REGISTRATION REPORT

Part B

Section 7

Metabolism and Residues

Detailed summary of the risk assessment

Product code: Cymoxanil 33% + Zoxamide 33% WG

Product name(s): **Lieto 66 WG**

Chemical active substances:

Cymoxanil, 330 g/kg
Zoxamide, 330 g/kg

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(product re-registration)

Applicant: Sipcam Oxon S.p.A.

Submission date: 30/12/2020

MS Finalisation date: September 2021

Revision date: December 2021

DATA PROTECTION CLAIM

Under Article 59 of Regulation 1107/2009/EC, the applicant claims data protection for these studies. The data protection status and corresponding justification as valid for the respective country will be confirmed in the respective PART A.

STATEMENT FOR OWNERSHIP

The summaries and evaluations contained in this review report may be based on unpublished proprietary data submitted for the purpose of the assessment undertaken by the regulatory authority that prepared it. Other registration authorities should not grant, amend, or renew a registration on the basis of the summaries and evaluation of unpublished proprietary data contained in this review report unless they have received the data on which the summaries and evaluation are based, either –

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- following expiry of any period of exclusive use, by offering – in certain jurisdictions – mandatory compensation, unless the period of protection of the proprietary data concerned has expired.

Version history

When	What
30th December 2020	Submission of initial Version 0 by the applicant.
September 2021	Version evaluated by PL zRMS
December 2021	Revised version, addressing the comments of MSs.

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7 Metabolism and residue data (KCA section 6)

This document summarises the residues, consumer intake and risk assessment data for the plant protection product CYMOXANIL 33% + ZOXAMIDE 33% WG (trade name Lieto 66 WG), a WG formulation containing 330 g/kg zoxamide and 330 g/kg cymoxanil. It is for the renewal of the authorisation of the product CYMOXANIL 33% + ZOXAMIDE 33% WG according to article 43 of Regulation (EC) No 1107/2009, following the renewal of approval of the active substance zoxamide according to Regulation (EU) 2018/1981 of 13 December 2018.
The aim of this step of the art. 43 process is to update the existing dossier information with regard to and limited to the information on the active substance zoxamide as follows:

- To comply with data requirements or criteria which were not in force when the authorisation of the plant protection product was granted and
- to demonstrate that the product meets the requirements set out in the Regulation on the renewal of the approval of the active substance zoxamide to comply with provisions of article 29 of Regulation (EU) No 1107/2009.

This dossier contains the consolidated version of the previous assessment of the zRMS Spain for the parts which do not require a re-evaluation, including all assessments and data on cymoxanil. The consolidated text has been shaded in grey in the present dRR section.

As the consolidated text of the applicant is shaded in grey in the present dRR section, the evaluator text outside the grey commenting boxes is shaded in blue.

The risk assessment provided for cymoxanil contains the consolidated version of the previous product evaluation, since that part does not require any update. The risk assessment is therefore based on the application pattern related to the previous product GAP uses, which represent a worst-case.

Please note: There is an authorization available for Romania (belonging to the central EU zone) on tomatoes. Since during product authorisation this country (Romania) belonged to the southern EU zone with regard to residues, this GAP use is further defended during product re-registration. As a result, a common document Part B Section 7 for both Southern and Northern EU countries has been issued.

The dossier follows the data requirements of

- Regulation (EC) No. 544/2011 for the active substance cymoxanil,
- Regulation (EC) No. 283/2013 for the active substance zoxamide and
- Regulation (EC) No. 284/2013 for the plant protection product ‘Cymoxanil 33% + Zoxamide 33% WG’.

7.1 Summary and zRMS Conclusion

7.1.1 Critical GAP(s) and overall conclusion

Selection of critical uses and justification

The critical GAPs with respect to consumer intake and risk assessment for the preparation CYMOXANIL 33% + ZOXAMIDE 33% WG are presented in Table 7.1-1. They have been selected from the individual GAPs in the central EU zone. A list of all intended uses within the Central zone (and the EU) is given in Part B, Section 0.

The critical good agricultural practice (GAP) uses were selected considering the maximum single application rates, the maximum numbers of applications at the intended interval and the minimum pre-harvest intervals (PHIs) per crop/crop group. In case applicable, the residues behaviour in the northern and southern EU zone have been compared.

Overall conclusion

Cymoxanil data are not under evaluation in this submission.

