of the residue (up to 3% TRR), along with numerous polar metabolites, including two glucose conjugates and an aspartic acid conjugate.

Cucumbers

Reference:	CA 6.2.1/07 Sharma, A.K. (1999b) RH-117,281: Nature of Residue in cucurbits (Cucumber) Report no.: 34-99-57
Guideline(s):	US EPA Guideline OPPTS 860.1300
Deviations:	None
GLP:	Yes
Validity of the study:	Valid
Previous evaluations:	No; Submitted for the purpose of renewal of a.s. approval

Executive Summary

After foliar application of [^{14}C]-RH-7281 to cucumber plants at a 1 day PHI at a nominal rate of 3 x 1344 g a.s./ha, the total radioactive residue (TRR) was 1.53 mg/kg in cucumber fruit and 108.7 mg/kg in foliage.

The residue in cucumber fruit and foliage was readily solvent extractable and comprised mainly parent RH-7281, accounting for 1.327 mg/kg, 87% TRR in cucumber fruit and 99.6 mg/kg, 92% TRR in foliage.

In cucumber fruit, the metabolites RH-127450, RH-141288, RH-150721 and RH-141452 were identified as minor components of the residue (up to 4.8% TRR).

The degradation pathway in cucumbers proceeds via hydrolysis and photolysis, with the initial degradation products being transformed by subsequent oxidation/hydrolysis steps.

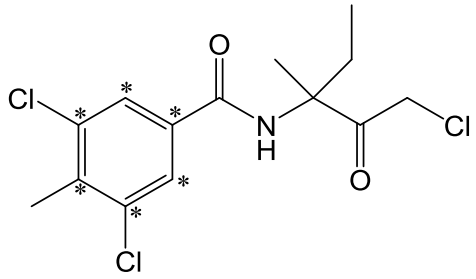
I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Description:	Zoxamide (RH-7281) [^{14}C]-RH-7281 [^{13}C]-RH-7281
Lot/batch No.:	942.0110 (^{14}C-label) CLM 3762/P6909 (^{13}C-label)
Purity:	Radiochemical purity: >95% (^{14}C-label)

Chemical purity: 96.8% (^{13}C-label)
Specific activity: 35.48 µCi/mg (1313 kBq/mg) (^{14}C-label)
CAS No.: 156052-68-5
Development Code: RH-7281
Position of Radiolabel: Uniformly isotopically labelled in the phenyl ring



*position of ^{14}C and ^{13}C labels

The key reference materials used in this study are the same as those used in the tomato metabolism study. Refer to point 6.2.1.2, Table 6.2.1.2/1 for the purity and structures of the reference materials.

2. Test Commodity
Crop: Cucumber
Type: Not applicable
Variety: Bush Champion
Botanical Name: *Cucumis sativus*
Crop Part or Processed Commodity: Cucumber fruit, foliage
Sample Size: 3.93-5.04 kg (fruit), 2.65-2.70 kg (foliage)

3. Soil
Type: Sandy loam

Table B.7.2.1.1-13 Soil physicochemical properties

Texture	Sandy Loam
Sand	74 %
Silt	12 %
Clay	14 %
CEC	8.2 meq/100g
pH	6.5
Organic matter	4.3 %
1/3 Bar moisture	19.0 %
Disturbed bulk density	1.12 g/cm^3

B. STUDY DESIGN

1. Test Procedure

The field phase was carried out in 1998-1999 at Grayson Research in North Carolina, USA.

The metabolism of zoxamide (RH-7281) was studied in cucumber plants. Cucumber plants, grown from seed by Rohm and Haas Co., were transplanted into the test plots. The control and treated plots each consisted of 4 cucumber plants in a plastic lined aluminium container (2 feet wide, 8 feet long, 2 feet deep) of sandy loam soil. Test plots were maintained in a greenhouse, and were located in separate greenhouse cells after test substance application. Plants were fertilized with a 10-10-10 (NPK) fertiliser, and watered and pollinated as needed. Systhane, Nova and Bravo were applied for the control of powdery mildew, and Diazinon was used for the control of aphids.

^{14}C-RH-7281 was isotopically diluted with ^{13}C-RH-7281 to a final specific activity of 17.691 µCi/mg (655 kBq/mg). This was formulated as a 2F formulation (containing approximately 6% a.s.) and applied as a foliar spray application to cucumber plants at an application rate of 3 x 1344 g as/ha (3 x 1.2 lb/Acre), corresponding to a total application rate of 4030 g as/ha (3.6 lb/Acre), with 7 days between each application. The water volume was 465 L/ha (49.7 gallons per acre). A second control cucumber plot was not treated. Plants were maintained in a greenhouse.

The stability and purity of the formulated radiolabelled test substance was confirmed at the time of each application.

2. Sampling

Cucumber fruit and foliage (entire plant except fruit) were harvested 1 day after the final application. Small, immature cucumber fruits were collected as a separate sample from the mature fruit, and were not analysed further. Samples were weighed and frozen within 5 minutes of sampling.

The cucumbers were partially defrosted, sliced and then homogenised with dry ice. The frozen foliage was broken into small pieces and then homogenised with dry ice. Aliquots of the homogenised samples were combusted and analysed by LSC for the determination of the total radioactive residue (TRR).

3. Extraction and analysis

Sub-samples of homogenised fruit and foliage were extracted twice by homogenisation with acetonitrile, and then once with acetonitrile/water (9:1 v/v). After each extraction, the extract was centrifuged, and the supernatant collected. The combined acetonitrile/water extract was rotary evaporated to an aqueous remainder and then partitioned three times against ethyl acetate. The ethyl acetate fractions were combined and concentrated to a small volume, to give an ethyl acetate extract and an aqueous extract.

Aliquots of each extract were analysed by LSC. The post-extraction solids (PES) were analysed for radioactivity by combustion and LSC.

The ethyl acetate extracts were analysed by reverse-phase HPLC (LC-18DB) with UV and radiochemical detection, or fraction collection and LSC for radiochemical detection. Metabolite identification was performed by HPLC and TLC with co-chromatography against reference standards and by GC-MS and LC-MS/MS analysis.

4. Radiovalidation of the residue analytical method

Sub-samples of the treated cucumber fruit sample were extracted following the procedure described in 'Residue Analytical Method for RH-7281 in Cucumbers'. This method uses an acetonitrile extraction followed by analysis by GC-ECD.

II. RESULTS AND DISCUSSION

A. TOTAL RADIOACTIVE RESIDUES (TRR)

No phytotoxicity was observed on the cucumber foliage or fruit following application of the 2F formulation of RH-7281.

The average total radioactive residues (TRR) determined by combustion were 1.530 mg/kg for cucumber fruit and 108 mg/kg for foliage. The standard deviations in the TRRs obtained by combustion were high, as summarised in Table B.7.2.1.1-14 below.

Table B.7.2.1.1-14 Summary of TRR in cucumber

Crop part	Total radioactive residue (TRR) by combustion (mg/kg)
Cucumber fruit	1.530 ± 0.703 (n=8)
Cucumber foliage	108 ± 139 (n=6)

B. EXTRACTION AND CHARACTERISATION OF RESIDUES

1. Extraction and characterisation of residues

The distribution of the radioactive residues in the solvent extracts and post-extraction solids of the tomato fruit is shown in Table B.7.2.1.1-15. The radioactive residues were completely solubilised by acetonitrile/water extraction, with no radioactivity remaining in the post-extraction solids.

Partitioning of the cucumber fruit acetonitrile/water extracts showed that 97.4% TRR was organo-soluble in the ethyl acetate fraction and 2.6% was water soluble. As the residue in the aqueous fraction was low, no further analysis was performed on the aqueous extract.

Partitioning of the cucumber foliage acetonitrile/water extracts gave similar results, showing that 98.9% TRR was organo-soluble in the ethyl acetate fraction and 1.2% was water soluble.

The ethyl acetate fractions were subjected to chromatographic analysis. As the % TRR values in the aqueous fractions were low, no further analysis was performed on the aqueous extracts.

Table B.7.2.1.1-15 Distribution of radioactive residues in cucumber fruit and foliage

Compound	^{14}C-Residues in Cucumber Fruit		^{14}C-Residues in Cucumber Foliage	
	mg/kg	% TRR	mg/kg	% TRR
Extractable ($MeCN/H_2O$)[1]	1.530	100	108.7	100
Liquid-liquid partition of primary $MeCN/H_2O$ extract				
Volatile residues	*0.000*	*0.0*	*0.000*	*0.0*
EtOAc fraction	*1.490*	*97.4*	*106.8*	*98.9*
Aqueous fraction	*0.040*	*2.60*	*1.244*	*1.2*
Unextracted residue	0.000	0	0.000	0
Total	1.530	100	108.7	100

[1] Combined acetonitrile and acetonitrile/water extracts

2. Identification of metabolites

The ethyl acetate extracts of cucumber fruit and foliage were profiled by radio-HPLC and TLC (see Table B.7.2.1.1-16).

In both fruit and foliage, parent RH-7281 was the main component of the residue, comprising 1.327 mg/kg, 86.7% TRR in fruit and 99.6 mg/kg, 92.2% TRR in foliage. The identity of RH-7281 was confirmed by LC/MS.

In cucumber fruit ethyl acetate extracts, the remainder of the residue was made up of a number of minor metabolites. No individual metabolite fraction exceeded 4.8% TRR. The metabolites RH-150721, RH-141452 and RH-127450 were identified by HPLC and the identity confirmed by LC/MS, and RH-141288 was identified by HPLC and confirmed by TLC against the reference standard. RH-141453 was also identified, however this was also found in the test material and is therefore not a metabolite of RH-7281.

A similar pattern of minor metabolites was also seen in cucumber foliage ethyl acetate extracts, with RH-129151, RH-139432 and RH-24549 also being identified in addition to the metabolites found in the fruit.

Table B.7.2.1.1-16 Identification of metabolites in cucumber

Compound	^{14}C-Residues in Cucumber Fruit		^{14}C-Residues in Cucumber Foliage	
	mg/kg	% TRR	mg/kg	% TRR
RH-150721/RH-141452[1]	0.073	4.80	0.209	0.19
RH-141453[2]	0.018	1.16	0	0
RH-139432	0	0	1.007	0.93
RH-141288	0.006	0.41	0.467	0.43
RH-24549	0	0	0.301	0.28
RH-119231[1]	0.007	0.47	0	0
RH-127450	0.030	1.96	1.627	1.51
RH-7281 (zoxamide)[3]	1.327	86.74	99.623	92.24
RH-129151	0	0	1.771	1.64
All other fractions	0.029	1.87	1.753	1.62
Total	1.490	97.40	106.758	98.85

[1] The identified components represented only a portion of the activity in this fraction
[2] RH-141453 was present in the test material used at ~1%, and therefore this is not a metabolite
[3] Some RH-7281 transformed into RH-129151 and RH-141288 during identification

3. Storage stability of residues

All analysis was completed within 2 months of harvest. Analysis of the fruit ethyl acetate extract at the end of the analytical phase showed no change from the initial analysis. The nature of the residues was therefore shown not to have been impacted by the period of storage of the samples.

4. Radiovalidation

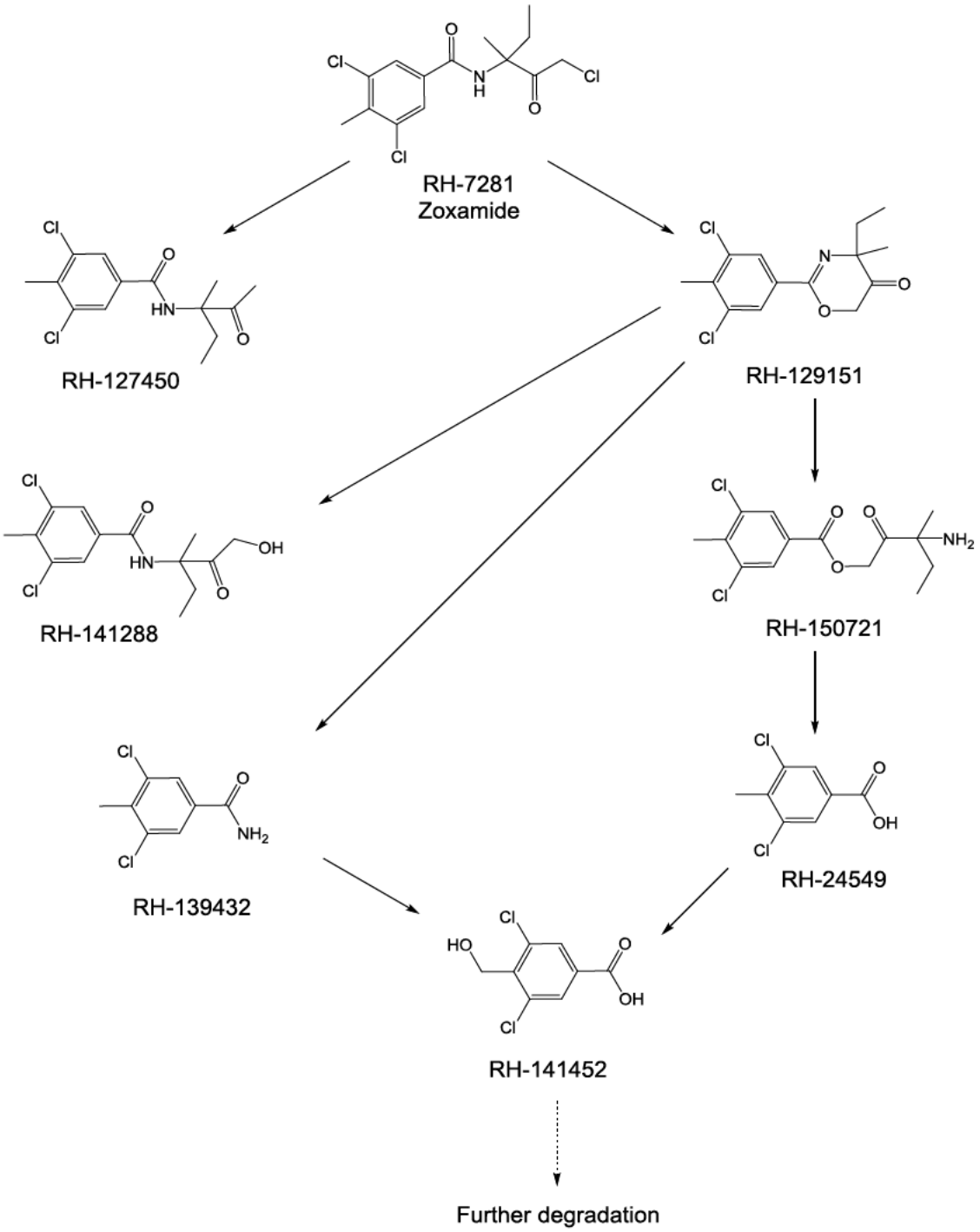
There is a residue analytical method for cucumbers that employs an acetonitrile extraction and analysis by GC-ECD. Analysis of a cucumber fruit sample using this method gave a recovery of RH-7281 of 1.28 mg/kg, which is comparable to the value obtained from the metabolism study by HPLC of 1.33 mg/kg.

5. Proposed metabolic pathway

RH-7281 is of low systemicity, and most of the residue stays on the plant surface. RH-7281 did not readily degrade in/on cucumber, and the majority of the residue was parent RH-7281.

The degradation pathway of RH-7281 in cucumbers proceeds via hydrolysis and photolysis. Photolytic dechlorination resulted in the formation of RH-127450, and cyclisation to form RH-129151. Subsequent hydrolysis and/or oxidisation formed RH-141288, RH-139432, RH-150721, RH-24549 and RH-141452. A metabolic pathway is proposed as follows:

Figure B.7.2.1.1-17 Proposed metabolic pathway for zoxamide in cucumber



III. CONCLUSION

After foliar application of [^{14}C]-RH-7281 to cucumber plants at a 1 day PHI at a nominal rate of 3 x 1344 g a.s./ha, the total radioactive residue (TRR) was 1.53 mg/kg in cucumber fruit and 108.7 mg/kg in foliage.

The residue in cucumbers was readily solvent extractable and comprised mainly parent RH-7281, accounting for 1.327 mg/kg, 87% TRR in cucumber fruit and 99.6 mg/kg, 92% TRR in foliage.

In cucumber fruit, the metabolites RH-127450, RH-141288, RH-150721 and RH-141452 were identified as minor components of the residue (up to 4.8% TRR).

B.7.2.1.2 Root and tuber vegetables

Potato

Reference:	CA 6.1/02 Reibach, PH and Spencer, WO, ^{14}C-RH-117,281: Nature of Residue in Potatoe, 1998 ER ref. No. 14.3
Guideline(s):	US EPA 40 CFR 157.3.240: Residue Chemistry Subdivision O, series 171-4 (a) and FAO Guidelines GIFAP C/91 TD/23217 April 1991
Deviations:	None
GLP:	Yes
Validity of the study:	Valid
Previous evaluations:	In DAR (2002)

In a 1995 study, white potatoes (variety Kennebec) grown in North Carolina USA were treated with ^{14}C phenyl labelled RH-7281 (Radiochemicalpurity 94.8%). Three foliar broadcast applications were made at the rate of 0.9 kg a.s./ha per application (6N), to give a total seasonal use rate of 2.7 kg. a.s./ha (1.8N for the season). The first application was made at 39 days after planting. The second and the third applications were made at 21 and 17-day intervals respectively. ^{14}C RH-7281 treated plants were grown in elliptical galvanised steel tanks containing loamy sand soil.

The test substance was formulated as a 5% w/w active substance (a.s.) emulsifiable concentrate (EC) and applied as an aqueous suspension. Samples of spray solutions were analysed using LSC to confirm the amount of ^{14}C applied and the stability of the spray solution.

There was transient and insignificant potato leaf injury caused by the EC formulation used but the Applicant stated that this did not affect the yield of the potatoes.

The harvest of mature potato tubers was at 14 days after the last spray. The potato tubers were hand dug. Diseased, damaged, or immature tubers were discarded. Freshly dug tubers were washed lightly to remove any soil and allowed to dry. The tubers were diced into cubes, weighed, and stored frozen until required for analysis.

Potato samples were prepared for analysis by cryogenic milling. The total 14C activity was determined by LSC following combustion. Based on the specific activity of the RH- 7281 used for treatment, the total radioactive residues (TRR) at harvest were determined to be 0.178 mg/kg for the homogenised potato tubers. The final harvest potato samples were analyzed to determine the nature of the residues present.

The potato samples were extracted with methanol and the methanol removed by rotary evaporation. The residue was re-dissolved in ethyl acetate and partitioned with water. The aqueous fraction was acidified with 6N hydrochloric acid and partitioned with ethyl acetate. Following acidification, the aqueous

fraction was further fractionated by sequential elution from a C-18 solid phase extraction cartridge with methanol and water.

The total recovery of each analytical procedure and the distribution of TRR in each fraction were determined via liquid scintillation counting or combustion radio-assay. The results of this analysis are presented in tables B.7.2.1.2-1/3

Table B.7.2.1.2-1 Radioactive residues extracted with methanol

	%Total	mg/kg
Total methanol extract	72.4	0.129
Post extraction solids	31.1	0.055
Total recovered	103.5	0.184

Table B.7.2.1.2-2 Fractionation of methanol extract

	%Total	mg/kg
Total extract	72.4	0.129
Ethyl acetate fraction	47.9	0.085
Water fraction	22.7	0.040

Table B. 7.2.1.2-3 Fractionation of water fraction using C18 column

	%Total	mg/kg
Eluted by methanol	14.3	0.025
Eluted by water	7.6	0.013

The post extraction solids (PES) resulting from the initial methanol extraction were hydrolysed using amyloglucosidase, a starch degrading enzyme. The hydrolysis procedure released an additional 25.1% of the TRR, resulting in a second PES that contained only 6% of the TRR. This second PES was not fractionated further. Following acidification, the hydrolysed fraction was further fractionated by means of a C-18 solid phase extraction cartridge. This procedure resulted in a fraction which was eluted with methanol containing 3.7% of the TRR, and an aqueous fraction containing 15.9% of the TRR.

The ethyl acetate fraction, the C-18 methanol fraction and the aqueous fraction were concentrated via rotary evaporation and compared to reference standards using normal phase TLC. The ethyl acetate fraction was first analysed by TLC using chloroform/methanol/acetic acid followed by development in a second system containing butanol/methanol/water/ acetic acid. Standards of RH-7281, RH-141452, RH-141288, RH-24549, RH-129432 and RH-141,455 were co-spotted with the sample and also spotted along the origin. The ethyl acetate

fraction was further analysed by two dimensional TLC using chloroform/ methanol/acetic acid followed by butanol/methanol/water/ acetic acid as solvent systems. No parent RH-7281 was found. RH-141,452 and RH-141,455 were identified as the major components of the residue. Further confirmation was obtained using reverse phase HPLC using a C-18 column, UV detection and acetonitrile, water and phosphoric acid as solvent.