The data available are considered sufficient for the risk assessment.

An exceedance of the current MRL of 5.0 mg/kg for zoxamide in wine and table grapes and 0.02 mg/kg in potatoes - as laid down in Reg. (EU) 396/2005 - is not expected.

An exceedance of the current MRL of 0.3 mg/kg for cymoxanil in wine and table grapes and 0.01* mg/kg in potatoes and as laid down in Reg. (EU) 396/2005 is not expected.

MRL (0,05) exceedance in honey cannot be excluded in melliferous crops. However, in zRMS opinion this case does not affect the intended GAP. According to the technical guidelines SANTE/11956/2016 rev. 9 potato is definitely not melliferous crop. But while grapes are listed as crop with some melliferous capacity, on the other hand EFSA (EJ 2013;11(7):3295) points out that despite having melliferous capacity, vines have low attractiveness to honeybees. Hence, we can consider residues in honey as not expected from the intended uses.

To make it clear, it is stated that this assessment is for Central Zone only, and tomato, as the subject of the specific zonal situation and the use not included in the evaluated GAP table 7.1-1, is excluded from this re-registration assessment.

The chronic intake of zoxamide and chronic and short-term intakes of cymoxanil residues are unlikely to present a public health concern.

As far as consumer health protection is concerned, zRMS agrees with the authorization of the intended uses (Table 7.1-1).

According to the available data, no specific mitigation measures should apply.

Data gaps

In the context of this re-registration request noticed data gaps are:

- None.

Table 7.1-1: Acceptability of critical GAPs (and respective fall-back GAPs, if applicable)

1	2	3	4	5	6	7		8				9			10	11
GAP no. (see part B.0)*	**Crop and/ or situation ****	**Zone**	**Product code**	**F, Fn, Fpn G, Gn, Gpn or I*****	**Pests or Group of pests controlled**	**Formulation**		**Application**				**Application rate per treatment**			**PHI (days)**	**Conclusion**
						Type	**Conc. of as**	**method kind**	**growth stage & season**	**number min max**	**interval between applications (min)**	**kg as/hL min max**	**water L/ha min max**	**kg as/ha min max**		
13-16	Wine grapes	Central EU	Cymoxanil 33% + Zoxamide 33% WG	F	Grape downy mildew	WG	330 g/kg zoxamide 330 g/kg cymoxanil	foliar spraying, overall	March to Oct./ BBCH 14 till PHI of 28 days	2-3	7-10	0.01155–0.07425 zoxamide + cymoxanil	200-1000	0.1155-0.1485 zoxamide + cymoxanil	28	
13-16	Table grapes	Central EU	Cymoxanil 33% + Zoxamide 33% WG	F	Grape downy mildew	WG	330 g/kg zoxamide 330 g/kg cymoxanil	foliar spraying, overall	March to Oct./ BBCH 14 till PHI of 28 days	2-3	7-10	0.01155–0.07425 zoxamide + cymoxanil	200-1000	0.1155-0.1485 zoxamide + cymoxanil	28	
1-12	Potatoes	Central EU	Cymoxanil 33% + Zoxamide 33% WG	F	potato late blight	WG	330 g/kg zoxamide 330 g/kg cymoxanil	foliar spraying, overall	Feb. to Sept./ BBCH 21 until PHI of 7 days	3	7-10	0.01155–0.07425 zoxamide + cymoxanil	200-1000	0.115-0.1485 zoxamide + cymoxanil	7	

* Use number(s) in accordance with the list of all intended GAPs in Part B, Section 0 should be given in column 1

** Use also code numbers according to Annex I of Regulation (EU) No 396/2005

*** F: professional field use, Fn: non-professional field use, Fpn: professional and non-professional field use, G: professional greenhouse use, Gn: non-professional greenhouse use, Gpn: professional and non-professional greenhouse use, I: indoor application

Explanation for Column 11 “Conclusion”

A	Exposure acceptable without risk mitigation measures, safe use
R	Further refinement and/or risk mitigation measures required
N	Exposure not acceptable, no safe use

7.1.2 Summary of the evaluation

The plant protection product preparation CYMOXANIL 33% + ZOXAMIDE 33% WG (trade name Lieto 66 WG) is a WG formulation containing 330 g/kg of each zoxamide and cymoxanil.