RH-141,452 and RH-141,455 were isolated from the ethyl acetate fraction using preparative TLC (butanol/methanol/water/ acetic acid as solvent). The identity was confirmed by comparison of the isolated samples with non-radiolabelled standards using TLC. Structures proposed for the two major isolated metabolites were confirmed by mass spectrometry following reaction with diazomethane to convert any carboxyl groups to methyl esters.

The aqueous fractions contained a metabolite that may have been RH-141,455 as judged by the similar Rf value, but due the polar nature of the fraction, the results were not conclusive. Since this fraction contained only 7.6% of the TRR (≈0.01 mg/kg), further analysis was not pursued.

The methanol and aqueous fractions obtained folowing hydrolysis of the initial PES with amyloglucosidase were also analyzed by TLC. The results of these analyses suggest that the material retained by the C18 cartridge was additional RH-141,452 and RH-141455, solubilized by the enzyme treatment. Analysis of the aqueous fraction suggested formation of radiolabelled sugars, most probably glucose released from the hydrolysis of starch.

The overall results of the fractionation of the methanol extraction of residues from potato are summarised in table B. 7.2.1.2-4

Table B. 7.2.1.2-4 Summary of results of the fractionation of the methanol extraction of residues from potato (TRR = 0.173 mg/kg)

Identity of fraction	%TRR	mg/kg[1]
RH-141455	39.0	0.069
RH-141452	20.9	0.037
Glucose or other sugars	15.9	0.028
Enzyme hydrolysis unknowns	1.7	0.003
Aqyueous unknowns	5.2	0.009
Organic unknowns	6.5	0.012
PES[2]	6.0	0.011
Total accounted for	95.2	0.169

(1) mg/kg calculated as parent equivalents
(2) Calculated by subtraction

Storage stability was confirmed by comparing analytical results obtained 3 months following harvest and those obtained 4 months later using methodologies that were essentially the same. The results obtained were very similar indicating that samples stored frozen were stable.

B.7.2.1.3 Pulses and oilseeds

Peas

Reference:	CA 6.2.1/08 Hein, W. (2014a) [Phenyl-UL-^{14}C] Zoxamide: Plant Metabolism in Pea Report no.: AS290
Guideline(s):	OECD guideline No. 501; Metabolism in Crops (08/01/2007)
Deviations:	None
GLP:	Yes
Validity of the study:	Valid
Previous evaluations:	No; Submitted for the purpose of renewal of a.s. approval

Executive Summary

[^{14}C]-zoxamide was applied to pea plants by foliar spray at a nominal rate of 2 x 145 g a.s./ha (1x rate) or at an exaggerated application rate of 2 x 725 g a.s./ha (5x rate).

Following treatment at the 1x rate, the total radioactive residue (TRR) in immature whole plants harvested at 7 days after the last application (1st harvest, BBCH 65-75) was 4.72 mg/kg. At a PHI of 13 days (2nd harvest, BBCH 77) the TRR in fresh peas was 0.069 mg/kg, and the TRRs in straw and pods were 10.5 mg/kg and 0.311 mg/kg, respectively. At a PHI of 30 days (3rd harvest, BBCH 89) the TRR in dry peas was 0.161 mg/kg, and the TRRs in straw and pods were 47.0 mg/kg and 6.01 mg/kg, respectively.

Following treatment at the 5x rate, the total radioactive residue (TRR) in immature whole plants harvested at 7 days after the last application (1st harvest, BBCH 65-75) was 38.7 mg/kg. At a PHI of 13 days (2nd harvest, BBCH 77) the TRR in fresh peas was 0.135 mg/kg, and the TRRs in straw and pods were 99.3 mg/kg and 10.6 mg/kg, respectively. At a PHI of 30 days (3rd harvest, BBCH 89) the TRR in dry peas was 0.295 mg/kg, and the TRRs in straw and pods were 217 mg/kg and 35.2 mg/kg, respectively.

For pod and whole plant/straw samples, the majority of the residue was removed in the surface rinses and comprised mainly parent zoxamide. In total, zoxamide accounted for 87.6-97.0% TRR in immature whole plant, straw and pods. The metabolites RH-149736, RH-150721, RH-141455, RH-149737, RH-139432, RH-149288 and RH-129151 were identified as minor components of the residue (up to 3.4% TRR). Up to 12 unidentified metabolites were also detected, individually not exceeding 4.2% TRR.

For fresh and dry peas, the main component identified in the extractable residue was parent zoxamide, accounting for 18.1-31.6% TRR in fresh peas and 11.9-16.7% TRR in dry peas. The remainder of the residue in fresh and dry peas comprised unidentified polar residues and unextractable residues that were characterised as being associated with starch, protein, pectin, lignin, hemicellulose and cellulose. The metabolites 139432, RH-149288 and RH-129151 were found in dry peas at very low levels, and RH-149736, RH-150721 and RH-139432 were found in fresh peas at very low levels.

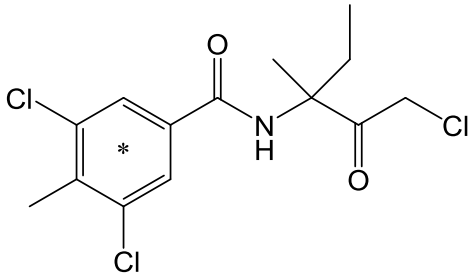
The degradation pathway in pea proceeds via hydrolysis/cyclisation to form RH-129151. This is hydrolysed to form RH-141288 and RH-150721. RH-139432 is probably formed by photolysis, and then oxidised to form RH-149736 and RH-149737.

I. MATERIAL AND METHODS

A. MATERIALS

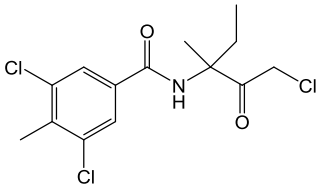
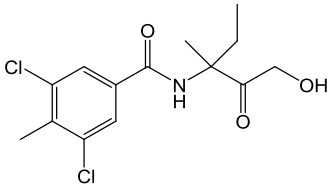
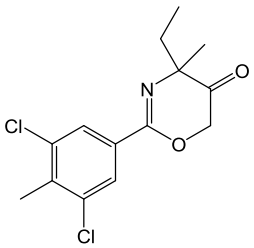
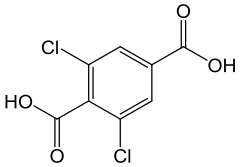
1. Test Material

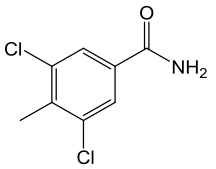
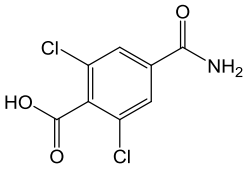
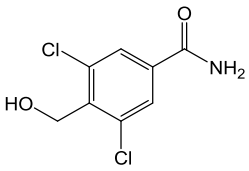
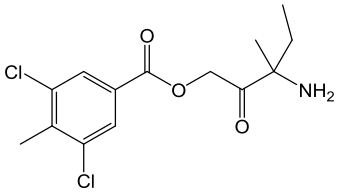
Description:	Zoxamide	(unlabelled)
	[Phenyl-UL-^{14}C]-zoxamide (phenyl label)	
Lot/batch No.:	SZBB136XV	(unlabelled)
	76045-06-35 (phenyl label)	
Purity:	Chemical purity: 99.6%	(unlabelled)
	Radiochemical purity: > 99% (phenyl label)	
Specific activity:	50 mCi/mmol (5.50 MBq/mg) (phenyl label as received)	
CAS No.:	156052-68-5	
Development Code:	RH-7281	
Position of Radiolabel:		



The purity and structures of the key reference materials used in this study are given in Table B.7.2.1.3-1 below.

Table B.7.2.1.3-1 Key reference materials used in the identification of metabolites

Reference Standard Name/code	**Structure**	**Batch Number Purity Molecular Weight (MW) Conversion Factor (CF)[1]**
Zoxamide		SZBB136XV 99.6% MW 336.64
RH-141288		TSN103694 99.0% MW 318.20 CF 0.9453
RH-129151		KY-00-25-17 96.5% MW 300.19 CF 0.8918
RH-141455		ELM-1775 98.8% MW 235.02 CF 0.6982

Reference Standard Name/code	Structure	Batch Number Purity Molecular Weight (MW) Conversion Factor (CF)[1]
RH-139432		TSN103193 96.8% MW 204.05 CF 0.6062
RH-149736		ELM-1774 95.7% MW 234.03 CF 0.6953
RH-149737		ELM-1772 95.2% MW 220.05 CF 0.6537
RH-150721		F1132-136 (methanesulphonate salt) 98.8% MW 318.19 CF 0.9453

[1] Conversion Factor (CF) is the molecular weight ratio of the metabolite/zoxamide. This value is used to convert residue values expressed as mg analyte/kg to mg parent equivalents/kg.

2. Test Commodity

Crop: Pea
Type: Not applicable
Variety: Karina
Botanical Name: *Pisum sativum*
Crop Part or Processed Commodity: Immature plants, fresh peas, dry peas, pods and straw
Sample Size: 87-91 g (immature whole plant), 50-70 g (fresh peas), 45-49 g (dry peas), 10-48 g (pods), 36-75 g (straw)

3. Soil

Type: Weak silty sand

Table B. 7.2.1.3-2 Soil Physicochemical Properties

Name	Birkenheide
Texture (DIN)	Weak silty sand
Clay <2 µm (%)	2.3
Silt 2-63 µm (%)	16.2
Sand 63-2000 µm (%)	81.5

pH ($CaCl_2$)	7.10
Organic matter (Corg, %)	0.41
%$CaCO_3$ (%)	<0.3

B. STUDY DESIGN

1. Test Procedure

The metabolism of zoxamide was studied in pea plants grown from seed in outdoor lysimeter plots (diameter 1.0 m, area 0.78 m^2) of silty sand. The study comprised three treatment plots, a control plot, one plot treated with ^{14}C-phenyl ring-labelled zoxamide at the 1x rate, and one plot treated with ^{14}C-phenyl ring-labelled zoxamide at the 5x rate. Plants were watered and fertilized according to normal agricultural practices to maintain healthy crops.

^{14}C-Phenyl ring-labelled zoxamide, with a specific activity of 5.5 MBq/mg, was formulated as a 240 g/L SC formulation (Zoxium 240 SC). For the 1x rate, this was applied to pea plants at an application rate of 140-146 g as/ha, corresponding to a total application rate of 286 g as/ha. For the 5x rate, this was applied to pea plants at a target application rate of 729-730 g as/ha, corresponding to a total application rate of 1459 g as/ha. The two foliar spray applications were made at BBCH 60-65 (1st application) and BBCH 60-69 (2nd application), with 7 days between each application. The water volume was equivalent to 400 L/ha. Control pea plants were not treated.

The field phase was carried out in 2013 at RLP AgroScience GmbH, Breitenweg 71, 67435 Neustadt, Rhineland-Palatinate, Germany.

2. Sampling

The 1st harvest was made 7 days after the last application. Immature pea plants at BBCH 65-75 were harvested from one third of each plot.

The 2nd harvest was made 13 days after the last application, at BBCH 77. Half the remaining plant material was harvested from each plot and separated into straw, pods and fresh peas.

The 3rd harvest was made 30 days after the last application, at BBCH 89. All the remaining plant material was harvested from each plot and separated into straw, pods and dry peas.

Each sample was weighed after collection. The control and treated immature whole plants, and treated straw and pods were rinsed three times with acetonitrile/water (1:1 v/v) to remove surface residues. Aliquots of the surface rinse were analysed by LSC and profiled by HPLC.

The rinsed samples were then frozen and lyophilised, and weighed after lyophilisation to obtain a dry weight. The samples were prepared by grinding in a mill. The control straw and pods samples and control and treated fresh and dry pea samples were not subjected to surface rinsing, but were frozen, lyophilised and milled directly. Aliquots of the homogenised samples were combusted and analysed by LSC for the determination of the total radioactive residue (TRR).

3. Extraction and analysis

Sub-samples of milled, lyophilised fresh peas, dry peas, and rinsed whole plant, straw and pods were extracted four times with methanol. After each extraction, the extract was centrifuged, and the supernatant decanted and filtered. Water was added to the combined methanol extracts, and the extraction mixture evaporated to an aqueous remainder. The resulting aqueous extract was partitioned twice against ethyl acetate, acidified and partitioned a further two times against ethyl acetate. The extracts were combined to give one water extract and one ethyl acetate extract. Aliquots of each extract

were analysed by LSC. The post-extraction solids (PES) were dried and analysed for radioactivity by combustion and LSC.

The surface rinse samples, water extracts and ethyl acetate extracts were analysed by reverse-phase (C18) HPLC to determine the metabolite profile, using UV and liquid scintillation cell radiochemical detection. Metabolite identification was performed by co-chromatography with authentic reference standards, and the results were confirmed by TLC of selected extracts.

The unextracted residue from immature whole plant, straw and pods contained <10% TRR, and therefore no further work was done on these samples. The unextracted residue from fresh peas and dry peas contained a significant proportion of the TRR, and these samples were subjected to exhaustive extraction by sequential treatment with amylase, protease, EDTA, sodium chlorite, 24% potassium hydroxide and 72% sulphuric acid. The amylase and protease extracts were profiled by HPLC. HPLC profiling was not performed on subsequent extracts due to the presence of large amounts of co-extractives.

All mg/kg values are expressed as parent equivalents in the tables below.

II. RESULTS AND DISCUSSION

A. TOTAL RADIOACTIVE RESIDUES (TRR)

The total radioactive residue in each treated fresh and dry pea sample was determined by combustion. The total radioactive residues in each treated whole plant, straw and pod sample was determined by liquid scintillation counting (LSC) of the surface rinses and combustion of the homogenised, lyophilised rinsed sample. The total radioactivity was determined as the sum of the radioactivity in the rinses and the rinsed sample.

Following treatment at the 1x rate, the total radioactive residue (TRR) in immature whole plants harvested at 7 days after the last application (1st harvest) was 4.72 mg/kg. At a PHI of 13 days (2nd harvest) the TRR in fresh peas was 0.069 mg/kg, and the TRRs in straw and pods were 10.5 mg/kg and 0.311 mg/kg, respectively. At a PHI of 30 days (3rd harvest) the TRR in dry peas was 0.161 mg/kg, and the TRRs in straw and pods were 47.0 mg/kg and 6.01 mg/kg, respectively.

Following treatment at the 5x rate, the total radioactive residue (TRR) in immature whole plants harvested at 7 days after the last application (1st harvest) was 38.7 mg/kg. At a PHI of 13 days (2nd harvest) the TRR in fresh peas was 0.135 mg/kg, and the TRRs in straw and pods were 99.3 mg/kg and 10.6 mg/kg, respectively. At a PHI of 30 days (3rd harvest) the TRR in dry peas was 0.295 mg/kg, and the TRRs in straw and pods were 217 mg/kg and 35.2 mg/kg, respectively.

The majority of the residue in the immature whole plant, pods and straw was removed in the surface rinses (86-97% TRR), as shown in Table B.7.2.1.3-3 below.

Table B. 7.2.1.3-3 Summary of TRR in peas

Crop part	PHI (days)	Location of residue	Total radioactive residue (TRR)			
			1x Rate		5x rate	
			mg/kg	% TRR	mg/kg	% TRR
Immature whole plant 1st harvest	7	Surface rinse	4.60	97.3	37.44	96.9
		Washed plant	0.13	2.7	1.21	3.1
		Total TRR	4.72	100	38.65	100
Straw 2nd harvest	13	Surface rinse	10.05	96.1	95.99	96.7
		Washed straw	0.41	3.9	3.30	3.3
		Total TRR	10.46	100	99.29	100
Pods	13	Surface rinse	0.27	85.9	10.15	96.0

Crop part	PHI (days)	Location of residue	Total radioactive residue (TRR)			
			1x Rate		5x rate	
			mg/kg	% TRR	mg/kg	% TRR
2nd harvest		Washed pods	0.04	14.1	0.42	4.0
		Total TRR	0.31	100	10.57	100
Fresh peas 2nd harvest	13	Surface rinse	Not performed		Not performed	
		Fresh peas	0.07	100	0.14	100
		Total TRR	0.07	100	0.14	100
Straw 3rd harvest	30	Surface rinse	43.47	92.5	200.77	92.6
		Washed straw	3.52	7.5	15.95	7.4
		Total TRR	46.98	100	216.72	100
Pods 3rd harvest	30	Surface rinse	5.54	92.1	33.51	95.1
		Washed pods	0.47	7.9	1.73	4.9
		Total TRR	6.01	100	35.24	100
Dry peas 3rd harvest	30	Surface rinse	Not performed		Not performed	
		Dry peas	0.16	100	0.30	100
		Total TRR	0.16	100	0.30	100

B. EXTRACTION AND CHARACTERISATION OF RESIDUES

1. Extraction and characterisation of residues

Fresh and dry peas were not subjected to surface washing as the peas are protected from direct contact with the spray by the pods. The fresh and dry peas were extracted with methanol, releasing 40.6% TRR in the 1x fresh peas, 48.5% TRR in the 5x fresh peas, 17.0% TRR in the 1x dry peas, and 26.1% TRR in the 5x dry peas. As a significant proportion of the TRR remained unextracted, the unextracted residue was subjected to sequential extraction with amylase, protease, EDTA, sodium chlorite, 24% KOH and 72% H_2SO_4. The extracts from the amylase and protease incubations were profiled by HPLC, and showed unresolved polar radioactivity. The unextractable residue from fresh and dry peas was therefore characterised as being associated with starch, protein, pectin, lignin, hemicellulose and cellulose.

The washed immature whole plant, straw and pods were extracted with methanol, and the methanol extracts partitioned to give an organo-soluble (ethyl acetate) phase and an aqueous soluble phase. This extraction released a further 1.9-2.3% TRR from immature plant, 2.7-5.7% TRR from straw and 1.7-7.7% TRR from pods. For whole plant, straw and pods, the unextracted residue remaining accounted for up to 4.6% TRR and was therefore not characterised further.

2. Identification of metabolites

The acetonitrile/water surface rinses, and ethyl acetate and water extracts were profiled by radio-HPLC (see Tables B.2.1.3-4 - B.2.1.3-17).

The majority of the radioactivity in the immature whole plant, straw and pod surface rinses was parent zoxamide (85.9-95.9% TRR). Two minor metabolites were identified in the surface rinses by co-chromatography with reference items as RH-139432 (1.4-3.6% TRR) and RH-129151 (0.4-1.5% TRR). Up to two unidentified metabolite fractions were found in the surface rinses, which individually did not exceed 1.3% TRR.

The radioactivity in the ethyl acetate extracts of the immature whole plant, straw and pod samples also comprised mainly parent zoxamide (0.6-4.7% TRR). Small amounts of the metabolites RH-149736, RH-150721, RH-141455, RH-149737, RH-139432, RH-149288 and RH-129151 were identified in the ethyl

acetate extracts at levels up to 1.4% TRR, and up to eight unidentified metabolites were also detected at low levels.

The radioactivity in the ethyl acetate extracts of the fresh and dry pea samples also comprised mainly parent zoxamide (18.1% TRR in 1x fresh peas, 31.6% TRR in 5x fresh peas, 11.9% TRR in 1x dry pea, 16.7% TRR in 5x dry peas). The metabolites RH-139432, RH-149288 and RH-129151 were found in dry peas, and RH-149736, RH-150721 and RH-139432 were found in fresh peas, but at very low levels (<0.01 mg/kg) even at the 5x rate.

For all samples, the water extracts contained principally unidentified metabolites that were polar in nature.

Table B.7.2.1.3-4 Identification of metabolites in immature whole plant, 7 day PHI, 1x rate

Compound	Residues of [^{14}C]-zoxamide in 1st harvest immature whole plant – 1x rate							
	Surface rinse		Ethyl acetate extract		Water extract		Total	
	mg/kg[1]	% TRR	mg/kg[1]	% TRR	mg/kg[1]	% TRR	mg/kg[1]	% TRR
Zoxamide	4.527	95.9	0.055	1.1			4.582	97.0
RH-149736			0.001	0.0			0.001	0.0
RH-150721			0.001	0.0			0.001	0.0
RH-141455			0.001	0.0			0.001	0.0
RH-149737			0.002	0.0			0.002	0.0
RH-139432	0.068	1.4	0.002	0.0			0.070	1.5
RH-141288			0.001	0.0			0.001	0.0
RH-129151[2]			0.009	0.2			0.009	0.2
Polar unknowns					0.015	0.3	0.015	0.3
Unknowns[3]			0.004	0.0			0.004	0.0
Unextracted							0.033	0.7
Total							4.719	99.9

- not detected

[1] Residue expressed as mg parent equivalents/kg

[2] RH-129151 represented only a portion of the radioactivity in this fraction

[3] Includes 4 unknowns, each individually 0.001 mg/kg, 0.0% TRR.

Table B.7.2.1.3-5 Identification of metabolites in straw, 13 day PHI, 1x rate

Compound	Residues of [^{14}C]-zoxamide in 2nd harvest straw – 1x rate							
	Surface rinse		Ethyl acetate extract		Water extract		Total	
	mg/kg[1]	% TRR	mg/kg[1]	% TRR	mg/kg[1]	% TRR	mg/kg[1]	% TRR
Zoxamide	9.881	94.5	0.189	1.8			10.070	96.3
RH-149736			0.001	0.0			0.001	0.0
RH-150721			0.003	0.0			0.003	0.0
RH-141455			0.001	0.0			0.001	0.0
RH-149737			0.009	0.1			0.009	0.1
RH-139432	0.172	1.6	0.005	0.0			0.177	1.7
RH-141288			0.005	0.0			0.005	0.0
RH-129151			0.050	0.5			0.050	0.5
Polar unknowns					0.024	0.2	0.024	0.2
Unknowns[2]			0.007	0.0	0.014	0.1	0.021	0.1
Unextracted							0.124	1.2
Total							10.484	100.2

- not detected
[1] Residue expressed as mg parent equivalents/kg
[2] Includes 5 unknowns, the largest accounting for 0.014 mg/kg, 0.1% TRR.

Table B.7.2.1.3-6 Identification of metabolites in pods, 13 day PHI, 1x rate

Compound	Residues of [^{14}C]-zoxamide in 2nd harvest pods – 1x rate							
	Surface rinse		Ethyl acetate extract		Water extract		Total	
	mg/kg[1]	% TRR	mg/kg[1]	% TRR	mg/kg[1]	% TRR	mg/kg[1]	% TRR
Zoxamide	0.267	85.9	0.005	1.7			0.272	87.6
RH-149736								
RH-150721								
RH-141455								
RH-149737								
RH-139432								
RH-141288								
RH-129151			0.004	1.4			0.004	1.4
Polar unknowns					0.013	4.2	0.013	4.2
Unknowns[2]			0.001	0.4			0.001	0.4
Unextracted							0.015	4.6
Total							0.306	98.2

- not detected
[1] Residue expressed as mg parent equivalents/kg
[2] Includes 1 unknown at 0.001 mg/kg, 0.4% TRR.

Table B.7.2.1.3-7 Identification of metabolites in fresh peas, 13 day PHI, 1x rate

Compound	Residues of [^{14}C]-zoxamide in 2nd harvest fresh peas – 1x rate					
	Ethyl acetate extract		Water extract		Total	
	mg/kg[1]	% TRR	mg/kg[1]	% TRR	mg/kg[1]	% TRR
Zoxamide	0.013	18.1			0.013	18.1
RH-149736						
RH-150721						
RH-141455						
RH-149737						
RH-139432						
RH-141288						
RH-129151						
Polar unknowns			0.016	22.5	0.016	22.5
Unknowns						
Unextracted					*0.040*	*59.4*
Amylase: starch fraction					0.011	15.9
Protease: Protein fraction					0.009	13.8
EDTA: pectin fraction					0.005	6.5
Sodium chlorite: lignin fraction					0.003	4.3
24% KOH: hemicellulose fraction					0.012	17.4
72% H_2SO_4: cellulose fraction					0.005	7.2
Total					0.073	105.8

- not detected

[1] Residue expressed as mg parent equivalents/kg

Table B.7.2.1.3-8 Identification of metabolites in straw, 30 day PHI, 1x rate

Compound	Residues of [^{14}C]-zoxamide in 3rd harvest straw – 1x rate							
	Surface rinse		Ethyl acetate extract		Water extract		Total	
	mg/kg[1]	% TRR	mg/kg[1]	% TRR	mg/kg[1]	% TRR	mg/kg[1]	% TRR
Zoxamide	41.202	87.7	2.211	4.7	0.008	0.0	43.421	92.4
RH-149736			0.006	0.0	0.003	0.0	0.010	0.0
RH-150721			0.040	0.1			0.040	0.1
RH-141455								
RH-149737			0.013	0.0			0.013	0.0
RH-139432	1.460	3.1	0.125	0.3			1.585	3.4
RH-141288			0.068	0.1			0.068	0.1
RH-129151	0.361	0.8	0.062	0.1			0.423	0.9
Polar unknowns					0.028	0.1	0.028	0.1
Unknowns[2]	0.439	0.9	0.053	0.1	0.047	0.1	0.539	1.1
Unextracted							1.092	2.3
Total							47.219	100.5

- not detected

[1] Residue expressed as mg parent equivalents/kg

[2] Includes 9 unknowns, the largest accounting for 0.439 mg/kg, 0.9% TRR.

Table B.7.2.1.3-9 Identification of metabolites in pods, 30 day PHI, 1x rate

Compound	Residues of [^{14}C]-zoxamide in 3rd harvest pods – 1x rate							
	Surface rinse		Ethyl acetate extract		Water extract		Total	
	mg/kg[1]	% TRR	mg/kg[1]	% TRR	mg/kg[1]	% TRR	mg/kg[1]	% TRR
Zoxamide	5.245	87.2	0.110	1.8			5.354	89.0
RH-149736								
RH-150721								
RH-141455								
RH-149737								
RH-139432	0.170	2.8					0.170	2.8
RH-141288			0.005	0.1			0.005	0.1
RH-129151	0.068	1.1	0.024	0.4			0.092	1.5
Polar unknowns								
Unknowns[2]	0.058	1.0	0.021	0.4			0.079	1.4
Unresolved					0.015	0.3	0.015	0.3
Unextracted							0.154	2.6
Total							5.869	97.6

- not detected
[1] Residue expressed as mg parent equivalents/kg
[2] Includes 4 unknowns, the largest accounting for 0.079 mg/kg, 1.4% TRR.

Table B.7.2.1.3-10 Identification of metabolites in dry peas, 30 day PHI, 1x rate

Compound	Residues of [^{14}C]-zoxamide in 3rd harvest dry peas – 1x rate					
	Ethyl acetate extract		Water extract		Total	
	mg/kg[1]	% TRR	mg/kg[1]	% TRR	mg/kg[1]	% TRR
Zoxamide	0.019	11.9			0.019	11.9
RH-149736						
RH-150721						
RH-141455						
RH-149737						
RH-139432						
RH-141288						
RH-129151	0.002	1.1			0.002	1.1
Polar unknowns			0.007	4.0	0.007	4.0
Unknowns						
Unextracted					*0.133*	*83.0*
Amylase: starch fraction					0.034	20.8
Protease: Protein fraction					0.026	16.1
EDTA: pectin fraction					0.014	8.7
Sodium chlorite: lignin fraction					0.009	5.3
24% KOH: hemicellulose fraction					0.039	24.2
72% H_2SO_4: cellulose fraction					0.008	4.7
Total					0.156	96.9

- not detected
[1] Residue expressed as mg parent equivalents/kg

Table B.7.2.1.3-11 Identification of metabolites in immature whole plant, 7 day PHI, 5x rate

Compound	Residues of [^{14}C]-zoxamide in 1st harvest immature whole plant – 5x rate							
	Surface rinse		Ethyl acetate extract		Water extract		Total	
	mg/kg[1]	% TRR	mg/kg[1]	% TRR	mg/kg[1]	% TRR	mg/kg[1]	% TRR
Zoxamide	36.075	93.4	0.449	1.1			36.524	94.5
RH-149736			0.018	0.0			0.018	0.0
RH-150721			0.005	0.0			0.005	0.0
RH-141455			0.003	0.0			0.003	0.0
RH-149737			0.009	0.0	0.002	0.0	0.011	0.0
RH-139432	0.610	1.6	0.028	0.1			0.638	0.1
RH-141288			0.016	0.0			0.016	0.0
RH-129151[2]	0.172	0.4	0.119	0.3			0.291	0.3
Polar unknowns			0.035	0.1	0.044	0.1	0.079	0.2
Unknowns[3]	0.581	1.5	0.046	0.1	0.090	0.2	0.719	1.8
Unextracted							0.305	0.8
Total							38.608	99.9

- not detected
[1] Residue expressed as mg parent equivalents/kg
[2] RH-129151 represented only a portion of the radioactivity in this fraction
[3] Includes 11 unknowns, the largest accounting for 0.296 mg/kg, 0.8% TRR.

Table B.7.2.1.3-12 Identification of metabolites in straw, 13 day PHI, 5x rate

Compound	Residues of [^{14}C]-zoxamide in 2nd harvest straw – 5x rate							
	Surface rinse		Ethyl acetate extract		Water extract		Total	
	mg/kg[1]	% TRR	mg/kg[1]	% TRR	mg/kg[1]	% TRR	mg/kg[1]	% TRR
Zoxamide	92.736	93.4	1.652	1.7			94.388	95.1
RH-149736			0.038	0.0			0.038	0.0
RH-150721			0.013	0.0			0.013	0.0
RH-141455			0.005	0.0			0.005	0.0
RH-149737			0.026	0.0			0.026	0.0
RH-139432	2.323	2.3	0.055	0.1			2.378	2.4
RH-141288			0.022	0.0			0.022	0.0
RH-129151[2]			0.403	0.1			0.403	0.4
Polar unknowns			0.003	0.0	0.193	0.2	0.197	0.2
Unknowns[3]	0.931	0.9	0.108	0.1	0.161	0.2	1.200	1.2
Unextracted							0.880	0.9
Total							99.548	100.3

- not detected
[1] Residue expressed as mg parent equivalents/kg
[2] RH-129151 represented only a portion of the radioactivity in this fraction
[3] Includes 9 unknowns, the largest accounting for 0.931 mg/kg, 0.9% TRR.

Table B.7.2.1.3-13 Identification of metabolites in pods, 13 day PHI, 5x rate

Compound	Residues of [^{14}C]-zoxamide in 2nd harvest pods – 5x rate							
	Surface rinse		Ethyl acetate extract		Water extract		Total	
	mg/kg[1]	% TRR	mg/kg[1]	% TRR	mg/kg[1]	% TRR	mg/kg[1]	% TRR
Zoxamide	9.954	94.2	0.059	0.6			10.013	94.7
RH-149736								
RH-150721								
RH-141455								
RH-149737								
RH-139432	0.116	1.1					0.116	1.1
RH-141288								
RH-129151[2]			0.056	0.5			0.056	0.5
Polar unknowns			0.003	0.0	0.014	0.1	0.018	0.2
Unknowns[3]	0.080	0.8	0.029	0.3	0.036	0.3	0.145	1.4
Unextracted							0.168	1.6
Total							10.515	99.5

- not detected
[1] Residue expressed as mg parent equivalents/kg
[2] RH-129151 represented only a portion of the radioactivity in this fraction
[3] Includes 10 unknowns , the largest accounting for 0.080 mg/kg, 0.8% TRR.

Table B.7.2.1.3-14 Identification of metabolites in fresh peas, 13 day PHI, 5x rate

Compound	Residues of [^{14}C]-zoxamide in 2nd harvest fresh peas – 5x rate					
	Ethyl acetate extract		Water extract		Total	
	mg/kg[1]	% TRR	mg/kg[1]	% TRR	mg/kg[1]	% TRR
Zoxamide	0.043	31.6			0.043	31.6
RH-149736	0.001	1.0			0.001	1.0
RH-150721	0.002	1.4			0.002	1.4
RH-141455						
RH-149737						
RH-139432	0.003	1.9			0.003	1.9
RH-141288						
RH-129151						
Polar unknowns			0.010	7.7	0.010	7.7
Unknowns[2]	0.002	1.6	0.004	3.5	0.006	5.1
Unextracted					*0.069*	*51.5*
Amylase: starch fraction					0.034	25.2
Protease: Protein fraction					0.017	12.6
EDTA: pectin fraction					0.007	4.8
Sodium chlorite: lignin fraction					0.008	5.9
24% KOH: hemicellulose fraction					0.014	10.0
72% H_2SO_4: cellulose fraction					0.003	1.9
Total					0.147	108.9

- not detected
[1] Residue expressed as mg parent equivalents/kg
[2] Includes 3 unknowns , the largest accounting for 0.003 mg/kg, 2.5% TRR.

Table B.7.2.1.3-15 Identification of metabolites in straw, 30 day PHI, 5x rate

Compound	Residues of [^{14}C]-zoxamide in 3rd harvest straw – 5x rate							
	Surface rinse		Ethyl acetate extract		Water extract		Total	
	mg/kg[1]	% TRR	mg/kg[1]	% TRR	mg/kg[1]	% TRR	mg/kg[1]	% TRR
Zoxamide	188.486	86.9	9.705	4.5	0.017	0.0	198.208	91.4
RH-149736			0.033	0.0			0.033	0.0
RH-150721			0.044	0.0			0.044	0.0
RH-141455								
RH-149737			0.033	0.0			0.033	0.0
RH-139432	6.866	3.2	0.418	0.2			7.284	3.4
RH-141288			0.214	0.1			0.214	0.1
RH-129151	1.185	0.5	0.440	0.2			1.624	0.8
Polar unknowns					0.152	0.1	0.152	0.1
Unknowns[2]	4.256	1.9	0.115	0.1	0.227	0.1	4.598	2.1
Unextracted							4.214	2.0
Total							216.406	99.9

- not detected
[1] Residue expressed as mg parent equivalents/kg
[2] Includes 8 unknowns, the largest accounting for 2.851 mg/kg, 1.3% TRR.

Table B.7.2.1.3-16 Identification of metabolites in pods, 30 day PHI, 5x rate

Compound	Residues of [^{14}C]-zoxamide in 3rd harvest pods – 5x rate							
	Surface rinse		Ethyl acetate extract		Water extract		Total	
	mg/kg[1]	% TRR	mg/kg[1]	% TRR	mg/kg[1]	% TRR	mg/kg[1]	% TRR
Zoxamide	31.047	88.1	0.322	0.9			31.369	89.0
RH-149736								
RH-150721								
RH-141455								
RH-149737								
RH-139432	1.260	3.6					1.260	3.6
RH-141288			0.022	0.1			0.022	0.1
RH-129151	0.523	1.5	0.106	0.3			0.629	1.8
Polar unknowns					0.018	0.1	0.018	0.1
Unknowns[2]	0.677	1.9	0.082	0.3	0.051	0.1	0.810	2.3
Unextracted							0.678	1.9
Total							34.785	98.7

- not detected
[1] Residue expressed as mg parent equivalents/kg
[2] Includes 9 unknowns, the largest accounting for 0.439 mg/kg, 1.2% TRR.

Table B.7.2.1.3-17 Identification of metabolites in dry peas, 30 day PHI, 5x rate

Compound	Residues of [^{14}C]-zoxamide in 3rd harvest dry peas – 5x rate					
	Ethyl acetate extract		Water extract		Total	
	mg/kg[1]	% TRR	mg/kg[1]	% TRR	mg/kg[1]	% TRR
Zoxamide	0.049	16.7			0.049	16.7
RH-149736						
RH-150721						
RH-141455						
RH-149737						
RH-139432	0.005	1.6			0.005	1.6
RH-141288	0.002	0.7			0.002	0.7
RH-129151						
Polar unknowns			0.014	4.7	0.014	4.7
Unknowns[2]	0.005	1.6	0.002	0.8	0.007	2.4
Unextracted					*0.218*	*73.9*
Amylase: starch fraction					0.090	30.3
Protease: Protein fraction					0.040	13.6
EDTA: pectin fraction					0.013	4.2
Sodium chlorite: lignin fraction					0.016	5.3
24% KOH: hemicellulose fraction					0.023	7.8
72% H_2SO_4: cellulose fraction					0.004	1.2
Total					0.261	88.5

- not detected

[1] Residue expressed as mg parent equivalents/kg

[2] Includes 3 unknowns, the largest accounting for 0.003 mg/kg, 0.9% TRR.

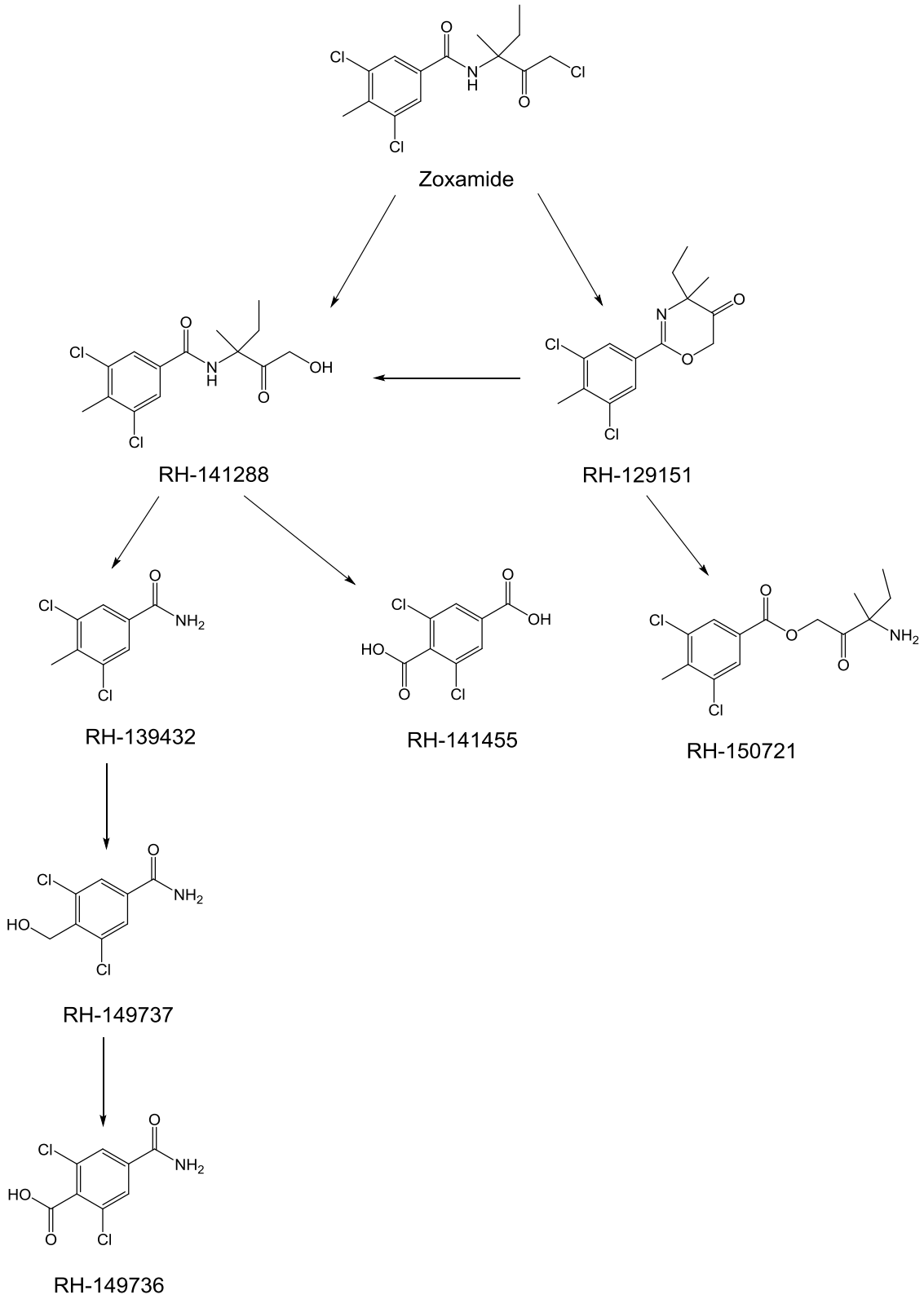
3. Storage stability of residues

Samples were stored frozen, and initial profiling of the extracts was completed within 6 months of harvest, therefore further storage stability investigations are not required.