Table 7.1-2: Toxicological reference values for the dietary risk assessment of zoxamide and cymoxanil

Reference value	Source	Year	Value	Study relied upon	Safety factor
Zoxamide - Parent compound					
ADI	EFSA	2017	0.5 mg/kg bw/d	90 d oral rat / one-year dog	100
ARfD	Not allocated, not necessary.				
RH-150721 – Zoxamide metabolite					
ADI	Satish Kumar*	2020	0.56 mg/kg bw/d	90 d oral rat	200
~~ARfD~~					
Cymoxanil - Parent compound					
ADI	EFSA	2008	0.013 mg/kg bw	1 year dog study	100
ARfD	EFSA	2008	0.08 mg/kg bw/day	Developmental NOAEL in the rabbit	100

* see also Strupp (2020; report no. CS13072020) for derivation of ADI

7.1.2.1 Summary for zoxamide

Table 7.1-3: Summary for zoxamide

Use-No.*	Crop, **	Plant metabolism covered?	Sufficient residue trials?	PHI sufficiently supported?	Sample storage covered by stability data?	MRL compliance	Chronic risk for consumers identified?	Acute risk for consumers identified?
17-23	Wine grapes, F	Yes	Yes (25 trials [5x 180 g a.s/ha]; 12 trials NEU, 13 trials SEU)	Yes	Yes	Yes	No	No
24-30	Table grapes, F	Yes	Yes (25 trials [5x 180 g a.s/ha]; 12 trials NEU, 13 trials SEU)	Yes	Yes	Yes		No
31-37	Tomatoes, F	Yes	Yes (25 trials [5x 150 or 5x 180 g a.s./ha])	Yes	Yes	Yes		No
38-40	Eggplants, F	Yes	Yes (Extrapolation from tomato)	Yes	Yes	Yes		No
41-47	Potatoes	Yes	Yes	Yes	Yes	Yes		No

Use-No.*	Crop, **	Plant metabolism covered?	Sufficient residue trials?	PHI sufficiently supported?	Sample storage covered by stability data?	MRL compliance	Chronic risk for consumers identified?	Acute risk for consumers identified?
			(8 trials [5x 150 or 5x 180 g a.s./ha]); residues ND or < LOQ					

* Use number(s) in accordance with the list of all intended GAPs in Part B, Section 0 should be given in column 1
** F: professional field use, Fn: non-professional field use, Fpn: professional and non-professional field use, G: professional greenhouse use, Gn: non-professional greenhouse use, Gpn: professional and non-professional greenhouse use, I: indoor application

The effects of processing on the nature of zoxamide residues have been investigated. These data were considered for risk assessment.

Residues in succeeding crops have been sufficiently investigated taking into account the specific circumstances of the cGAP uses being considered here. It is very unlikely that residues will be present in succeeding crops.

Considering dietary burden and based on the intended uses, no significant modification of the intake for livestock is expected. Further investigation of residues in commodities of animal origin is therefore not necessary for authorisation of CYMOXANIL 33% + ZOXAMIDE 33% WG.

7.1.2.2 Summary for cymoxanil

Table 7.1-4: Summary for cymoxanil

Use-No.*	Crop, **	Plant metabolism covered?	Sufficient residue trials?	PHI sufficiently supported?	Sample storage covered by stability data?	MRL compliance	Chronic risk for consumers identified?	Acute risk for consumers identified?
13-16	Wine grapes, F	Yes	Yes	Yes	Yes	Yes	No	No
13-16	Table grapes, F	Yes	Yes	Yes	Yes	Yes		No
1-12	Potatoes	Yes	Yes	Yes	Yes	Yes		No

* Use number(s) in accordance with the list of all intended GAPs in Part B, Section 0 should be given in column 1
** F: professional field use, Fn: non-professional field use, Fpn: professional and non-professional

As residues of Cymoxanil do not exceed the trigger values defined in Reg (EU) No 283/2013, there is no need to investigate the effect of industrial and/or household processing

Residues in succeeding crops have been sufficiently investigated taking into account the specific circumstances of the cGAP uses being considered here. It is very unlikely that residues will be present in succeeding crops.

Considering dietary burden and based on the intended uses, no significant modification of the intake for livestock is expected. Further investigation of residues in commodities of animal origin is therefore not

necessary for authorisation of CYMOXANIL 33% + ZOXAMIDE 33% WG.