4. Proposed metabolic pathway

The degradation pathway in peas proceeds via photochemical degradation on the surface of the plant to form RH-129151. This is then hydrolysed to form RH-141288 and RH-150721. Hydrolysis of zoxamide and RH-141288 forms RH-139432, and this is then further oxidised to form RH-149737 and RH-149736. A metabolic pathway is proposed as follows:

Figure B.7.2.1.3-17 Proposed metabolic pathway for zoxamide in pea



III. CONCLUSION

After foliar application of [^{14}C]-zoxamide to pea plants at a nominal rate of 2 x 145 g a.s./ha (1x rate), the total radioactive residue (TRR) in immature whole plants harvested at 7 days after the last application (1st

harvest, BBCH 65-75) was 4.72 mg/kg. At a PHI of 13 days (2nd harvest, BBCH 77) the TRR in fresh peas was 0.069 mg/kg, and the TRRs in straw and pods were 10.5 mg/kg and 0.311 mg/kg, respectively. At a PHI of 30 days (3rd harvest, BBCH 89) the TRR in dry peas was 0.161 mg/kg, and the TRRs in straw and pods were 47.0 mg/kg and 6.01 mg/kg, respectively

After foliar application of [^{14}C]-zoxamide to pea plants at an exaggerated application rate of 2 x 725 g a.s./ha (5x rate), the total radioactive residue (TRR) in immature whole plants harvested at 7 days after the last application (1st harvest, BBCH 65-75) was 38.7 mg/kg. At a PHI of 13 days (2nd harvest, BBCH 77) the TRR in fresh peas was 0.135 mg/kg, and the TRRs in straw and pods were 99.3 mg/kg and 10.6 mg/kg, respectively. At a PHI of 30 days (3rd harvest, BBCH 89) the TRR in dry peas was 0.295 mg/kg, and the TRRs in straw and pods were 217 mg/kg and 35.2 mg/kg, respectively.

The majority of the residue in immature whole plant, pods and straw was removed in the surface rinses and comprised mainly parent zoxamide. In these crop parts, zoxamide was the only significant component of the residue, accounting for 87.6-97.0% TRR.

For fresh and dry peas, the main component identified in the extractable residue was parent zoxamide, accounting for 18.1-31.6% TRR in fresh peas and 11.9-16.7% TRR in dry peas. The remainder of the residue in fresh and dry peas comprised unidentified polar residues and unextractable residues that were characterised as being associated with starch, protein, pectin, lignin, hemicellulose and cellulose.

The metabolites RH-149736, RH-150721, RH-141455, RH-149737, RH-139432, RH-149288 and RH-129151 were identified as minor components of the residue.

Reference:	CA 6.2.1/09 Hein, W. (2014b) Extraction Efficiency of [phenyl-UL-14C] Zoxamide from Plant Metabolism Samples (Pea) Report no.: AS362
Guideline(s):	OECD Guidance document No 72 (ENV/JM/MONO(2007)17 SANCO/825/00 rev.8.1, 16/11/2101 OECD guideline No. 501; Metabolism in Crops (08/01/2007)
Deviations:	None
GLP:	Yes
Validity of the study:	Valid
Previous evaluations:	No; Submitted for the purpose of renewal of a.s. approval

Executive Summary

Samples from the pea metabolism study summarised above (CA 6.2.1/09, report AS290) were used to radiovalidate the extraction method used in the QuEChERS method (See point B.5.1.2, study 4.1.2/01).

Samples of immature whole plant (5-fold dose, after surface washing) and dry peas (1-fold dose) containing incurred residues of zoxamide were extracted using the QuEChERS method extraction with acetonitrile and a salt solution. The organic extracts were profiled by radio-TLC and HPLC, and the profiles compared with those obtained in the metabolism study.

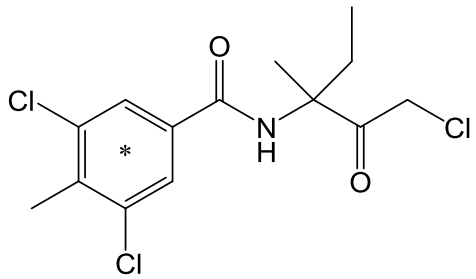
The amount of zoxamide extracted using the QuEChERS extraction method and the metabolism study extraction method was found to be in good agreement. The recovery of zoxamide using the QuEChERS method was 98.4% for immature whole plant and 68.4% for dry peas, compared to the amount extracted using the metabolism study extraction method. The lower recovery obtained for the dry pea sample is attributed to the low absolute concentration of zoxamide in the sample.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Description:	Zoxamide	(unlabelled)
	[Phenyl-UL-^{14}C]-zoxamide (phenyl label)	
Lot/batch No.:	SZBB136XV	(unlabelled)
	76045-06-35 (phenyl label)	
Purity:	Chemical purity: 99.6%	(unlabelled)
	Radiochemical purity: > 99% (phenyl label)	
Specific activity:	50 mCi/mmol (5.50 MBq/mg) (phenyl label as received)	
CAS No.:	156052-68-5	
Development Code:	RH-7281	
Position of Radiolabel:		



2. Test Commodity

Crop:	Pea
Type:	Not applicable
Variety:	Karina
Botanical Name:	*Pisum sativum*
Crop Part or Processed Commodity:	Immature whole plant (5-fold dose), dry peas (1-fold dose)
Sample Size:	5 g

B. STUDY DESIGN

Samples of immature whole plant (5-fold dose, after surface washing) and dry peas (1-fold dose) remaining from study AS 290 (CA 6.2.1/08) were extracted according to the QuEChERS method. As the samples had been freeze-dried during sample preparation in study AS290, they were treated as dry commodities in the QuEChERS method.

5g samples were weighed into a centrifuge tube and 10 mL of water added. The samples were extracted with 10 mL acetonitrile by shaking for one minute. A salt mixture was added (4g magnesium sulphate, 1g sodium chloride, 1g trisodium citrate dihydrate, 0.5g disodium hydrogen citrate sesquihydrate) and the sample shaken for a further minute. After extraction, the sample was centrifuged for at least 2 minutes at 4000 rpm to separate the phases. An additional aliquot of 5 mL water and 5 mL acetonitrile was added to the immature whole plant to achieve phase separation. The resulting organic and aqueous phases were collected separately and the radioactivity extracted measured by LSC. The organic phase was analysed by radio-TLC using the method from the metabolism study (AS290), and by radio-HPLC using the chromatographic conditions from the QuEChERS method.

All mg/kg values are expressed as parent equivalents in the tables below.

II. RESULTS AND DISCUSSION

The QuEChERS extraction method extracted a lower proportion of the total radioactive residue (TRR) from each sample, compared to the extraction method used in the pea metabolism study (AS290).

In total, the QuEChERS extraction method extracted 43.6% of the TRR from the immature whole plant sample, compared to 71.4% TRR using the metabolism study extraction method. For the dry peas sample, the QuEChERS method extracted a total of 9.2% TRR, compared to 17.3% TRR using the metabolism study extraction method (see Table **B.7.2.1.3-19**).

The organic phase from each sample was profiled by radio-TLC for direct comparison with the equivalent extracts from the metabolism study. Confirmatory analysis was performed by radio-HPLC. Although the total amount of radioactivity extracted was lower using the QuEChERS method, the amount of zoxamide extracted was similar using the two extraction methods (see Table 6.2.1.4/19).

The recovery of zoxamide from the immature whole plant sample was 0.442 mg/kg (36.5% TRR) using the QuEChERS extraction compared to 0.449 mg/kg (37.0% TRR) in the metabolism study. Therefore, for immature whole plant, the Quechers method gave a recovery of zoxamide of 98.4% of that obtained using the metabolism study extraction method.

For dry peas, the recovery of zoxamide using the QuEChERS method was 0.013 mg/kg (7.9% TRR), compared to 0.019 mg/kg (11.8% TRR) using the metabolism study extraction method. Therefore, for dry peas, the QuEChERS method gave a recovery of zoxamide of 68.4% of that obtained using the metabolism study extraction method. This lower recovery is attributed to the low absolute residue in the dry pea sample, whereby a small difference in the amount of zoxamide extracted makes a large difference to the % recovery value.

Table B.7.2.1.3-18 Comparison of distribution of residues following extraction

Matrix	Fraction	QuEChERS extraction		Metabolism study extraction	
		mg/kg fresh weight	% of initial TRR	mg/kg fresh weight	% of initial TRR
DAT 7 Immature whole plant (5-fold dose)	Organic phase	0.523	43.1	0.727	60.0
	Water phase	0.006	0.5	0.138	11.4
	Unextracted residue	0.683[1]	56.4[1]	0.305[2]	25.2[2]
	Sum	1.212	100	1.170	96.5
DAT 30 Dry peas (1-fold dose)	Organic phase	0.013	7.9	0.021	13.0
	Water phase	0.002	1.3	0.007	4.3
	Unextracted residue	0.146[1]	90.8[1]	0.130[2]	79.8[2]
	Sum	0.161	100	0.156	96.9

[1] Unextracted residue determined by calculation from the sample TRR value

[2] Determined by combustion

[3] Unextracted residue subjected to exhaustive extraction. This figure represents the sum of the residues in these extracts.

Table B.7.2.1.3-19 Recovery of [^{14}C]-zoxamide following extraction

Matrix	Extraction method	Recovery of [^{14}C]-zoxamide[1]	
		mg/kg fresh weight	% of initial TRR
DAT 7 Immature whole plant (5-fold dose)	QuEChERS extraction	0.442	36.5
	AS290 Metabolism study extraction	0.449	37.0
DAT 30 Dry peas (1-fold dose)	QuEChERS extraction	0.013	7.9
	AS290 Metabolism study extraction	0.019	11.8

[1] Recoveries based on TLC analysis

III. CONCLUSION

The extraction from the QuEChERS method was radiovalidated using immature whole plant and dry pea samples from the pea metabolism study.

The amount of zoxamide extracted using the QuEChERS extraction method and the metabolism study extraction method was found to be in good agreement. The recovery of zoxamide using the QuEChERS method was 98.4% for immature whole plant and 68.4% for dry peas, compared to the amount extracted using the metabolism study extraction method. The lower recovery obtained for the dry pea sample is attributed to the low absolute concentration of zoxamide in the sample.

B.7.2.2 Animals

B.7.2.2.1 Poultry

As the trigger value of 0.004 mg/kg bw/day is not reached for poultry considering representatives uses, metabolism studies are not necessary for this kind of livestock.

The representative uses on potatoes and grapevines do not result in significant residues occurring in the diet of poultry, and therefore a poultry metabolism study is not required or submitted.

B.7.2.2.2 Lactating ruminants

As the trigger value of 0.004 mg/kg bw/day is not reached for ruminants considering representatives uses, metabolism studies are not necessary for this kind of livestock. However a goat metabolism study was evaluated for Annex I inclusion and considered to be acceptable. This study is summarized below.

Reference:	CA 6.2.1 [redacted] Metabolism of 14C-RH-117,281 in lactating goats Report No. 34-97-166, September 10, 1998, ER ref. No. 16.1
Guideline(s):	US EPA 40 CFR 157.3.240: Residue Chemistry Subdivision O, series 171-4 (b) and EC Draft Guidelines 4699/VI/94 Rev.4, 4700/VI/94, Rev.4
Deviations:	None
GLP:	Yes
Validity of the study:	Acceptable
Previous evaluations:	In DAR (2002)

RH-7281, labelled uniformly on the aromatic (phenyl) ring with ^{14}C (isotopically diluted with [^{12}C]RH-7281 and [^{13}C]RH-7281) was administered orally in gelatin capsules to a lactating goat once a day for 7 consecutive days. The test material was dosed at levels equivalent to a dietary concentration of 60.7mg/kg. A second goat served as a control animal. Urine, faeces, and milk samples were collected twice daily (A.M. and P.M.). The urine and faeces samples were each pooled after the A.M. and P.M. collection intervals. A cage rinse was collected at the end of each 24-hour sampling period. Blood samples were taken from both animals on days 0 (control), 1, 3, and 7. Both goats were sacrificed approximately 23 hours after the final dose. Omental fat, liver, kidney, and muscle samples were removed at necropsy for further analysis.

Daily urine, daily cage rinse, and final bile samples were analysed for radioactivity content by direct liquid scintillation counting (LSC). Total radioactive residue (TRR) levels, expressed as mg/kg equivalents of RH-7281 in milk were determined by direct LSC. TRR levels in faeces, muscle (combination of leg and loin), liver, kidney, and blood samples were determined by combusting samples to ^{14}C-carbon dioxide and counting by LSC. TRR levels in omental fat were determined by tissue solubilisation.

The total dose recovered was 77.5%. Radioactivity analyses of urine and faeces samples from the treated goat showed values accounting for 37% and 36%, respectively, of the total administered dose. Individual tissues and cumulative milk samples on day 7 each amounted to <0.3% of the administered dose. A summary of the distribution of radioactivity and total terminal residues is presented in table B.7.2.2.2-1

Table B.7.2.2.2-1 Summary of the distribution of radioactivity, expressed as a percent of the administered dose and total terminal residues.

Matrices	% administered dose	Total terminal residues (mg/kg)
Urine*	37.14%	
Cage Rinse *	3.77%	

Faeces *	36.11 %	
Bile	0.10%	
Milk *	0.27%	0.236 (Day 4, pm)
Liver	0.05%	0.450
Kidney	0.01 %	0.365
Leg Muscle	0.01 %	0.046
Loin Muscle	<0.01 %	0.044
Omental Fat	0.02%	0.197
Blood	<0.01 %	0.101 (at sacrifice)
Total	~ 77.48%	

(*) Values expressed as a cumulative percent of dose administered over 7 days. -

Milk samples (Day 3 A.M. and day 4 P.M) were extracted with acetone and the extract partitioned between acetonitrile and hexane. >80% of the TRR went into the acetonitrile phase with the remaining residues distributed between hexane and bound residue (post extraction solids [PES]) fractions.

Muscle, liver, and kidney samples were extracted with methanol/water and chloroform. Methanol extracted the majority of the radioactivity from liver and kidney, whereas most of the radioactive residues in muscle, which were organosoluble, resided in the chloroform extract. The chloroform extracts were concentrated and subjected to acetonitrile/hexane solvent partition, which resulted in a majority of radioactive residues residing in the acetonitrile fraction. The majority of radioactivity in omental fat was extractable with hexane. The hexane extract was subjected to solvent partitioning with acetonitrile, resulting in removal of a majority of the radioactivity.

With the exception of liver, radioactivity remaining as bound residues (PES) was low for all samples, accounting for less than 10% of TRR (<0.05 mg/kg). In liver samples, however, 11.75% of TRR (0.053 mg/kg) remained as bound residues. Treatment of the liver PES fraction with protease enzyme released 5.12% of TRR (0.023 mg/kg).

Fractions containing significant TRR levels (>10% of TRR, >0.01 mg/kg) were analyzed for their metabolite profiles by reversed-phase HPLC (ODS 20 column with UV and/or radioactivity detection) and/or normal-phase TLC. Metabolites that contributed a significant portion to the total terminal residues in milk and tissues were identified by comparative chromatography and co-chromatography with the unlabeled reference standards using reversed-phase HPLC and normal-phase TLC and/or by liquid chromatography/ electrospray ionization mass spectrometry (LC/ESI-MS).

The total contribution of major metabolites in extractable fractions from milk and tissues is summarized in table B.7.2.2.2-2.

Table B.7.2.2.2-2 Major metabolites in extractable fractions from milk and tissues

Milk - Day 4

Metabolite	% TRR	mg/kg (parent equivalents)
M-12a, M-12b	37.87	0.090
RH-141454	18.00	0.043
RH-141288	11.88	0.028
RH-127450	20.24	0.048
Others	2.54	0.006
Total	90.53	0.215

Fat

Metabolite	% TRR	mg/kg (parent equivalents)
RH-141288	15.75	0.031
RH-127450	65.18	0.129
Others	4.07	0.008
Total	85.00	0.168

Liver

Metabolite	% TRR	mg/kg (parent equivalents)
M-1, M-2, M-3, M-4*	17.27	0.078
M-5	14.53	0.065
M-5, M-6	16.72	0.075
M-7	23.17	0.104
M-8	1.55	0.007
M-9	1.54	0.007
RH-141288	1.72	0.008
M_10	0.87	0.004
RH-127450	2.01	0.009
M-11 (unknown)	1.97	0.009
Others	4.36	0.020
Total	85.71	0.386

* M-2 and M-4 major.

Kidney

Metabolite	% TRR	mg/kg (parent equivalents)
M-1, M-2, M-3, M-4*	17.82	0.065
M-5	13.61	0.050
M-5, M-6	20.11	0.074
M-7	10.54	0.039
M-8	4.99	0.018
RH-141288	3.95	0.014
RH-127450	0.85	0.003
M-11 (unknown)	0.64	0.002
M-12a, M-12b	11.72	0.043
Others	4.20	0.015
Total	88.43	0.323

* M-2 and M-4 major.

Muscle

Metabolite	% TRR	mg/kg (parent equivalents)
M-1, M-2, M-3, M-4	2.96	0.001
M-5	5.03	0.002
M-5, M-6	8.34	0.004
M-7	5.20	0.002
M-8	12.26	0.006
RH-141288	12.64	0.006
M-10	1.16	0.001
RH-127450	15.13	0.007
M-12a, M-12b	25.82	0.012
Others	3.49	0.002
Total	92.03	0.043

Key to metabolites

	M1	3,5-dichloro-N-(1-ethyl-1-methyl-2-oxopropyl)-benzamide-4-carboxylic acid.
	M2 and M4	structurally isomeric glucuronic acid conjugates of 3,5-dichloro-N-(3-hydroxy-1-ethyl-1-methyl-2-oxopropyl)-4- hydroxymethylbenzamide
	M3	glucuronic acid conjugate of 3,5-dichloro-N (2,3-dihydroxy-1-ethyl-1-methylpropyl)-4-hydroxymethylbenzamide
	M5, M6, and M7	glucuronic acid conjugates of 4-hydroxymethyl-RH-141,643, RH-141,454, and RH-141,288, respectively
	M8 and M10	methyl sulfone metabolites related to RH-141,453 and RH-117,281, respectively
	M9	The methyl sulfoxide derivative of dechlorinated RH-117,281
	Ml2a and Ml2b	Positional isomers of a dihydroxylated analogue of RH-127,450.

B.7.2.2.3. Pigs

The representative uses on potatoes and grapevines do not result in significant residues occurring in the diet of pigs, intake is not expected to exceed 0.004mg/kg bw/day and the metabolism of zoxamide is qualitatively similar in the goat and the rat, and therefore a pig metabolism study is not required or submitted.

B.7.2.2.4. Fish

Information on the crops used for the preparation of fish feedstuffs is provided in the draft guidance document on the nature or residues in fish (SANCO/11187/2013, 31.01.2013 rev.3). Grapes are not used for the preparation of fish feedstuffs. Root and tuber vegetables are used only in small quantities for fish feedstuffs, and potato protein comprises a maximum of 3% of the diet for carp, and is not used in trout feed. Residues of zoxamide (and RH 141455 and RH 141452) in potatoes are <LOQ, therefore residues in fish feed will be <0.1 mg/kg diet.

Zoxamide has a log P_{OW} of 3.76, and therefore has potential to bioaccumulate, however as residues of zoxamide in fish feed will be <0.1 mg/kg diet, a fish metabolism study is not required. A radiolabelled bioaccumulation study in Bluegill sunfish (*Lepomis macrochirus*) was previously evaluated in the DAR (B9.2.2.1) (Robinson, 1998, Report no. 34-98-145) which provides information on the metabolism of zoxamide in fish. In this study, bluegill sunfish were exposed to ^{14}C-RH-7281 at 0, 0.5 or 5 µg/L for 28 days in a flow-through system, followed by a 14-day depuration period. A steady state was reached within 2 days, and depuration of total residues was >92% from all tissues within 14 days. Metabolism was extensive and no parent RH-7281 was found in fillet or viscera. The major metabolite was RH-127450 which comprised 40% TRR in fillet on day 21 and 33% on day 28 (18% and 17% in viscera respectively). No other component of the residue reached 10% TRR.

Grapes are not expected to form a significant part of a fish diet, and potatoes have been shown to contain <0.02 mg/kg (<LOQ) and <0.005mg/kg (<LOQ) in available trials. It can therefore be expected that there is no potential for residues in commercial fish diet and therefore that no data are required.

B.7.2.3 Summary of metabolism

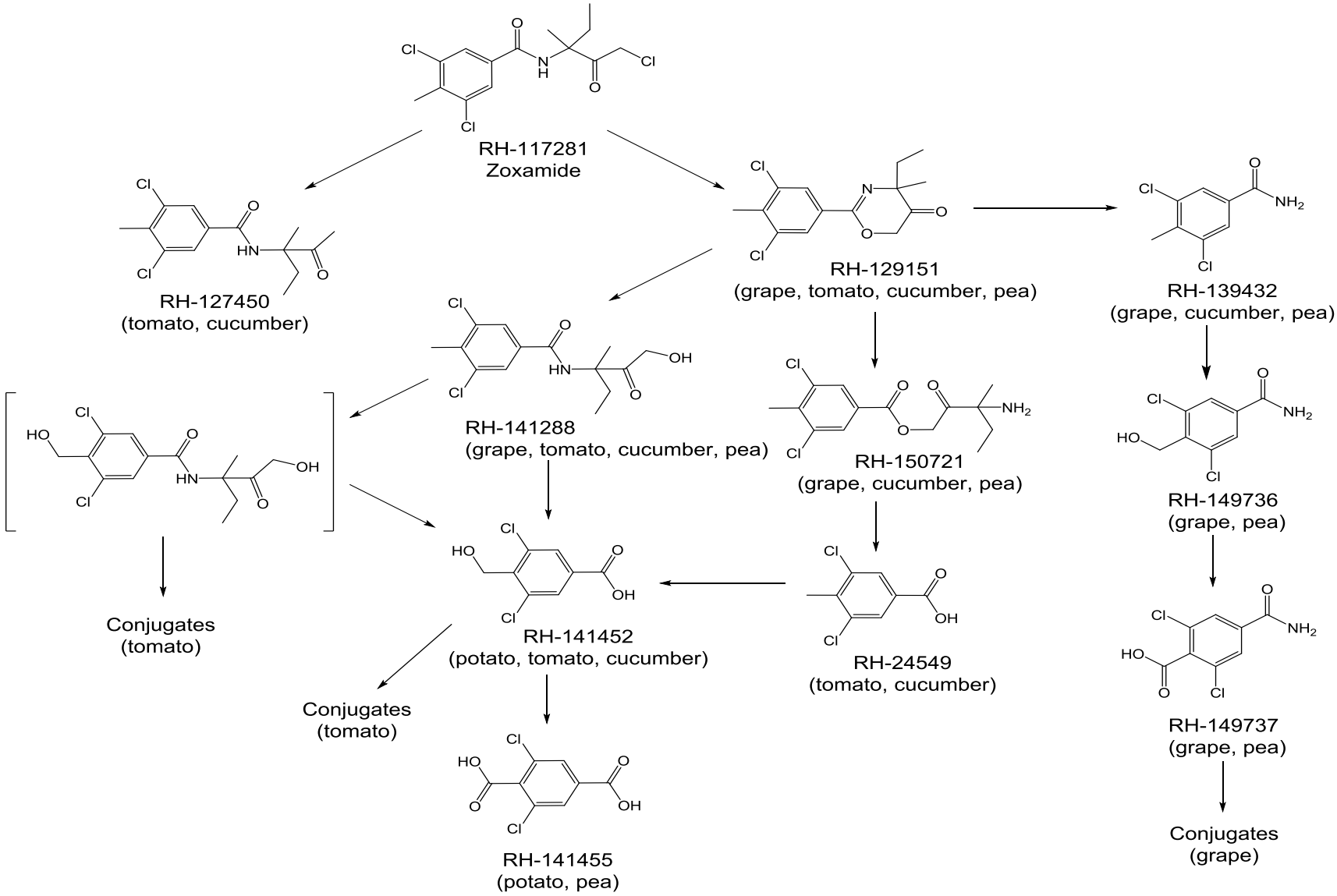
Zoxamide is of low systemicity, therefore after foliar application to crops, most of the applied material remains on the surface of the plants. In the metabolism studies conducted in grapes, tomato, cucumber and peas, the major component of the residue is unchanged zoxamide (RH-7281). Degradation is by photolysis on the crop surface and hydrolysis or oxidation, and results in a number of minor metabolites.

In the metabolism study conducted in potato, no parent zoxamide was found in the potato tubers as the tubers were not in direct contact with the spray, and zoxamide is minimally translocated. The main components of the residue in potato tubers were the metabolites RH-141452 and RH-141455, probably as a result of uptake of residues from the soil.

In the metabolism study conducted in peas, low residues of zoxamide were found in the fresh peas and dry peas as the pea seed was protected by the pods and therefore not in direct contact with the spray. The remainder of the residue in fresh and dry peas comprised unidentified polar residues and unextractable residues that were characterised as being associated with starch, protein, pectin, lignin, hemicellulose and cellulose.

An overall metabolic pathway for zoxamide in crops is proposed in Figure B.7.2.3-1. This shows that the metabolites found in potato are also found in other crops and are part of the same overall metabolic pathway.

Figure B.7.2.3-1 Proposed metabolic pathway for zoxamide in plants



B.7.3 Magnitude of residue trials in plants

Table B.7.3-1 Summary of the critical GAPs for the representative uses of Zoxium 240 SC

Crop	Region	Outdoor/ Protected	Growth stage	Maximum Number of Applications	Min. Interval (days)	Maximum		Min. PHI (days)
						Rate g as/ha	Water L/ha	
Wine grapes	~~Central~~ North South	O	BBCH 15-79	5	8	180	1000	28
Table grapes	~~Central~~ North South	O	BBCH 15-79	5	8	180	1000	28
Potato	North ~~Central~~ South	O	BBCH 20-80	5	8	180	1000	7

B.7.3.1 Potatoes

In the DAR (B7.6 and B7.6.3), residue trials on potatoes were presented that were carried out from 1996-1999 in Germany, UK, Netherlands, France (North and South), Italy, Greece and Spain.

For the WG and WP formulations, 15 trials were presented for EU-N and 13 trials for EU-S that supported the EU critical GAP (10 x 150 g as/ha). For the 2F formulation (equivalent to the 240 SC formulation), 8 trials were presented for EU-N and 8 trials for EU-S that supported the EU critical GAP (10 x 150 g as/ha). At a PHI of 7 days, in all trials and for all formulations, residues of zoxamide (RH-7281) and the metabolites RH-1452 and RH-1455 were <0.02 mg/kg.

The trials data supporting a 7 day PHI are summarised in Table B.7.3.1-1 below.

Table B.7.3.1-1 Summary of residue data for potatoes from Annex I submission

Crop	Residue region	GAP	Formulation	PHI (days)	Crop part analysed	Residue (mg/kg)			Report reference
						RH-7281 (zoxamide)	RH-1452	RH-1455	
Potato	EU-N	10 x 150 g as/ha or 7 x 150 + 3 x 200 g as/ha	75 WG 75 WP	7	Tubers	<0.02 x 15	<0.02 x 15	<0.02 x 15	CA 6.3.1/01 (R66.4/R66.5) CA 6.3.1/02 (R70.3/R70.4) CA 6.3.1/03 (R63.3) CA 6.3.1/05 (R64.4/R64.5) CA 6.3.1/06 (R65.5/R65.6) CA 6.3.1/07 (R64.1) CA 6.3.1/10 (R68.1/R68.2) CA 6.3.1/11 (R68.3/R68.4) CA 6.3.1/14 (R72.5) CA 6.3.1/15 (R72.9) CA 6.3.1/16 (R72.4)
Potato	EU-N	10 x 150 g as/ha or 7 x 150 + 3 x 200 g as/ha	2F	7	Tubers	<0.02 x 8	<0.02 x 8	<0.02 x 8	CA 6.3.1/01 (R66.4/R66.5) CA 6.3.1/02 (R70.3/R70.4) CA 6.3.1/03 (R63.3) CA 6.3.1/05 (R64.4/R64.5) CA 6.3.1/06 (R65.5/R65.6) CA 6.3.1/07 (R64.1)

Crop	Residue region	GAP	Formulation	PHI (days)	Crop part analysed	Residue (mg/kg)			Report reference
						RH-7281 (zoxamide)	RH-1452	RH-1455	
Potato	EU-S	10 x 150 g as/ha or 7 x 150 g as/ha or 4 x 150 + 3 x 200 g as/ha	75 WG 75 WP	7	Tubers	<0.02 x 13	<0.02 x 13	<0.02 x 13	CA 6.3.1/04 (R67.5/R67.6) CA 6.3.1/03 (R63.3) CA 6.3.1/08 (R65.3/R65.4) CA 6.3.1/09 (R64.2/R64.3) CA 6.3.1/07 (R64.1) CA 6.3.1/12 (R66.6/R66.7) CA 6.3.1/17 (R73.2)
Potato	EU-S	10 x 150 g as/ha or 7 x 150 g as/ha or 4 x 150 + 3 x 200 g as/ha	2F	7	Tubers	<0.02 x 8	<0.02 x 8	<0.02 x 8	CA 6.3.1/04 (R67.5/R67.6) CA 6.3.1/03 (R63.3) CA 6.3.1/08 (R65.3/R65.4) CA 6.3.1/09 (R64.2/R64.3) CA 6.3.1/07 (R64.1)

In this submission, the proposed GAP for the representative formulation, Zoxium 240 SC, is 5 applications at 180 g as/ha (total application rate 900 g as/ha), PHI 7 days. This is less critical than the previously evaluated GAP as the total application rate is lower. The residue data for potatoes evaluated in the DAR may therefore be used to support this GAP and a less critical use. At the representative GAP in this submission, residues of zoxamide, and the metabolites RH-1452 and RH-1455, will be <0.02 mg/kg and below the current EU MRL for zoxamide of 0.02 mg/kg.

In addition to the data in the DAR, two residue trials have been conducted on potatoes in 2010, using the product Zoxium 240 SC according to the representative GAP in this submission. These trials are presented below and show that residues of zoxamide at this GAP are below the LOQ (<0.005 mg/kg).

Reference:	CA 6.3.1/18 Luciani, G.P. (2010a) Determination of Zoxamide residues after five application of ELECTIS MZ and ZOXIUM 240 SC on potato – Italian trial, year 2010 Report no.: AGRI 012/10 GLP DEC
Guideline(s):	Commission Directive 96/68/EC amending Council Directive 91/414/EEC Working document 7029/VI/95 Rev.5: Appendix B Italian Decrees: D.L. n. 194/95, D.L. n. 50/07 and D.M. 5 Agosto 1999
Deviations:	None
GLP:	Yes (certified laboratory)
Validity of the study:	Valid
Previous evaluations:	Submitted for the purpose of renewal

Executive Summary

Two trials were conducted on potatoes in Italy in 2010 in which potato plants were treated either with 5 applications of zoxamide formulated as Electis MZ at 150 g as/ha, or with 5 applications of zoxamide formulated as Zoxium 240 SC at 180 g as/ha.

At a PHI of 7 days, residues of zoxamide were below the limit of quantification (<0.005 mg/kg) in all treated and untreated samples of potato tubers.

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material-1:** Zoxamide (RH-7281) formulated as Electis MZ
   Description: Mancozeb/Zoxamide WG formulation
   Lot/batch No.: EM20004668
   Purity: Zoxamide 8.78% (87.8 g/kg)
   Expiry date: December 2011
   Development Code: Not reported

   Test Material-2: Zoxamide (RH-7281) formulated as Zoxium 240 SC
   Description: Zoxamide SC formulation
   Lot/batch No.: 19052010
   Purity: Zoxamide 246.1 g/L
   Expiry date: Not reported

Development Code: Not reported

CAS No.: 156052-68-5
Spiking levels: 0.005 and 0.20 mg/kg

2. Test Commodity
Crop: Potato
Type: Not applicable
Variety: Agata, Elvira
Botanical Name: *Solanum tuberosum*
Crop Part or Processed Commodity: Potato tubers
Sample Size: 2.65-4.76 kg

B. STUDY DESIGN

1. Test Procedure

Two trials were conducted on potatoes in 2010 in Italy. Each trial consisted of three plots: a control (untreated) plot and two treated plots. One treated plot (T1) received 5 applications of Electis MZ (87.8 g/kg zoxamide) at a nominal application rate of 150 g as/ha, and the second treated plot (T2) received 5 applications of Zoxium 240 SC (246 g/L zoxamide) at a nominal application rate of 180 g as/ha. Applications were made using a water volume of about 1000 L/ha.

The first application was made at BBCH 40-42 and subsequent applications were made at intervals of 9 ± 1days. The final application was made at BBCH 47-48, and potato tuber samples collected at BBCH 49, 7 days after the last application.

Samples were stored frozen after collection.

2. Description of analytical procedures

Samples were analysed for zoxamide using method Agri BPL 015 Rev.2 'Determination of zoxamide residues in tomato, potato and grape'. The method was validated on potatoes in this study, and the method validation data are presented in M-CA, Section 4, Point CA 4.1.2/04.

Samples were extracted with acetonitrile, after addition of magnesium sulphate, sodium chloride and buffering citrate salts. The mixture was shaken vigorously and centrifuged to separate the phases. The final extract was analysed directly by HPLC-MS/MS. The limit of quantification (LOQ) for potatoes was 0.005 mg/kg. Mean procedural recoveries are given in Table 6.3.1/2. The zoxamide residues in the treated samples were not corrected for the recovery values.

Samples were stored for up 2 months prior to analysis for zoxamide.

II. RESULTS AND DISCUSSION

Residues of zoxamide were below the method limit of quantification (<0.005 mg/kg) in all the untreated and treated potato samples.

Results of the residue trials are summarised in Table B.7.3.1-2.

III. CONCLUSION

Potatoes were treated with 5 applications of zoxamide formulated either as Electis MZ at 150 g as/ha, or as Zoxium 240 SC at 180 g as/ha.

At a PHI of 7 days, residues of zoxamide were below the limit of quantification (<0.005 mg/kg) in all treated and untreated samples of potato tubers.

RMS:
In Study AGRI 012/10 GLP DEC, final application on potatoes was made at BBCH 47-48, whereas last application is intended to be made at BBCH 80. Therefore these two trials cannot be used to support intended uses on potatoes. Sampes were not tested for metabolites RH-141452 and RH-141455, therefore residue data for those metabolites are not available.

Table B.7.3.1-2 Residues in Potatoes

Trial details	Crop Variety	Country	Product	Application rate			Crop growth stage	Portion analyzed	Zoxamide residue (mg/kg)	PHI (days)	Recovery data
Trials 2010				**g as/ha**	**Water l/ha**	**g as/hl**					
Report Number: AGRI 012/10 GLP DEC Field code RA 10 059 BPL IT 01	Potato/ Agata	Italy Abruzzo, Trasacco (AQ)	Electis MZ	149 154 149 146 145	990 1027 992 970 952	15	BBCH 40 BBCH 43 BBCH 45 BBCH 47 BBCH 48	Tubers	< 0.005	7	Mean recovery at 0.005 mg/kg: 95.8%, CV 14.9%
			Zoxium 240 SC	185 173 177 180 173	1027 960 985 999 958	18	BBCH 40 BBCH 43 BBCH 45 BBCH 47 BBCH 48	Tubers	< 0.005	7	Mean recovery at 0.20 mg/kg: 83.5%, CV 11.1%
Report Number: AGRI 012/10 GLP DEC Field code RA 10 059 BPL IT 02	Potato/ Elvira	Italy Puglia, Chieuti (FG)	Electis MZ	143 147 155 155 155	951 976 1035 1022 1005	15	BBCH 42 BBCH 43 BBCH 45 BBCH 46 BBCH 47	Tubers	< 0.005	7	Mean recovery at 0.005 mg/kg: 95.8%, CV 14.9%
			Zoxium 240 SC	177 186 177 174 183	982 1032 971 1033 1016	18	BBCH 42 BBCH 43 BBCH 45 BBCH 46 BBCH 47	Tubers	< 0.005	7	Mean recovery at 0.20 mg/kg: 83.5%, CV 11.1%

B.7.3.2 Grapes

In the DAR (B7.6 and B7.6.3) and DAR Addendum 1 (Point 6), residue trials on wine and table grapes were presented that were carried out from 1996-1999 in Germany, France (North and South), Italy, Greece and Spain. The data in the DAR are for the WG and WP formulations. Additional data for the 2F formulation (equivalent to the 240 SC formulation) were presented in the Annex I dossier.

For the WG and WP formulations, in Northern Europe (EU-N), 8 trials were presented supporting the German GAP (6 x 15 g as/hL) and a PHI of 28 days, and 15 trials were presented supporting the GAP in Northern France (10 x 150 g as/ha). For Southern Europe (EU-S), 11 trials were presented supporting the GAP in Southern France (10 x 150 g as/ha) and 16 trials were presented supporting the GAP for other southern European countries (10 x 15 g as/hL). For these GAPs, at a PHI of 28 days, residues of zoxamide (RH-7281) were in the range 0.09-1.55 mg/kg in EU-N, and 0.21-2.84 mg/kg in EU-S.

For the 2F formulation, in Northern Europe (EU-N), 8 trials were presented supporting the German GAP (10 x 15 g as/hL) and a PHI of 28 days, and 6 trials were presented supporting the GAP in Northern France (10 x 150 g as/ha). For Southern Europe (EU-S), 2 trials were presented supporting the GAP in Southern France (10 x 150 g as/ha) and 10 trials were presented supporting the GAP for other southern European countries (10 x 15 g as/hL). For these GAPs, at a PHI of 28 days, residues of zoxamide (RH-7281) were in the range 0.47-2.65 mg/kg in EU-N, and 0.30-1.53 mg/kg in EU-S. Four additional trials were presented which were performed in EU-S with the 2F formulation at a GAP of 6 x 150 g as/ha, which gave residues of zoxamide (RH-7281) in the range 0.22-1.86 mg/kg.

The trials data supporting a 28 day PHI are summarised in Table B.7.3.2-1 below.

Table B.7.3.2-1 Summary of residue data for grapes from Annex I submission

Crop	Residue region	GAP	Formulation	PHI (days)	Crop part analysed	Residue (mg/kg)	Report reference
						RH-7281 (zoxamide)	
Grapes	EU-N	6 x 15 g as/hL	75 WG 75 WP	28	Grapes	0.41, 0.45, 0.55, 0.59, 0.60, 0.72, 0.89, 0.93	CA 6.3.2/06 (R71.1/R71.2) CA 6.3.2/11 (R67.2/R67.3)
Grapes	EU-N	10 x 15 g as/hL	2F	28	Grapes	0.76, 0.78, 0.83, 1.02, 1.37, 1.51, 1.67, 2.65	CA 6.3.2/01 (R69.4/R69.5) CA 6.3.2/06 (R71.1/R71.2) CA 6.3.2/11 (R67.2/R67.3)
Grapes	EU-N	10 x 25-43 g as/hL (10 x 125-150 g as/ha)	75 WG 76.25 WG	28	Grapes	0.09, 0.17, 0.19, 0.19, 0.33, 0.35, 0.45, 0.48, 0.50, 0.56, 0.77, 0.77, 0.88, 1.31, 1.55	CA 6.3.2/02 (R60.1) CA 6.3.2/07 (R62.3) CA 6.3.2/12 (R63.1) CA 6.3.2/19 (R 73.3)
Grapes	EU-N	6 x 25-30 g as/hL (6 x 125-150 g as/ha)	76.25 WG	28	Grapes	0.05, 0.11, 0.39, 0.42, 0.43, 0.46, 0.47, 0.48	CA 6.3.2/07 (R62.3) CA 6.3.2/12 (R63.1)
Grapes	EU-N	10 x 25-43 g as/hL (10 x 125-150 g as/ha)	2F	28	Grapes	0.47, 0.50, 0.51, 0.55, 0.67, 0.81	CA 6.3.2/02 (R60.1) CA 6.3.2/07 (R62.3)
Grapes	EU-S	10 x 15 g as/hL (10 x 150 g as/ha)	75 WG 75 WP	28	Grapes	0.24, 0.27, 0.28, 0.29, 0.33, 0.34, 0.36, 0.48, 0.53, 0.54, 0.59, 0.65, 0.81, 1.17, 1.56, 1.92	CA 6.3.2/03 (R70.1/R70.2) CA 6.3.2/08 (R68.5/R68.6) CA 6.3.2/09 (R69.2/R69.3) CA 6.3.2/13 (R66.2/R66.3) CA 6.3.2/14 (R67.4) CA 6.3.2/04 (R71.3/R71.4) CA 6.3.2/05 (R70.5/R70.6)
Grapes	EU-S	6 x 15 g as/hL (6 x 150 g as/ha)	75 WG	28	Grapes	0.30, 0.32, 0.36, 0.38, 0.46, 0.51	CA 6.3.2/08 (R68.5/R68.6) CA 6.3.2/09 (R69.2/R69.3) CA 6.3.2/14 (R67.4)

Crop	Residue region	GAP	Formulation	PHI (days)	Crop part analysed	Residue (mg/kg)	Report reference
						RH-7281 (zoxamide)	
Grapes	EU-S	10 x 15 g as/hL (10 x 150 g as/ha)	2F	28	Grapes	0.30, 0.32, 0.48, 0.56, 0.64, 0.66, 0.82, 1.21, 1.37, 1.42	CA 6.3.2/03 (R70.1/R70.2) CA 6.3.2/08 (R68.5/R68.6) CA 6.3.2/09 (R69.2/R69.3) CA 6.3.2/04 (R71.3/R71.4) CA 6.3.2/05 (R70.5/R70.6)
Grapes	EU-S	10 x 25-43 g as/hL (10 x 125-150 g as/ha)	75 WG 76.25 WG	28	Grapes	0.21, 0.21, 0.33, 0.42, 0.46, 0.49, 0.54, 0.58, 0.63, 1.07, 2.84	CA 6.3.2/02 (R60.1) CA 6.3.2/07 (R62.3) CA 6.3.2/12 (R63.1) CA 6.3.2/20 (R 73.4)
Grapes	EU-S	6 x 25-30 g as/hL (6 x 125-150 g as/ha)	76.25 WG 75 WG	28	Grapes	0.13, 0.21, 0.22, 0.24, 0.25, 0.29, 0.42, 0.43, 0.44, 0.52, 0.52, 0.56, 0.58, 0.61, 0.62, 0.87	CA 6.3.2/07 (R62.3) CA 6.3.2/12 (R63.1) CA 6.3.2/10 (R65.1/R65.2) CA 6.3.2/15 (R66.1) CA 6.3.2/16 (R69.1)
Grapes	EU-S	10 x 30-43 g as/hL (10 x 150 g as/ha)	2F	28	Grapes	1.11, 1.53	CA 6.3.2/02 (R60.1)
Grapes	EU-S	6 x 30 g as/hL (6 x 150 g as/ha)	2F	28	Grapes	0.22, 0.46, 0.63, 1.86	CA 6.3.2/10 (R65.1/R65.2)

In this submission, the proposed GAP for the representative formulation, Zoxium 240 SC, is 5 applications at 180 g as/ha (total application rate 900 g as/ha), PHI 28 days. At a water volume of 1000 L/ha, the application rate is equivalent to 18 g as/hL. This is less critical than the previously evaluated trials data as the reduced number of applications results in a lower total application rate. The residue data for grapes evaluated in the DAR may therefore be used to support this GAP and a less critical use.