7.1.2.3 Summary for CYMOXANIL 33% + ZOXAMIDE 33% WG

Table 7.1-5: Information on CYMOXANIL 33% + ZOXAMIDE 33% WG (KCA 6.8)

Crop, ***	PHI for CYMOXANIL 33% + ZOXAMIDE 33% WG proposed by applicant	PHI/Withholding period* sufficiently supported for		PHI for CYMOXANIL 33% + ZOXAMIDE 33% WG proposed by zRMS	zRMS Comments (if different PHI proposed)
		Zoxamide	Cymoxanil		
Wine grapes, F	28	Yes	Yes		
Table grapes, F	28	Yes	Yes		
Potatoes, F	7	Yes	Yes		

NR: not relevant
* Purpose of withholding period to be specified
** F: PHI is defined by the application stage at last treatment (time elapsing between last treatment and harvest of the crop).
*** F: professional field use, Fn: non-professional field use, Fpn: professional and non-professional field use, G: professional greenhouse use, Gn: non-professional greenhouse use, Gpn: professional and non-professional greenhouse use, I: indoor application

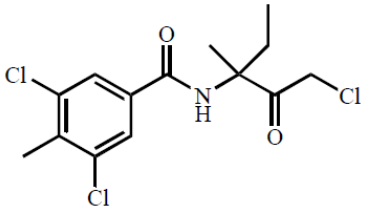
Due to the fast degradation of both active substances in soil a waiting period between last application and sowing or planting of succeeding crops is not required.

Assessment

7.2 Zoxamide

General data on zoxamide are summarised in the table below (last updated 2020/09/30)

Table 7.2-1: General information on zoxamide

Active substance (ISO Common Name)	Zoxamide
IUPAC	(RS)-3,5-dichloro-N-(3-chloro-1-ethyl-1-methyl-2-oxopropyl)-p-toluamide
Chemical structure	
Molecular formula	$C_{14}H_{16}NO_2Cl_3$
Molar mass	336.65 g/mol
Chemical group	Benzamide
Mode of action (if available)	Inhibition of germ tube development and mycelium growth by inhibiting cell division.
Systemic	No
Company (ies)	Gowan Crop Protection Ltd. as legal successor of Gowan Comercio Internacional e Servicos Limitada*
Rapporteur Member State (RMS)	Latvia
Approval status	Approved since 01/07/2018 (Commission Implementing Regulation (EU) 2018/692)
Restriction (e.g. is restricted to use as "...")	None.
Review Report	SANTE/10052/2018 Rev 2 23 March 2018
Current MRL regulation	Regulation (EU) 2017/171
Peer review of MRLs according to Article 12 of Reg No 396/2005 EC performed	No
EFSA Journal: Conclusion on the peer review	Yes, EFSA Peer Review Conclusion, 2017
EFSA Journal: conclusion on article 12	No
Current MRL applications on intended uses	EFSA-Q-2019-00404 Dry onion bulbs Status: Additional data request EFSA-Q-2015-00078 Various crops Status: Finished (EFSA Journal 2016;14(7):4527) EFSA-Q-2008-649 All existing crops Status: Ongoing

* Notifier in the EU process to whom the a.s. belong(s)

7.2.1 Stability of Residues (KCA 6.1)

7.2.1.1 Stability of residues during storage of samples

Available data

New data is submitted in the framework of this application, it is included in Appendix 1 (reference list) and 2 (study summaries).
The available data on freezer storage stability of sample residues are summarised in the following table.

Table 7.2-2: Summary of stability data achieved at ≤ -18 °C (unless stated otherwise)

Matrix	Characteristics of the matrix	Acceptable maximum storage duration (Test substance)	Reference
Data relied on in EU			
Plant products			
Potato tubers	High water content, high starch content	24 months (zoxamide, RH-141452, RH-141455)	EFSA, 2017
Grape berries	High acid content	18 months (zoxamide, RH-150721)	EFSA, 2017
Grape juice		24 months (zoxamide)	EFSA, 2017
Wine		24 months (zoxamide, RH-150721)	EFSA, 2017
Raisins		24 months (zoxamide)	EFSA, 2017
Animal Products			
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New data			
Plant products			
Potato tubers	High water content, high starch content	22 months (zoxamide [R, S, sum]) 18 months (RH-141452, RH-141452)	Sala A. (2020), report no. BPL-STUDY-18-000047, interim report
Animal Products			
Honey	High sugar content	85 days (zoxamide [R, S, sum])	Poráczki, K. (2020), report no. 19 48 BTR 0003
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Conclusion on stability of residues during storage

The active substance and its metabolites RH-141455 and RH-141452 were shown to be stable under frozen conditions for 24 months in potato tubers (high water content, high starch content).