In addition to the data in the DAR, two residue trials have been conducted on grapes in 2010, using the product Zoxium 240 SC according to the representative GAP in this submission, and also for comparison the product Electis MZ, a mancozeb/zoxamide WG formulation. These trials are presented below and gave residues of zoxamide at the GAP for Zoxium 240 SC in EU-S of 0.24-0.64 mg/kg. These trials demonstrate that zoxamide residues from the use of Zoxium 240 SC on grapes will be lower than the residues obtained at the more critical GAP previously evaluated for Annex I approval, and well below the current EU MRL of 5 mg/kg.

Reference:	CA 6.3.2/21 Luciani, G.P. (2010b) Determination of Zoxamide residues after five application of ELECTIS MZ and ZOXIUM 240 SC on wine grape and table grape under field conditions – Italian trial, year 2010 Report no.: AGRI 010/10 GLP DEC
Guideline(s):	Commission Directive 96/68/EC amending Council Directive 91/414/EEC Working document 7029/VI/95 Rev.5: Appendix B Italian Decrees: D.L. n. 194/95, D.L. n. 50/07 and D.M. 5 Agosto 1999
Deviations:	None
GLP:	Yes (certified laboratory)
Validity of the study:	Valid
Previous evaluations:	Submitted for the purpose of renewal

Executive Summary

Two trials were conducted on grape vines in Italy in 2010 in which table and wine grape vines were treated either with 5 applications of zoxamide formulated as Electis MZ at 150 g as/ha, or with 5 applications of zoxamide formulated as Zoxium 240 SC at 180 g as/ha.

At a PHI of 28 days, residues of zoxamide from the treatment with Electis MZ were 0.18-0.49 mg/kg, and residues of zoxamide from the treatment with Zoxium 240 SC were 0.24-0.64 mg/kg.

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material-1:** Zoxamide (RH-7281) formulated as Electis MZ
   Description: Mancozeb/Zoxamide WG formulation
   Lot/batch No.: EM20004668
   Purity: Zoxamide 8.78% (87.8 g/kg)
   Expiry date: December 2011
   Development Code: Not reported

   Test Material-2: Zoxamide (RH-7281) formulated as Zoxium 240 SC
   Description: Zoxamide SC formulation

Lot/batch No.:	19052010
Purity:	Zoxamide 246.1 g/L
Expiry date:	Not reported
Development Code:	Not reported
CAS No.:	156052-68-5
Spiking levels:	Wine grapes: 0.01 and 0.75 mg/kg Table grapes: 0.01 and 1.00 mg/kg

2. Test Commodity

Crop:	Grape vines
Type:	Wine grape, table grape
Variety:	Montepulciano d'Abruzzo, Italia
Botanical Name:	*Vitis vinefera*
Crop Part or Processed Commodity:	Grapes bunches
Sample Size:	2.52-10.0 kg

B. STUDY DESIGN

1. Test Procedure

Two trials were conducted on grape vines in 2010 in Italy. Each trial consisted of three plots: a control (untreated) plot and two treated plots. One treated plot (T1) received 5 applications of Electis MZ (87.8 g/kg zoxamide) at a nominal application rate of 150 g as/ha, and the second treated plot (T2) received 5 applications of Zoxium 240 SC (246 g/L zoxamide) at a nominal application rate of 180 g as/ha. Applications were made using a water volume of 1000 L/ha.

The first application was made at BBCH 73-77 and subsequent applications were made at intervals of 9 +/- 1days. The final application was made at BBCH 83-85, and grape bunches collected at BBCH 89, 28 days after the last application.

Samples were stored frozen after collection.

2. Description of analytical procedures

Samples were analysed for zoxamide using method Agri BPL 015 Rev.2 'Determination of zoxamide residues in tomato, potato and grape'. The method was validated on grapes in this study, and the method validation data are presented in M-CA, Section 4, Point CA 4.1.2/04.

Samples were extracted with acetonitrile by shaking using a vortex mixer. Anhydrous magnesium sulphate, sodium chloride and buffering citrate salts (trisodium citrate dehydrate and disodium hydrogencitrate sesquihydrate) were added and the mixture vortex mixed and centrifuged to separate the phases. The final extract was analysed directly by HPLC-MS/MS. The limit of quantification (LOQ) for table and wine grapes was 0.01 mg/kg. Mean procedural recoveries are given in Table 6.3.2/2. The zoxamide residues in the treated samples were not corrected for the recovery values.

Samples were stored for up 1 month prior to analysis for zoxamide.

II. RESULTS AND DISCUSSION

Residues of zoxamide were below the method limit of quantification (<0.01 mg/kg) in all the untreated grape samples.

Following application of zoxamide at 150 g as/ha formulated as Electis MZ, residues of zoxamide in grapes were 0.18-0.49 mg/kg.

Following application of zoxamide at 180 g as/ha formulated as Zoxium 240 SC, residues of zoxamide in grapes were 0.24-0.64 mg/kg. The higher residue levels in these samples reflect the higher application rate of zoxamide in the Zoxium 240 SC treatments.

Results of the residue trials are summarised in Table B.7.3.2-2.

III. CONCLUSION

Table and wine grape vines were treated with 5 applications of zoxamide formulated either as Electis MZ at 150 g as/ha, or as Zoxium 240 SC at 180 g as/ha.

At a PHI of 28 days, residues of zoxamide following application of Electis MZ were 0.18-0.49 mg/kg, and residues of zoxamide following application of Zoxium 240 SC were 0.24-0.64 mg/kg.

Table 7.3.2-2 Residues in Wine and Table Grapes

trial details	Crop Variety	Country	Product	Application rate			Crop growth stage	Portion analyzed	Zoxamide residue (mg/kg)	PHI (days)	Recovery data
Trials 2010				**g as/ha**	**Water l/ha**	**g as/hl**					
Report Number: AGRI 010/10 GLP DEC Field code RA 10 057 BPL IT 01	Wine grape/ Moltepulciamìno d'Abruzzo	Italy Abruzzo, Corropoli (TE)	Electis MZ	147 149 149 156 146	979 992 989 1040 969	15	BBCH 73 BBCH 75 BBCH 77 BBCH 79 BBCH 83	Bunches	0.18	28	Mean recovery at 0.01 mg/kg: 106.0%, CV 3.5%
			Zoxium 240 SC	187 172 188 188 172	1040 951 1045 1041 953	18	BBCH 73 BBCH 75 BBCH 77 BBCH 79 BBCH 83	Bunches	0.24	28	Mean recovery at 0.75 mg/kg: 98.9%, CV 11.5%
Report Number: AGRI 010/10 GLP DEC Field code RA 10 057 BPL IT 02	Table grape/ Italia	Italy Abruzzo, Roseto degli Abruzzi (TE)	Electis MZ	151 144 155 157 143	1005 961 1029 1045 952	15	BBCH 77 BBCH 79 BBCH 81 BBCH 83 BBCH 85	Bunches	0.49	28	Mean recovery at 0.01 mg/kg: 100.5%, CV 9.0%
			Zoxium 240 SC	182 178 188 189 173	1014 989 1045 1050 962	18	BBCH 77 BBCH 79 BBCH 81 BBCH 83 BBCH 85	Bunches	0.64	28	Mean recovery at 1.00 mg/kg: 105.2%, CV 4.4%

RMS:

Three open literature studies were submitted as potential relevant.

- Česnik H.B, Velikonja-Bolta S, Gregorčič A, 2012

Pesticide residues in samples of apples, lettuce and potatoes from integrated pest management in Slovenia from 2005-2009
Analytical survey of pesticide residues in selected crops. No data on zoxamide.
No impact on human health assessment.

- Česnik H.B, Velikonja-Bolta S, Gregorčič A, 2010

Pesticide residues in cauliflower, eggplant, endive, lettuce, pepper, potato and wheat of the slovene origin found in 2009.
Analytical survey of pesticide residues in selected crops. No MRL excedences identified.
No impact on human health assessment.

- Česnik H.B, Velikonja-Bolta S, Gregorčič A., 2011

Pesticide residues in agricultural products of the slovene origin found in 2007
Analytical survey of pesticide residues in selected crops. No useable data on zoxamide as no information provided on GAPs etc.
No impact on human health assessment.

B.7.4 Feeding studies

Grape pomace is not fed to livestock. Potatoes may be fed to livestock, however residues of zoxamide in potatoes were <0.02 mg/kg in all trials and therefore will not result in significant residues occurring in the diets of livestock. The proposed residue definition in crops is parent zoxamide only, therefore the dietary burden calculations presented below have been performed using the residue levels of zoxamide only.

Table 7.4-1 Zoxamide residue values used for calculations of dietary burdens

Commodity	Crop group	STMR (mg/kg)	HR (mg/kg)
Potatoes	Roots and tubers	<0.02	<0.02

The dietary burden calculations for beef and dairy cattle, lamb, poultry and pigs are presented in Table B.7.4-2. These demonstrate that the dietary burdens are all below 0.004 mg/kg bw/day, and therefore livestock feeding studies are not required.

Table B.7.4-2 Theoretical maximum daily intakes of zoxamide residues by domestic animals calculated with OECD animal burden calculator

Animal	Intake	
	mg/kg bw/day	mg/kg diet (DM)
Dairy cattle	0.001	0.03
Beef cattle	0.001	0.03
Ram/Ewe	0.001	0.03
Lamb	0.001	0.03
Swine breeding	0.001	0.05
Swine finishing	0.002	0.05
Broiler	0.001	0.01
Poultry layer	0.001	0.01
Poultry turkey	0.001	0.02

B.7.4.1 Poultry

The representative uses on potatoes and grapevines do not result in significant residues occurring in the diet of poultry, and therefore a poultry feeding study is not required or submitted.

B.7.4.2 Ruminants

The representative uses on potatoes and grapevines do not result in significant residues occurring in the diet of ruminants. The goat metabolism study shows that no significant residue will occur in any edible animal tissue as a result of residues in feedstuffs at the 1x rate, and therefore a cow feeding study is not required or submitted.

B.7.4.3 Pigs

The representative uses on potatoes and grapevines do not result in significant residues occurring in the diet of pigs. In addition, the metabolism of zoxamide is qualitatively similar in the goat and the rat, and therefore pig metabolism and feeding studies are not required or submitted.

B.7.4.4 Fish

Information on the crops used for the preparation of fish feedstuffs is provided in the draft guidance document on the nature or residues in fish (SANCO/11187/2013, 31.01.2013 rev.3). Grapes are not used for the preparation of fish feedstuffs. Root and tuber vegetables are used only in small quantities for fish feedstuffs, and potato protein comprises a maximum of 3% of the diet for carp, and is not used in trout feed. Residues of zoxamide (and the metabolites RH-141455 and RH-141452) in potatoes are <LOQ, therefore residues in fish feed will be <0.1 mg/kg diet.

Zoxamide has a log P_{OW} of 3.76, and therefore has potential to bioaccumulate, however as residues of zoxamide in fish feed will be <0.1 mg/kg diet, fish metabolism and feeding studies are not required.

B.7.5 Effects of processing

B.7.5.1 Nature of the residue

Processing studies are not required for potato as residues of zoxamide (and the metabolites RH-141455 and RH-141452) are <0.02 mg/kg (<LOQ).

To investigate the nature of residues following processing of grapes, a radiolabelled vinification study was performed.

Reference:	CA 6.5.1/01 Mamouni, A., (1998) 14C-RH-117281: Vinification Study Report no.: 34-98-151, December 3
Guideline(s):	Commission Directive 96/68/EC Section 6.5
Deviations:	
GLP:	Yes
Validity of the study:	
Previous evaluations:	In DAR (2001)

In a vinification study ^{14}C phenyl labelled RH-7281 (Radiochemical purity 97.81%)in solution in acetone was sprayed uniformly onto bunches of fresh white or red grapes in a glass aquarium at the rate of 3 mg ^{14}C-RH-7281 per kg grapes. The spray bottle was rinsed again with acetone and re-applied onto the

grapes. After evaporation of the acetone, the grapes were crushed by hand. The must (fresh juice) was separated from the mixture by filtration through a nylon bag. After filtration, the remaining skins, pips and stalks in the nylon bag were hand pressed to force out practically all of the juice. The musts were then transferred into 1 litre fermenting flasks (also with skins and stems), where they fermented in a closed system, under nitrogen at 20 °C for 20-30 days in the presence of yeast. Wine was clarified and decanted, bottled, and stored at approximately 12 °C for 8 months. The following six types of wine were prepared:

Wine I:	White grapes
Wine II:	White grapes, heated must
Wine IX:	White grapes, air dried before crushing and pressing (repeat of Wine I with thorough removal of acetone)
Wine V:	Red grapes, pressed after crushing (Rosé)
Wine VI:	Red grapes, fermented with skins 4 days before pressing
Wine VII:	Red grapes, pressed after crushing, heated must

In all cases yeast was added following the process described above. In addition, three control wines (two white and one red) were prepared from grapes treated only with acetone.

Samples of the must were taken before fermentation and at the end of fermentation. Wine samples were taken after 2, 4, and 8 months of storage at 12 °C. Radioactive residues were determined by LSC (Grapes and debris were homogenised and combusted prior to LSC). The nature of the residue determined by HPLC analysis using a a C 18 column with 14C and/ or UV detection. Selected results were confirmed by TLC. The distribution of radioactivity through the pressing step for each wine is shown in tables B.7.5.1-1 and B.7.5.1-2. Only the levels of parent RH-7281 and the degradation product RH-150721 are provided. Ten other compounds were present in the HPLC. However, none comprised more than 3.8% of the total applied radioactivity at any time point in any sample. During fermentation, very low amounts of radioactive carbon dioxide were detected (<0.1 % of the initial dose). No radioactive residues were detected in the control musts.

Table B.7.5.1-1 Recovery of radioactive residues after processing the treated grapes

Wine Sample	Residues in fresh juice %	Residues in air dried marc %	Residues in Washings[1] %	Total Recovery %
Wine I & II	49.6	32.2	8.5	90.2
Wine IX	36.3	45.2	11.4	92.8
Wine V	25.0	58.2	5.1	88.3
Wine VI	20.8	58.9	12.2	91.9
Wine VII	17.1	69.6	2.5	89.2

[1] Radioactivity recovered in the rinsings from the aquarium and application devices

Table B.7.5.1-2 Radioactive residues in must and wine from treated grapes

	Fresh juice without filtration (used for fermentation)	Fresh juice after filtration for HPLC	At end of fermentation	After 2 months ageing	After 4 months ageing	After 8 months ageing
	mg/l %	mg/l %	mg/l %	mg/l %	mg/l %	mg/l %
White Wine I						
TRR	2.300 49.4	0.927 19.9	1.565 33.6	1.385 29.8	1.460 31.4	1.379 29.6
Parent RH-7281	-	0.816 17.5	0.617 13.3	0.352 7.6	0.267 5.7	0.124 2.7
RH-150721	-	-	0.647 13.9	0.702 15.1	0.828 17.8	0.903 19.4
White Wine II						
TRR	2.300 49.4	0.927 19.9	1.432 30.8	1.365 29.3	1.325 28.5	1.286 27.6
Parent RH-7281	-	0.816 17.5	0.640 13.8	0.403 8.7	0.309 6.6	0.139 3.0
RH-150721	-	-	0.454 9.8	0.591 12.7	0.656 14.1	0.788 16.9
White Wine IX						
TRR	1.702 36.3	0.768 16.4	1.130 24.1	1.133 24.1	1.153 24.6	1.085 23.1
Parent RH-7281	-	0.667 14.2	0.512 10.9	0.476 10.1	0.294 6.3	0.124 2.6
RH-150721	-	-	0.322 6.9	0.493 10.5	0.490 10.4	0.542 11.5
	Mg/l %	mg/l %	mg/l %	mg/l %	mg/l %	mg/l %

Rose Wine V

TRR	1.121 25.0	-	0.616 13.7	0.622 13.9	0.656 14.6	0.639 14.3
Parent RH-7281	0.955 21.3	-	0.244 5.4	0.142 3.2	0.108 2.4	0.077 1.7
RH-150721	-	-	0.249 5.6	0.330 7.4	0.368 8.2	0.400 8.9
	Fresh juice without filtration (used for fermentation)	Fresh juice after filtration for HPLC	At end of fermentation	After 2 months ageing	After 4 months ageing	After 8 months ageing
	mg/l %	mg/l %	mg/l %	mg/l %	mg/l %	mg/l %

Red Wine VI

TRR	0.897 20.8	0.0394 9.1	0.553 12.8	0.529 12.2	0.499 11.6	0.418 9.7
Parent RH-7281	0.695 16.1	0.129 3.0	0.136 3.2	0.082 1.9	0.027 0.6	
RH-150721	0.029 0.7	0.094 2.2	0.268 6.2	0.204 4.7	0.266 6.2	0.134 3.1

Red Wine VII

	fresh juice before the start of fermentation	Before the end of the fermentation	at end of fermentation	after 2 months storage	after 4 months storage	after 8 months storage
TRR	0.780 17.1	0.373 8.2	0.488 10.7	0.525 11.5	0.526 11.6	0.399 8.8
Parent RH-7281	0.546 12.0	0.125 2.8	0.103 2.3	0.064 1.4	0.034 0.7	
RH-150721	0.036 0.8	0.139 3.1	0.225 5.0	0.269 5.9	0.228 5.0	0.172 3.8

RMS:

Potato – Supervised residue trials data from Annex I submission and residue trials submitted for purpose of renewal of active substance, shows that no residues above the LOQ are detected in potato samples. Therefore RMS agrees with applicant that processing studies are not required for potato.

Grapes – Studies are required as residues in grapes exceeds trigger value of 0.1mg/kg. Radiolabelled vinification study was performed and evaluated for Annex I inclusion.This study is also reported in RAR. No studies simulating hydrolytic conditions for industrial processing (pasteurisation, baking, brewing, boiling, sterilisation) are submitted. Such studies are required according to EU Regulation No 283/2013 and OECD document 507 „Nature of the pesticide residues in processed commodities – high temperature hydrolysis". **Therefore RMS is not able to conclude on Nature of residues for processed commodities.**

B.7.5.2 Distribution of the residue in peel and pulp

Residues in peel and pulp are not relevant to grapes. Peeling studies are not required for potato as residues of zoxamide (and the metabolites RH 141455 and RH 141452) are <0.02 mg/kg (<LOQ). Nevertheless, peeling was conducted in two trials (CA 6.3.1/16 and CA 6.3.1/17) which showed that residues of zoxamide, RH 141455 and RH 141452 were <0.02 mg/kg (<LOQ) in potato RAC, peel and peeled potatoes.