The active substance and its metabolite RH-150721 were shown to be stable under frozen conditions for 18 months in grape fruits (high water content, high acidic), and for 24 months in grape juice and wine.
The active substance was shown to be stable under frozen conditions for 24 months in raisins.

7.2.1.2 Stability of residues in sample extracts (KCA 6.1)

Available data

The stability of residues of zoxamide and its metabolites in sample extracts was checked as part of individual studies in case necessary (i.e. stored for > 24 hours in the refrigerator). All other extracts were analysed within 24h after extraction. In addition, addition, a separate study on the storage stability of RH-129151 has been provided and was regarded valid.

The new study on the stability of the stability of the zoxamide metabolite RH-129151 (A, B and sum) in the final sample extract of grape (grape, grape juice, wine, raisin), potato (potato tuber, fried potato, potato flakes), tomato (tomato, peeled tomato), and cucumber commodities is available (Longhi D., 2020; report no. GLP-STUDY-20-77) and is summarised hereunder, the reference is provided in Appendix 1, the study summary in Appendix 2.

The objective of this study was the evaluation of the stability of the enantiomers of the Zoxamide metabolite RH-129151 when stored for 8 and 24 hours under dark and refrigerated conditions at 4°C (± 2°C). The obtained results of the spiking experiments are summarised in the following tables.
Please note: Since the enantiomers of RH-129151 could not be assigned as the R- and R-form (individual reference standards missing), they were assigned as “A” and “B”.

Table 7.2-3: Results summary for RH-129151 (A) - Longhi D., 2020; report no. GLP-STUDY-20-77

Commodity/processed	Commodity type	% Recovery analyte	
		T 8h	T 24 h
Grape	High acid content	102.1	101.1
Grape Juice	Processed	98.8	95.8
Wine	Processed	98.3	94.7
Raisin	Processed	98.6	97.1
Potato tuber	High water content	97.4	87.4
Fried potato	Processed	94.3	107.2
Potato flakes	Processed	106.9	95.4
Tomato	High water content	107.0	104.6
Peeled tomato	Processed	99.9	96.7
Cucumber	High water content	100.9	106.3

Table 7.2-4: Results summary for RH-129151 (B) - Longhi D., 2020; report no. GLP-STUDY-20-77

Commodity/processed	Commodity type	% Recovery analyte	
		T 8h	T 24 h
Grape	High acid content	96.4	93.8
Grape Juice	Processed	99.3	99.5
Wine	Processed	96.8	92.4
Raisin	Processed	100.2	101.0
Potato tuber	High water content	101.1	101.0
Fried potato	Processed	97.3	106.9
Potato flakes	Processed	99.7	93.8
Tomato	High water content	105.2	103.3
Peeled tomato	Processed	98.4	97.1

Commodity/processed	Commodity type	% Recovery analyte	
		T 8h	T 24 h
Cucumber	High water content	105.5	109.0

No significative differences in the recovery values were noticed after storage of the sample extracts at 4°C (± 2°C) in the dark for at least 8 and 24 hours compared to the initial "time 0" values for all the tested commodities: the % residue analyte after 8 and 24 hours is within the range of 70-120%, that is within the SANCO/825/00 rev. 8.1 guideline requirement to consider a chemical as stable. Therefore, RH-129151 (sum) as well as its enantiomer's ratio was considered stable for 24 hours in the final sample extracts of the tested commodities when stored at 4°C (± 2°C) in dark.

Conclusion on stability of residues in sample extracts

The stability of residues of zoxamide and its metabolites in sample extracts was checked as part of individual studies in case necessary (i.e. stored for > 24 hours in the refrigerator). All other extracts were analysed within 24h after extraction.

In addition, addition, a separate study on the storage stability of RH-129151 has been provided and was regarded valid. This study demonstrates the stability of RH-129151 (sum) as well as its enantiomer's ratio in sample extracts of relevant crop commodities for 24 hours when stored at 4°C (± 2°C) in dark.

7.2.2 Nature of residues in plants, livestock and processed commodities

7.2.2.1 Nature of residue in primary crops (KCA 6.2.1)

Available data

No new data submitted in the framework of this application.

The metabolism of zoxamide in primary crops was investigated after foliar spray application in the categories fruit (covering grapes and fruiting vegetables), pulses and oilseeds (i.e. peas) and root crops (covering root and tuber vegetables [i.e. potato] and bulb vegetables [i.e. onion]) using zoxamide ^{13}C- and/or ^{14}C-labelled in the phenyl ring (EFSA, 2017). The characteristics of these studies are summarised in Table 7.2-3 below:

Table 7.2-5: Summary of plant metabolism studies

Crop Group	Crop	Label position	Application and sampling details					Reference
			Method, F or G (a)	Rate (g a.s./ha)	No. / interval	Sampling (DAT)	Remarks	
EU data								
Fruits	Grape	^{14}C-phenyl ring labelled zoxamide	foliar application, F	1867	3 / 26-28 days	1	only zoxamide > 10% AR (0.43 mg/kg)	EFSA, 2017
		^{14}C-phenyl ring labelled zoxamide	foliar application, F	500	3 / 9-11 days	28	only zoxamide > 10% AR (3.67 mg/kg)	EFSA, 2017
	Tomato	^{14}C-phenyl ring labelled zoxamide	foliar application, G	860	3 / 18-19 days	1	only zoxamide > 10% AR (0.22 mg/kg) in red tomato	EFSA, 2017

	Cucumber	^{14}C-phenyl ring labelled zoxamide	foliar application, G	1344	3 / 7 days	1	only zoxamide > 10% AR (1.33 mg/kg)	EFSA, 2017
Leafy crops	--	--	--	--	--	--	--	
Root crops	Potato	^{13}C- and ^{14}C-phenyl ring labelled zoxamide	foliar application, F	900	3 / 21 and 17 days	14	RH-141452 (0.037 mg/kg) and RH-141455 (0.069 mg/kg) > 10% AR	EFSA, 2017
Pulses and oilseeds	Peas	^{14}C-phenyl ring labelled zoxamide	foliar application, F	145	2 / 7 days	7, 13, 30	only zoxamide > 10% AR (5.35 mg/kg in pods and 0.019 mg/kg in dry peas at a phi of 30 days)	EFSA, 2017
			foliar application, F	725	2 / 7 days	7, 13, 30	only zoxamide > 10% AR (e.g. 36.52 mg/kg in immature whole plants at 7 days phi; 10.01 mg/kg in pods at 13 days phi; 0.04 mg/kg in fresh peas at 13 days phi)	EFSA, 2017
Cereals	--	--	--	--	--	--	--	
New data								
--	--	--	--	--	--	--	--	

Summary of plant metabolism studies reported in the EU

Zoxamide is not systemic; therefore, following foliar application to crops, most of the applied material remains on the surface of the plants.

Fruits (including grapes and fruiting vegetables [tomato, cucumber, melon, zucchini, etc.])

In total four metabolism studies are available on fruits and fruiting vegetables, giving a good insight.

Two metabolism studies are available on grapes, one older study of Reibach & Spencer (1998) with a PHI of 1 day and a younger study of Staffa & Möndel (2014) with a PHI of 28 days. Both studies were performed outdoors. The grape metabolism study with a PHI of 1 day does not reflect the intended GAP of zoxamide products with a PHI of 28 days and is therefore only regarded as supportive.

After foliar application of [^{14}C]-zoxamide to grape vines with 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 detailed results of the metabolism study in grapes are described in the RAR (2017).

In the metabolism study on tomato (Sharma, 1999) zoxamide was applied to the foliage at a 1-day PHI at a nominal rate of 3 x 860 g a.s./ha. The study was performed in the greenhouse. The metabolism study on tomatoes has been performed with longer application intervals (18-19 days compared to the intended 8-10

days) and a shorter PHI (1 day instead of the here intended 3 days) but exaggerated single application rates of 860 g a.s./ha compared to the intended 157.5 g a.s./ha zoxamide. It is therefore regarded applicable for the intended tomato (and eggplant) uses with zoxamide.

A metabolism study on cucumber is available in the RAR (2017). In this metabolism study, zoxamide was applied to the foliage at nominally 3 x 1344 g a.s./ha with an interval of 7 days and a PHI of one day.