B.7.5.3 Magnitude of residues in processed commodities

Potato

Processing studies are not required for potato as residues of zoxamide (and the metabolites RH 141455 and RH 141452) are very low, <0.02 mg/kg (<LOQ) in studies for AnnexI inclusion and <0.005mg/kg (<LOQ) in studies performed in 2010 using the product Zoxium 240 SC according to the representative GAP in this submission.

Grapes

Reference:	CA 6.5.3/01 Graves, DD. (1998) RH-117281 80W and 2F Residue Studies in Grapes and Grape Process Fractions 1996 and 1997 Trials Report no.: 34-98-154
Guideline(s):	US EPA-OPPTS 860.1520
Deviations:	None
GLP:	Yes
Validity of the study:	
Previous evaluations:	In DAR (2001)

In US supervised field trials, grapes were treated with RH-7281 80W formulation. 10 applications were made at rates of either 1.4 or 2.8 kg a.s/ha. Grapes were harvested 14 days after the final application. Samples were either processed into juice on the day of harvest or processed into raisins over the following few weeks. Processing was stated by the Applicant to be in accordance with commercial practice. Processed fractions were then frozen until required for analysis. Residues in the grapes and processed

fractions were measured by the following validated analytical methods: Grapes, Martin, 1996 (DP81821), Grape juice, Kendi 1998a (DP81824) and Raisins, Kendi 1998b

(DP 81825). For each method, the LOQ was determined to be 0.01 mg/kg. Residues in the processed components are summarised in table B.7.5.3-1

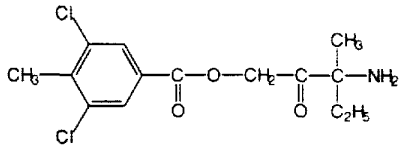
Table 7.5.3-1 Summary of processing data for grape juice and raisins

Commodity	Application at 1.4 kg/ha, PHI 14 days		Application at 2.8 kg/ha, PHI 14 days		Average PF	Reference
	mg/kg	PF	mg/kg	PF		
Grapes (RAC)	0.31	-	0.39	-	-	CA 6.5.2/02 (R62.1)
Unclarified juice	0.050	0.16	0.039	0.10	0.13	
Clarified juice	0.014	0.05	0.021	0.05	0.05	
Raisins	0.70	2.2	1.4	3.5	2.9	

PF = processing factor

Twelve grape processing trials were also presented in the DAR (2001) in which grapes from residue trials conducted in 1996 and 1997 were processed to wine. Samples of grapes, must and pomace were analysed for residues of zoxamide (RH-7281), and samples of wine were analysed for residues of zoxamide (RH-7281) and the metabolite RH-150721. Wine samples were analysed at the end of fermentation and after 6 months to 1 year's ageing.

In a vinification study 14C-RH-7281 in solution in acetone was sprayed onto bunches of fresh white or red grapes. The grapes were crushed by hand and the filtered musts were fermented into wine. Wine was clarified and decanted, bottled, and stored at approximately 12 °C for 8 months. In the filtrated must, parent 14C-RH-7281 represented the main radioactive fraction. The total radioactive residue at the end of fermentation was 24-34% of the applied activity in the white wines and 11-14% of the applied activity in the red wines, with parent RH-7281 and RH-150721 at roughly equal concentrations. Total residues declined slightly during ageing (approximately 10%, average). Parent RH-7281 declined by 69-100% (average 84%) over 8 months. In general, RH-150721 increased in concentration over the ageing period. However, in red wines where concentrations of parent were low at the end of the fermentation, residues of RH-150721 began to decline toward the end of the ageing period.



RH-150721

It was observed that clarifying the wine by Bentonite and centrifugation removed significant amounts of residues (up to 49% of the residues in white wines and 31 % of the residues in red wines).

Table B.7.5.3-2 Summary of processing data for wine

Commodity	Zoxamide (RH-7281)			RH-150721		Reference
	Residue range mg/kg	STMR mg/kg	Median PF	Residue range mg/kg	STMR mg/kg	
Grapes	0.23-3.06	0.61	-			CA 6.3.2/01 (R69.4/R69.5) CA 6.3.2/02 (R60.1) CA 6.3.2/03 (R70.1/R70.2) CA 6.3.2/06 (R71.1/R71.2) CA 6.3.2/07 (R62.3) CA 6.3.2/08 (R68.5/R68.6)
Pomace	0.02-2.04	0.26	0.13			
Must	0.02-1.66	0.16	0.29			
Young wine	<0.01	<0.01	<0.02	<0.01-0.49	0.03	
Aged wine	<0.01	<0.01	<0.02	0.01-0.49	0.04	

PF = processing factor

Residues in grapes ranged from 0.23 - 3.06 mg/kg. In musts, residues were in the range 0.02 – 1.66. In only two sample of young wine were residues found at levels above the LOQ (0.06 and 0.07 mg/kg). When wine was aged residues of the parent RH-7281 declined and residues of the metabolite RH 150721 increased slightly. Levels of RH-150721 were in the range 0.01 to 0.49 mg/kg.

Reference:	CA 6.5.3/02 Wais, A. (2001) Determination of residues of RH-117,281 and mancozeb in/on vine grapes (RAC grapes and processing products) following treatment with RH-7281/mancozeb 75WG from a field trial (semi residue decline study) in Italy; 1999 Report no.: 734580. ER Ref: R77.10
Guideline(s):	Commission Directive 96/68/EC Working document 1607/VI/97 Rev.1 and 7029/VI/95 Rev.5
Deviations:	None
GLP:	Yes
Validity of the study:	Valid
Previous evaluations:	Submitted for the purpose of renewal

Executive Summary

A trial was conducted on grape vines in Italy in 1999 in which wine grape vines were treated with 10 applications of a 75 WG formulation at 15 g zoxamide/hL + 120 g mancozeb/hL. Grapes were collected at 0, 14 and 28 days after the last application, and the 28 day PHI samples were processed to pomace, must and wine.

At a PHI of 28 days, residues of zoxamide were 1.32 mg/kg in grapes, 1.18 mg/kg in pomace, 1.23 mg/kg in must and <0.01 mg/kg in wine. Residues of the metabolite RH-150721 in wine were 0.15 mg/kg.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Zoxamide (RH-7281) formulated as a 75 WG
Description: Mancozeb/Zoxamide WG formulation
Lot/batch No.: 9803
Purity: Zoxamide 8.33% (nominal), 8.55% (certified)
Expiry date: 30 April 2000
Development Code: 50340

CAS No.: 156052-68-5
Spiking levels: RH-7281 in grapes, pomace, must, wine: 0.01 – 3.0 mg/kg
RH-150721 in wine: 0.01 and 2.0 mg/kg

2. Test Commodity
Crop: Grape vines
Type: Wine grape
Variety: Barbera
Botanical Name: *Vitis vinefera*
Crop Part or Processed Commodity: Grapes bunches, pomace, must, wine
Sample Size: Grapes: 1.1-1.4 kg
Grapes for processing: 37.5-40.5 kg
Must 21.6-22.3 kg
Pomace 3.5-3.6 kg
Wine 15-16 kg

B. STUDY DESIGN

1. Test Procedure

A trial was conducted on grape vines in 1999 in Italy. The trial consisted of two plots: a control (untreated) plot and a treated plot. The treated plot (T1) received 10 applications of a 75 WG formulation (8.33% zoxamide (RH-7281)/66.67% mancozeb) at a nominal application rate of 15 g as/hL (equivalent to 66-166 g as/ha). Applications were made using a water volume of 426-1075 L/ha.

The first application was made at BBCH 69 and subsequent applications were made at intervals of 7-10 days. The final application was made at BBCH 89, and grape bunches collected at BBCH 89, 0, 14 and

28 days after the last application. Leaves were also collected, but were not analysed. A large grape sample for processing was collected at a PHI of 28 days.

Samples for residue analysis were stored frozen after collection.

2. Processing

Grapes were crushed using a horizontal drum stemmer with stalk separator. Fresh must with skins was placed in a tank and inoculated with yeast. A sample of must (without skins) was collected.

Fermentation started after 6 hours and continued for 9 days, with the cap being punched down in days 3 to 8. A sample of pomace was collected at the end of the fermentation period.

14 days after must preparation, fresh wine was separated from the skins, decanted into a glass container and $K_2S_2O_5$ was added. After 16 days, the wine was decanted for a second time into a new glass container and stored in a cool place (11-12°C) for 76 days. A malo-lactic fermentation with heating was then performed, and the wine decanted. The wine was filtered, $K_2S_2O_5$ was added and the wine filled into bottles. A sample of wine was collected.

3. Description of analytical procedures

Samples of grapes, must, pomace and wine were analysed for zoxamide using validated method TR 34-98-150 (CA 4.2.1/08 (ER 15.3)). The method was validated on grapes (report 647177), must (report 676888) and pomace (report 673683). Samples of wine were analysed for zoxamide and RH-150721 using validated method TR 34-98-179 (CA4.2.1/12 (ER29.15)). The method was validated on wine (report 707310). The method validation data were presented in the DAR, Point B5.2.

Samples of grapes were extracted by homogenisation with methanol/water (80:20 v/v). The extract was filtered through celite and the filtrate concentrated by rotary evaporation. After the addition of 0.1M sodium chloride solution, the extract was partitioned twice against dichloromethane. The combined organic phases were dried with sodium sulphate, evaporated to dryness and redissolved in hexane. The extract was cleaned-up by SPE using an ENVI-Carb SPE column, followed by an LC-Alumina-B SPE column. The ethyl acetate/hexane eluate was collected, evaporated to dryness and redissolved in hexane for final determination by GC-ECD.

Grape pomace was extracted by homogenisation with methanol/water (80:20 v/v). The extract was filtered through celite and the filtrate concentrated by rotary evaporation. After the addition of 0.1M sodium chloride solution, the extract was partitioned twice against hexane and twice against dichloromethane. The combined organic phases were evaporated to dryness and redissolved in ethyl acetate/hexane (10:90 v/v). The extract was cleaned-up by SPE using a carbon cartridge, followed by a Florisil column and an LC-Alumina-B SPE column. The ethyl acetate/hexane eluate was collected, evaporated to dryness and redissolved in hexane for final determination by GC-ECD.

Must was extracted by adding 0.1M sodium chloride solution and partitioning twice against dichloromethane. The combined organic phases were evaporated to dryness and redissolved in ethyl acetate/hexane (5:95 v/v). The extract was cleaned-up by SPE using a carbon cartridge, followed by an LC-Alumina-B SPE column. The ethyl acetate/hexane eluate was collected, evaporated to dryness and redissolved in hexane for final determination by GC-ECD.

Wine was extracted by adding 1% potassium hydrogen carbonate and partitioning against ethyl acetate. The organic phase was rotary evaporated to a reduced volume and made up to a known volume with ethyl acetate. Final determination was by GC-ECD.

The limit of quantification (LOQ) for was 0.01 mg/kg for zoxamide in grapes, must, pomace and wine, and 0.01 mg/kg for RH-150721 in wine. Procedural recoveries are given in Table 6.5.3/2.

Grape samples were stored for up 5 months prior to analysis, and pomace, must and wine samples were stored for up to 4 months prior to analysis.

II. RESULTS AND DISCUSSION

Residues of zoxamide (RH-7281) were below the method limit of quantification (<0.01 mg/kg) in all the untreated grape, must pomace and wine samples. Residues of RH-150721 were also below the method limit of quantification (<0.01 mg/kg) in the untreated wine sample.

At a PHI of 0 days, residues of zoxamide were 2.09 mg/kg in grapes. At a PHI of 28 days, residues of zoxamide declined to 1.32 mg/kg in grapes. Following processing of the 28 day PHI grape sample, residues of zoxamide were 1.18 mg/kg in pomace, 1.23 mg/kg in must and <0.01 mg/kg in wine. Residues of the metabolite RH-0721 in wine were 0.15 mg/kg.

Results of the residue/processing trial are summarised in Table B.7.5.3-3.

III. CONCLUSION

Grape vines were treated with 10 applications of a 75 WG formulation at 15 g zoxamide/hL + 120 g mancozeb/hL.

At a PHI of 28 days, residues of zoxamide were 1.32 mg/kg in grapes, 1.18 mg/kg in pomace, 1.23 mg/kg in must and <0.01 mg/kg in wine. Residues of the metabolite RH-0721 in wine were 0.15 mg/kg.

Processing factors were 0.93 for must, 0.89 for pomace, and <0.01 for wine.

Table B.7.5.3-3 Residues in grapes and processed fractions

Trial details	Crop Variety	Country	Product	Application rate			Crop growth stage	Portion analyzed	Residue (mg/kg)		PHI (days)	Recovery data
Trial 1999				**g as/ha**	**Water l/ha**	**g as/hl**			**Zoxamide**	**RH-150721**		
Report Number: 734580 (R77.10) Field code A/IT/F/99/66	Wine grape/ Barbera	Italy Mongardino, Asti	75 WG	66 66 79 160 164 164 165 166 165 163	426 431 512 1039 1064 1063 1070 1075 1070 1057	15.4 15.4 15.4 15.4 15.4 15.4 15.4 15.4 15.4 15.4	BBCH 6:69 BBCH 7:71 BBCH 7:73 BBCH 7:76 BBCH 7:79 BBCH 8:81 BBCH 8:83 BBCH 8:84 BBCH 8:85 BBCH 8:87 BBCH 8:89	Grapes Grapes Pomace Must Wine	2.09 1.32 1.18 1.23 <0.01	0.15	0 28 28 28 28	Recoveries RH-7281: Grapes: 82.9-97.3% Pomace 76.4-109.7% Must 71.2-78.9% Wine: 91.5-103.3 Overall mean: 87.4% RH-150721 Wine: 99.3-100.8% Mean: 100.1%

RMS:

It is not possible to conclude about processing factors and magnitude of residues in processed commodities as long as nature of residues in processed commodities is not clearly demonstrated.

B.7.6 Residues in rotational crops

B.7.6.1 Metabolism in rotational crops

Reference:	CA 6.6.1/01 Kim-Kang, H., (1998) 14C-RH-117,281: Cofined Rotational Crop Study Report no.: 34-98-144, December 4
Guideline(s):	US EPA 40 CFR 158.290: Environmental Fate Subdivision N, series 165-1
Deviations:	
GLP:	Yes
Validity of the study:	Valid
Previous evaluations:	In DAR (2001)

In an outdoor confined rotation study conducted in North Carolina, mustard, radish, turnip, sorghum and soybean were planted at 30, 137, 210, 365 days following the last of four applications of ^{14}C phenyl labelled RH-7281 (Radiopurity of spray samples used for each treatment was determined to be >96% by 1-D TLC and HPLC). The active substance, formulated as an emulsifiable concentrate, was applied to bare soil between mid April and early June (18 day intervals) at a rate of 0.5 kg/ha. Due to crop failure resulting from extreme weather conditions, 30 DALA mustard and radish crops were replanted.

Crops were harvest at an intermediate stage and when mature. Crop and soil samples were processed within 1 month of harvest. Soil and plants samples were cryogenically milled and total radioactive residues (TRR) were determined by combustion analysis and LSC. Crop components (e.g., roots and tops, grain and straw) were analyzed separately. TRRs were very low for all samples at all plantback intervals. The results are shown in table 7.6.1-1

Table B.7.6.1-1 Total radioactive residues (mg/kg as parent equivalents) determined in rotational crops

Plantback Interval	Crop Component	Intermediate Harvest	Mature Harvest
30 days	Mustard	0.051[1], 0.078[2]	0.041
30 days	Radish root	<u>0.127[1],</u> 0.033[2]	0.023
30 days	Radish top	0.063[1], 0.043[2]	0.048
30 days	Sorghum stover	0.050	0.027
30 days	Sorghum grain	NA	0.026
30 days	Soybean hay	0.099	<u>0.189</u>
30 days	Soybean seed	NA	0.092

137 days	Mustard	0.020	< 0.01
137 days	Turnip root	0.023	0.042
137 days	Turnip top	0.011	< 0.01
137 days	Wheat grain	NA	< 0.01
137 days	Wheat straw	< 0.010	< 0.01
210 days	Mustard	0.042	0.030[3]
210 days	Radish root	0.024	0.026[3]
210 days	Radish top	0.038	0.031[3]
210 days	Sorghum stover	0.017	< 0.01
210 days	Sorghum grain	NA	0.011
210 days	Soybean hay	0.034	0.024
210 days	Soybean seed	NA	0.020
365 days	Mustard	0.019	0.011[3]
365 days	Radish root	0.014	0.011[3]
365 days	Radish top	0.012	0.011[3]
365 days	Sorghum stover	< 0.010	0.012
365 days	Sorghum grain	NA	0.014
365 days	Soybean hay	0.019	0.014
365 days	Soybean seed	NA	0.012

[1] Crop samples harvested from the first planting.
[2] Crop samples harvested from the second planting.
[3] 210 and 365 DALA mature mustard and radish samples thawed due to freezer failure
NA: not applicable: indicates that either no sample was harvested or the sample was not planted at the designated plant back interval.

The TRR found in soyabean, sorghum, and radish samples grown in [^{14}C]RH-117281-treated soil decreased significantly with increasing plantback time. The residue concentration in mustard leaf also decreased considerably with increasing plantback time. Although the residue concentration in mustard leaf grown in the 137 DALA plot was somewhat lower than the level in the 210 DALA sample, the diference was not considered significant. The residue concentrations in wheat grain and turnip top samples grown in the 137 DALA plot were ≤0.011 ppm. The TRR found in intermediate and mature turnip root grown in the 137 DALA plot were 0.023 and 0.042 ppm, respectively. Radish and mustard samples in the 30 DALA plot were replanted due to the

loss of crops after intermediate samples were harvested. The crop losses resulted from adverse weather conditions.
All crop samples with residue levels higher than 0.01 mg/kg, except for intermediate mustard leaf (137 DALA) and intermediate turnip root and top (137 DALA), were subjected to solvent extraction procedures and analysis by HPLC, TLC, and LC/MS.

Homogenized crop samples were extracted with MeOH:H2O:CHC13 and then with CHCl3 and the extracts were combined. Following separation, the CHCl3 fraction wase first concentrated and partitioned with a mixture of CH3CN and hexane to yield a CH3CN-soluble fraction and a hexane-soluble fraction. Duplicate aliquots of each fraction were taken for LSC. The post-extraction solids (PES) were allowed to dry and were then subjected to combustion analysis.

In general, the amount of extractable residues was low in all the crop samples. Between 7% and 40% of the TRR was distributed into the polar MeOH/H20 fractions for all the crops grown in treated soil. About 2-36% of the TRR was found in the organic extracts (CHCl3, CH3CN and hexane) of all the crops. The levels in these samples did not exceed 0.023 mg/kg. The extraction values for all the crop samples showed a significant percentage of unextractable residues; generally 49% or greater except for 365 DALA mature radish root which contained ≈35% of the TRR as unextractable residues.

Analyses of metabolites in various fractions were performed using HPLC with radiometric detection. The overall percent distribution of metabolites in the extractable fractions showed metabolite A-II and metabolite I as the major metabolites in most crop samples. However, only in samples of the 30 DALA soybean forage did levels exceed 0.01 mg/kg (Levels were 0.016 and 0.023 mg/kg, respectively). Other metabolites were detected at low levels in some crops. Metabolite I was proposed as RH-141,452 based on its comparable HPLC retention time to that of an authentic reference standard. However, given the low level of residue in all crops, the metabolites were not further characterized.

Only intermediate 30 DALA radish root samples from the first planting contained Metabolite A-1 at a level greater than 0.01 mg/kg.

Among the PES fractions, those containing greater than 0.050 mg/kg of the TRR were subjected to a series of hydrolyses using enzyme, acid, and base until the residue in the terminal PES fraction was below 0.050 mg/kg. The bound residue fraction from 30 DALA soybean forage was subjected to enzyme hydrolysis using cellulase, which yielded 7.68% (0.008 mg/kg) of the TRR in the hydrolysate. The hydrolysate was not further analyzed due to the low residue level.

B.7.6.2 Magnitude of residues in rotational crops

No supervised field trials were conducted to investigate residues in succeeding crops. However, in the confined rotational crop metabolism study, the only crops to contain total radioactive residues greater than 0.1 mg/kg were immature radish (0.127 mg/kg) and soybean hay (0.189 mg/kg). Both crops were planted 30 days after bare soil was treated (4 applications at 18 day intervals) at a rate of 500 g/ha. Therefore residues in succeeding crops are not considered to be of concern.

B.7.7 Other studies

B.7.7.1 Effect on the residue level in pollen and bee products

This is not applicable to the current application for renewal of approval of the a.s.

B.7.8 References relied on A literature review report on zoxamide has been submitted by the applicant in the framework of this renewal. Twenty two databases have been searched (AGRICOLA, AGRIS International, Aqualine, ASFA, BIOSIS® Toxicology , BIOSIS Previews® , CAB Abstracts, EMBASE, Environment Abstracts, Foodline®: SCIENCE, FSTA®, GEOBASE, GeoRef, MEDLINE, Meteorological and Geoastrophysical Abstracts, PASCAL, Pollution Abstracts, ToxFile, Toxicology Abstracts, TOXLINE, Water Resources Abstracts) and their choices have been justified.

The following key words were searched: zoxamide, CAS number 156052-68-5, company developmental name RH-117,281 and RH-7281, PPP Zoxium 240 SC, IUPAC names, chemical names and primary metabolites of concern, plus synonyms (RH-141,452, RH-141,455, RH-150,721, RH-24549, RH-139432, RH-127450, RH-163353).

Public literature

Studies published since 2004 have been looked for in order to include the most recent scientific peer-reviewed open literature.

Following assessment of titles and abstracts seven articles were obtained for review of the full text to assess their reliability and detailed relevance. Publications meeting the relevance criteria were those showing new/unknown effects or information potentially contradictory to the regulatory data package for the active substance, its relevant metabolites and/or the plant protection product on human health, animal health and/or the environment, which could impact the endpoints or the risk assessment parameters.

Following assessment of the output from these searches, none of the results met the relevant criteria for residue section.

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	Vertebrate study Y/N	Data Protection Claimed Y/N	Justification if data protection claimed	Owner
CA, 6.1/01	IIA, 6.	Ross, JR	1998a	Ross, J.R., Storage Stability of RH-117281 Residues in Grapes, Grape Juice, Raisins and Potatoes under Conditions of Frozen Storage, Rohm and Haas Technical Report No. 34-98-161, December 15, 1998, GLP, unpublished. ER ref. no. R 61.1	N	N	NA	Gowan
CA, 6.1/02	IIA, 6.	Ross, JR	1998b	Ross, J.R., Stability of RH-141455 and RH-141452 Residues in Potatoes, Potato Chips, and Potato Flakes under Conditions of Frozen Storage, Rohm and Haas Technical Report No. 34-98-162, December 15, 1998, GLP, unpublished. ER ref. no. R 61.2	N	N	NA	Gowan
CA, 6.1/03	IIA, 6 **See add. vol August 2000**	Reibach, P.H.	2000	Storage Stability of RH-117,281 Residue in Potato Samples under Conditions of Frozen Storage:Supplement to TR34-98-161 (ER 61.1) Rohm and Haas unpublished Technical Report No. 34-00-80 September 2, 2000 ER ref. no. R 77.11 (submitted with 44.7)	N	N	NA	Gowan
CA, 6.2.1/01	IIA, 6.1/01	Reibach, PH, Spencer, WO	1998a	Reibach, PH and Spencer, WO, 14C-RH-117,281: Nature of the Residue in Fruiting Grape Plants, Rohm and Haas Technical Report No. 34-98-49, October 1, 1998, GLP, unpublished. ER ref. no. 14.5	N	N	NA	Gowan
CA, 6.2.1/02	IIA, 6.1/02	Reibach, PH, Spencer, WO	1998b	Reibach, PH and Spencer, WO, 14C-RH-117,281: Nature of the Residue in Potato, Rohm and Haas Technical Report No. 34-98-50, September 17, 1998, GLP, unpublished. ER ref. no. 14.3	N	N	NA	Gowan

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CA, 6.2.1/03	IIA, 6.1/03 **Add study**	Graves D.D. , Reibach P.	2000	Consideration of the Difference in the Magnitude of the Residues of RH-7281 in Grapes from Supervised Field Residue Trials Compared to the 14C Grape Metabolism Study ER 14.5 Rohm and Haas unpublished Technical Report No. 34-00-83 September 11, 2000 ER ref. no. R 76.6	N	N	NA	Gowan
CA, 6.2.1/04	IIA, 6.1/04 **Add study**	Wolf S	2001	Determination of RH-0721 Residues in/on Grape (RAC Grape) from Field Trials in Europe (1997/1999) - to support ER 14.5 Rohm and Haas unpublished Technical Report No. 799773 February 13, 2001 ER ref. no. R 79.1	N	N	NA	Gowan
CA, 6.2.3/01	IIA, 6.2.1	[redacted]	1998	Robert A. Robinson, Metabolism of 14C-RH-117,281 in lactating goats, [redacted] Technical Report No. 34-97-166, September 10, 1998, GLP, unpublished. ER ref. no. 16.1	Y	N	NA	Gowan
CA, 6.3.1/01	IIA, 6.3.1/01	Wais, A.	1999a	Determination of residues of RH-117281 and mancozeb in/on potatoes (RAC tubers) following treatment with RH-7281 2F and Dithane /RH-117,281 75 DG Blend from field trials in Germany; 1996 Report no. 553002/649776, April 12, 1999 GLP, unpublished ER ref. no. R 66.4/R 66.5	N	N	NA	Gowan
CA, 6.3.1/02	IIA, 6.3.1/02	Wais, A.	1999b	Determination of residues of RH-117281 and mancozeb in/on potatoes (RAC tubers) following treatment with RH-7281 2F and Dithane /RH-117,281 75 DG Blend from field trials in the United Kingdom; 1996 Report no. 553300/649811, April 16, 1999 GLP, unpublished ER ref. no. R 70.3/R 70.4	N	N	NA	Gowan

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	Vertebrate study Y/N	Data Protection Claimed Y/N	Justification if data protection claimed	Owner
CA, 6.3.1/03	IIA, 6.3.1/03	Grolleau, G.	1999a	Magnitude of the residue of RH-7281 and its metabolites RH-1452 and RH-1455 in Potato Raw Agricultural Commodity. Northern and Southern France, 1996 Report no. EA960112, April 6, 1999 GLP, unpublished ER ref. no. R 63.3	N	N	NA	Gowan
CA, 6.3.1/04	IIA, 6.3.1/04	Wais, A.	1999c	Determination of residues of RH-117281 and mancozeb in/on potato (RAC tubers) following treatment with RH-7281 2F and Dithane /RH-117,281 75 DG Blend from field trials in Italy; 1996 Report no. 553103/649800, April 13, 1999 GLP, unpublished ER ref. no. R 67.5/R 67.6	N	N	NA	Gowan
CA, 6.3.1/05	IIA, 6.3.1/05	Wais, A.	1999d	Determination of residues of RH-117281 and mancozeb in/on potatoes (RAC tubers) following treatment with RH-7281 2F and Dithane /RH-117,281 75 DG Blend from field trials in Germany; 1997 Report no. 652252, March 18, 1999 GLP, unpublished ER ref. no. R 64.4/R 64.5	N	N	NA	Gowan
CA, 6.3.1/06	IIA, 6.3.1/06	Wais, A.	1999e	Determination of residues of RH-117281 and mancozeb in/on potatoes (RAC tubers) following treatment with RH-7281 2F and Dithane /RH-117,281 75 DG Blend from field trials in UK; 1997 Report no. 652263, March 23, 1999 GLP, unpublished ER ref. no. R 65.5/R 65.6	N	N	NA	Gowan

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CA, 6.3.1/07	IIA, 6.3.1/07	Wais, A.	1999f	Magnitude of the residue of RH-7281 and its metabolites RH-1452 and RH-1455 in Potato Raw Agricultural Commodity. Northern and Southern France, 1997 Report no. EA970131, April 6, 1999 GLP, unpublished ER ref. no. R 64.1	N	N	NA	Gowan
CA, 6.3.1/08	IIA, 6.3.1/08	Wais, A.	1999g	Determination of residues of RH-117281 and mancozeb in/on potatoes (RAC tubers) following treatment with RH-7281 2F and Dithane /RH-117,281 75 DG Blend from field trials in Italy; 1997 Report no. 652285, March 25, 1999 GLP, unpublished ER ref. no. R 65.3/R 65.4	N	N	NA	Gowan
CA, 6.3.1/09	IIA, 6.3.1/09	Wais, A.	1999h	Determination of residues of RH-117281 and mancozeb in/on potatoes (RAC tubers) following treatment with RH-7281 2F and Dithane/RH-117,281 75 DG Blend from field trials in Greece; 1997 Report no. 652307, March 17, 1999 GLP, unpublished ER ref. no. R 64.2/R 64.3	N	N	NA	Gowan
CA, 6.3.1/10	IIA, 6.3.1/10	Wais, A.	1999i	Determination of residues of RH-117,281 and mancozeb in/on potato (RAC tubers) following treatment with Dithane/RH-117,281 75 DG Blend (8:1) and Dithane/RH-117,281 75 WP Blend (8:1) from two field trials in Germany; 1998 Report no. 688904, April 13, 1999 GLP, unpublished,ER ref. no. R 68.1/R 68.2	N	N	NA	Gowan

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	Vertebrate study Y/N	Data Protection Claimed Y/N	Justification if data protection claimed	Owner
CA, 6.3.1/11	IIA, 6.3.1/11	Wais, A.	1999j	Determination of residues of RH-117,281 and mancozeb in/on potato (RAC tubers) following treatment with Dithane/RH-117,281 75 DG Blend (8:1) and Dithane/RH-117,281 75 WP Blend (8:1) from two field trials in UK; 1998 Report no. 688937, April 13, 1999 GLP, unpublished,ER ref. no. R 68.3/R 68.4	N	N	NA	Gowan
CA, 6.3.1/12	IIA, 6.3.1/12	Wais, A.	1999k	Determination of residues of RH-117,281 and mancozeb in/on potato (RAC tubers) following treatment with Dithane/RH-117,281 75 DG Blend (8:1) and Dithane/RH-117,281 75 WP Blend (8:1) from four field trials in Spain; 1998 Report no. 688926, April 13, 1999 GLP, unpublished ER ref. no. R 66.6/R 66.7	N	N	NA	Gowan
CA, 6.3.1/14	IIA, 6.3.1/14 **See add. vol. April 2000**	Wais, A.	2000	Determination of residues of RH-117,281 and its metabolites RH-141,452 and RH-141,455 in/on potatoes (RAC tubers) following treatment with RH-7281/mancozeb 75WG from a field trial (semi residue decline study) in the Netherlands; 1999 Report no. 734567, January 2000 GLP, unpublished ER ref. no. R 72.5	N	N	NA	Gowan
CA, 6.3.1/15	IIA, 6.3.1/15 **See add. vol. April 2000**	Wais, A.	2000	Determination of residues of RH-117,281 and its metabolites RH-141,452 and RH-141,455 in/on potatoes (RAC tubers and processing products) following treatment with RH-7281/mancozeb 75WG from a field trial (semi residue decline study) in Northern France; 1999 Report no. 734556, February 2000 GLP, unpublished ER ref. no. R 72.9	N	N	NA	Gowan

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CA, 6.3.1/16	IIA, 6.3.1/16 **See add. vol. April 2000**	Wais, A.	2000	Determination of residues of RH-117,281 and its metabolites RH-141,452 and RH-141,455 in/on potatoes (RAC tubers) following treatment with RH-7281/mancozeb 75WP from a field trial (semi residue decline study) in Northern France; 1999 Report no. 739001, March 2000 GLP, unpublished ER ref. no. R 72.4	N	N	NA	Gowan
CA, 6.3.1/17	IIA, 6.3.1/17 **See add. vol. April 2000**	Wais, A.	2000	Determination of residues of RH-117,281 and its metabolites RH-141,452 and RH-141,455 in/on potatoes (RAC tubers and processing products) following treatment with RH-7281/mancozeb 75WG from a field trial (semi residue decline study) in Italy; 1999 Report no. 734545, March 2000 GLP, unpublished, ER ref. no. R 73.2	N	N	NA	Gowan
CA, 6.3.2/01	IIA, 6.3.2/01	Wais, A.	1999	Determination of residues of RH-117281 and mancozeb in/on vine (RAC grapes) following treatment with RH-7281 2F and Dithane/RH-117,281 75 DG Blend from field trials in Germany, 1996 Report no. 553001/649765, April 16, 1999 GLP, unpublished ER ref. no. R 69.4/R 69.5	N	N	NA	Gowan
CA, 6.3.2/02	IIA, 6.3.2/02	Grolleau, G.	1999b	Magnitude of the Residue of RH-7281 and Mancozeb in Grape Raw Agricultural Commodity and of RH-7281 in Wine and Processed Fractions - Northern and Southern France - 1996 Report no. EA960110, March 15, 1999 GLP, unpublished ER ref. no. R 60.1	N	N	NA	Gowan

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CA, 6.3.2/03	IIA, 6.3.2/03	Wais, A.	1999m	Determination of residues of RH-117281 and mancozeb in/on vine (RAC grapes) following treatment with RH-7281 2F and Dithane/RH-117,281 75 DG Blend from field trials in Italy, 1996 Report no. 553101/649787, April 16, 1999 GLP, unpublished ER ref. no. R 70.1/R 70.2	N	N	NA	Gowan
CA, 6.3.2/04	IIA, 6.3.2/04	Wais, A.	1999n	Determination of residues of RH-117281 and mancozeb in/on table grapes (RAC grapes) following treatment with RH-7281 2F and Dithane/RH-117,281 75 DG Blend from field trials in Italy, 1996 Report no. 553102/649798, April 16, 1999 GLP, unpublished ER ref. no. R 71.3/R 71.4	N	N	NA	Gowan
CA, 6.3.2/05	IIA, 6.3.2/05	Wais, A.	1999o	Determination of residues of RH-117281 and mancozeb in/on table grapes (RAC grapes) following treatment with RH-7281 2F and Dithane/RH-117,281 75 DG Blend from field trials in Spain, 1996 Report no. 553200/620875, April 16, 1999 GLP, unpublished ER ref. no. R 70.5/R 70.6	N	N	NA	Gowan
CA, 6.3.2/06	IIA, 6.3.2/06	Wais, A.	1999p	Determination of residues of RH-117281 and mancozeb in/on vine (RAC grapes) following treatment with RH-7281 2F and Dithane/RH-117281 75 DG Blend from field trials in Germany, 1997 Report no. 652241, April 16, 1999 GLP, unpublished ER ref. no. R 71.1/R 71.2	N	N	NA	Gowan

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CA, 6.3.2/07	IIA, 6.3.2/07	Grolleau, G.	1999c	Magnitude of the Residue of RH-7281 and Mancozeb in Grape Raw Agricultural Commodity and of RH-7281 in Wine - Northern and Southern France - 1997 Report no. EA 970130, March 15, 1999 GLP, unpublished ER ref. no. R 62.3	N	N	NA	Gowan
CA, 6.3.2/08	IIA, 6.3.2/08	Wais, A.	1999q	Determination of residues of RH-117281 and mancozeb in/on vine (RAC grapes) following treatment with RH-7281 2F and Dithane/RH-117,281 75 DG Blend from field trials in Italy, 1997 Report no. 652274, April 14, 1999 GLP, unpublished ER ref. no. R 68.5/R 68.6	N	N	NA	Gowan
CA, 6.3.2/09	IIA, 6.3.2/09	Wais, A.	1999r	Determination of residues of RH-117281 and mancozeb in/on vine (RAC grapes) following treatment with RH-7281 2F and Dithane/RH-117281 75 DG Blend from field trials in Greece, 1997 Report no. 652296, April 14, 1999 GLP, unpublished ER ref. no. R 69.2/R 69.3	N	N	NA	Gowan
CA, 6.3.2/10	IIA, 6.3.2/10	Wais, A.	1999s	Determination of residues of RH-117281 and mancozeb in/on table grapes (RAC grapes) following treatment with RH-7281 2F and Dithane/RH-117,281 75 DG Blend from field trials in Italy, 1997 Report no. 660688, March 19, 1999 GLP, unpublished ER ref. no. R 65.1/R 65.2	N	N	NA	Gowan

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CA, 6.3.2/11	IIA, 6.3.2/11	Wais, A.	1999t	Determination of residues of RH-117,281 and mancozeb in/on vine grapes (RAC grapes) following treatment with Dithane/ RH-117,281 75 DG Blend (8:1), Dithane/ RH-117,281 75 WP Blend (8:1) and RH-7281 2F Experimental fungicide from four field trials in Germany, 1998 Report no. 688893, April 13, 1999 GLP, unpublished ER ref. no. R67.2/R 67.3	N	N	NA	Gowan
CA, 6.3.2/12	IIA, 6.3.2/12	Grolleau, G.	1999d	Magnitude of the Residue of RH-7281 and Mancozeb in Grape Raw Agricultural Commodity and of RH-7281 in Wine - Northern and Southern France - 1998 Report no. EA 980117, March 15, 1999 GLP, unpublished ER ref. no. R 63.1	N	N	NA	Gowan
CA, 6.3.2/13	IIA, 6.3.2/13	Wais, A.	1999u	Determination of residues of RH-117,281 and mancozeb in/on vine grapes (RAC grapes) following treatment with Dithane/RH-117,281 75 DG Blend (8:1) and Dithane/RH-117,281 75 WP Blend (8:1) from two field trials in Italy, 1998 Report no. 688961, April 12, 1999 GLP, unpublished ER ref. no. R 66.2/R 66.3	N	N	NA	Gowan
CA, 6.3.2/14	IIA, 6.3.2/14	Wais, A.	1999v	Determination of residues of RH-117,281 and mancozeb in/on vine grapes (RAC grapes) following treatment with Dithane/RH-117,281 75 DG Blend (8:1) from two field trials in Spain, 1998 Report no. 688915, April 14, 1999 GLP, unpublished ER ref. no. R 67.4	N	N	NA	Gowan

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CA, 6.3.2/15	IIA, 6.3.2/15	Wais, A.	1999w	Determination of residues of RH-117,281 and mancozeb in/on table grapes (RAC grapes) following treatment with Dithane/RH-117,281 75 DG Blend (8:1) from two field trials in Spain, 1998 Report no. 693674, April 12, 1999 GLP, unpublished ER ref. no. R 66.1	N	N	NA	Gowan
CA, 6.3.2/16	IIA, 6.3.2/16	Wais, A.	1999x	Determination of residues of RH-117,281 and mancozeb in/on table grapes (RAC grapes) following treatment with Dithane/RH-117,281 75 DG Blend (8:1) from two field trials in Portugal, 1998 Report no. 688948, April 14, 1999 GLP, unpublished ER ref. no. R 69.1	N	N	NA	Gowan
CA, 6.3.2/18	IIA, 6.3.2/18 **See add. vol. April 2000**	Wais, A.	2000	Determination of residues of RH-117,281 and mancozeb in/on grapes (RAC grapes) following treatment with RH-7281/mancozeb 75WG from a field trial in Germany, 1999 Report no. 734578, February 2000 GLP, unpublished ER ref. no. R 72.8	N	N	NA	Gowan
CA, 6.3.2/19	IIA, 6.3.2/19 **See add. vol. April 2000**	Grolleau, G.	2000	Magnitude of the Residue of RH-7281/mancozeb 76.25WG in grapes raw agricultural commodity - Northern France - 1999 Report no. EA990175, March 2000 GLP, unpublished ER ref. no. R 73.3	N	N	NA	Gowan
CA, 6.3.2/20	IIA, 6.3.2/20 **See add. vol. April 2000**	Grolleau, G.	2000	Magnitude of the Residue of RH-7281/mancozeb 76.25WG in grapes raw agricultural commodity - Southern France - 1999 Report no. EA990176, March 2000 GLP, unpublished ER ref. no. R 73.4	N	N	NA	Gowan

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CA, 6.5.1/01	IIA, 6.5.1	Mamouni, A	1998	Mamouni, A, 14C-RH-117281: Vinification Study, RCC Ltd., Rohm and Haas Technical Report No. 34-98-151, December 3, 1998, GLP, unpublished. ER ref. no. 30.17	N	N	NA	Gowan
IIA, 6.5.3/01	IIA, 6.5.2.2	Graves, DD	1998	Graves, D.D., RH-117281 80W and 2F Residue Studies in Grapes and Grape Process Fractions 1996 and 1997 Trials, Agri Business Group, Inc., Enviro-Test Laboratories, McKenzie Laboratories, California State University at Fresno, Rohm and Haas Technical Report No. 34-98-154, November 24, 1998, GLP, unpublished. ER ref. no. R 62.1	N	N	NA	Gowan
IIA, 6.6.1/01	IIA, 6.6/01	Kim-Kang, H	1998	Kim-Kang, H., 14C-RH-117,281: Confined Rotational Crop Study, XenoBiotic Laboratories, Inc., Rohm and Haas Technical Report No. 34-98-144, December 4, 1998, GLP, unpublished. ER ref. no. R 60.2	N	N	NA	Gowan