In fruits, zoxamide was the main component of the total radioactive residue (TRR) with 48% and 44% in green and red tomatoes and 92% in both cucumber and grape. The remaining TRR was extensively metabolised to a range of degradation products representing each less than 10% TRR – besides RH-141452, which was detected at 15% TRR in green tomato fruits and 11 % TRR in red tomato fruits at a PHI of 1 day.

However, the related metabolites RH-141455 and RH-141452 are not of toxicological relevance (see dRR Part B Section 6), they should therefore not be included in residue definitions. Instead, it is proposed to keep zoxamide in the residue definition for enforcement.

EFSA (2017) proposed to include RH-141452 in the residue definition for risk assessment of fruits (pending data gap on RH-141452). RH-141452 occurred both free and as (mainly glucose) conjugate in tomato fruits. The total amount of RH-141452 (free and conjugated) was calculated in the RAR (2017) at 9.1% TRR (0.045 mg/kg). RH-141452 can therefore be regarded as a minor metabolite in red tomatoes (and fruits in general). Besides, it needs to be noted that zoxamide has been applied in the tomato metabolism study with an exaggerated application rate. New residue trials confirmed that RH-141452 does not occur at levels above 0.01 mg/kg in tomato fruits when applied at the intended application pattern. And it occurs at or slightly above the LOQ (max. of 0.0188 mg/kg) in grape fruits. However, the new toxicological studies requested by EFSA (2017) confirmed that RH-145452 is not of toxicological relevance. As a result, this metabolite should not be included in the residue definition for enforcement and risk assessment of fruit crops.

The actual and GAP relevant metabolism study on grapes (Staffa & Möndel, 2014) showed that the parent zoxamide is the predominant compound in grape fruits with amounts of 92% of total residues at a PHI of 28 days. Numerous minor metabolites were determined at levels < 10% TRR, for example RH-129151 in grape fruits at < 3% TRR, but not considered relevant for risk assessment.

Pulses and oilseeds (i.e. peas)

[^{14}C]-zoxamide was applied to young pea plants by foliar spray at a nominal rate of 2 x 145 g a.s./ha or at an exaggerated application rate of 2 x 725 g a.s./ha with an interval of 7 days. Immature whole plants were harvested with a phi of 7 days at BBCH 65-75, and straw, pods and peas during 2nd harvest at a PHI of 13 days (BBCH 77) and 3rd harvest at a PHI of 30 days (BBCH 89).

The majority of the radioactivity on the immature whole plant, straw and pod surface could be rinsed off with water from the plant surfaces; it was mainly parent zoxamide (85.9-95.9% TRR).

The main compound in the immature whole plant, straw and pod samples was zoxamide (max. 1.8 % TRR). Besides, only minor metabolites were identified, at even lower levels. The rest of radioactivity was incorporated into the natural constituents of the plants.

Root crops (i.e. potatoes)

After foliar application of [^{14}C]-zoxamide to potatoes with a 14-days PHI at a nominal rate of 3 x 900 g a.s./ha, the total radioactive residue (TRR) in potato tubers (RAC) determined by combustion was 0.178 mg/kg. Two major metabolites, RH-141452 and RH-141455, were observed in potato tubers at 21% and 39% TRR, respectively. Parent zoxamide was not found.

The GAP used in the potato metabolism study was 3 x 900 g a.s./ha (total of 2700 g a.s./ha/year), applied

with a spray interval 17-21 days and a PHI 14 days. The future GAP of zoxamide for potatoes on EU level is at max 3 x 180 g a.s./ha, applied at 7-10 days interval with a PHI 7 days. Nevertheless, the metabolism study is regarded applicable to evaluate the metabolism of zoxamide in potato tubers. It represents a worse-case in terms of higher single and seasonal application rate and longer PHI - which allows more time for the formation and accumulation of metabolites in the edible part of potatoes. Besides, supervised residues trials confirmed a no residues situation in potato tubers for the worst-case future GAP use in potatoes. No residues of zoxamide, RH-141455 and RH-141452 $\geq$ 0.01 mg/kg were detected in potato tubers in a new residues data package to support the future GAP uses, which are going to be restricted to 3 applications to avoid resistance.

On the basis that the metabolites RH-141455 and RH-141452 are not detectable in potato tubers and are not of toxicological relevance, they should not be included in the residue definition. Instead, it is proposed to keep zoxamide in the residue definition for enforcement.

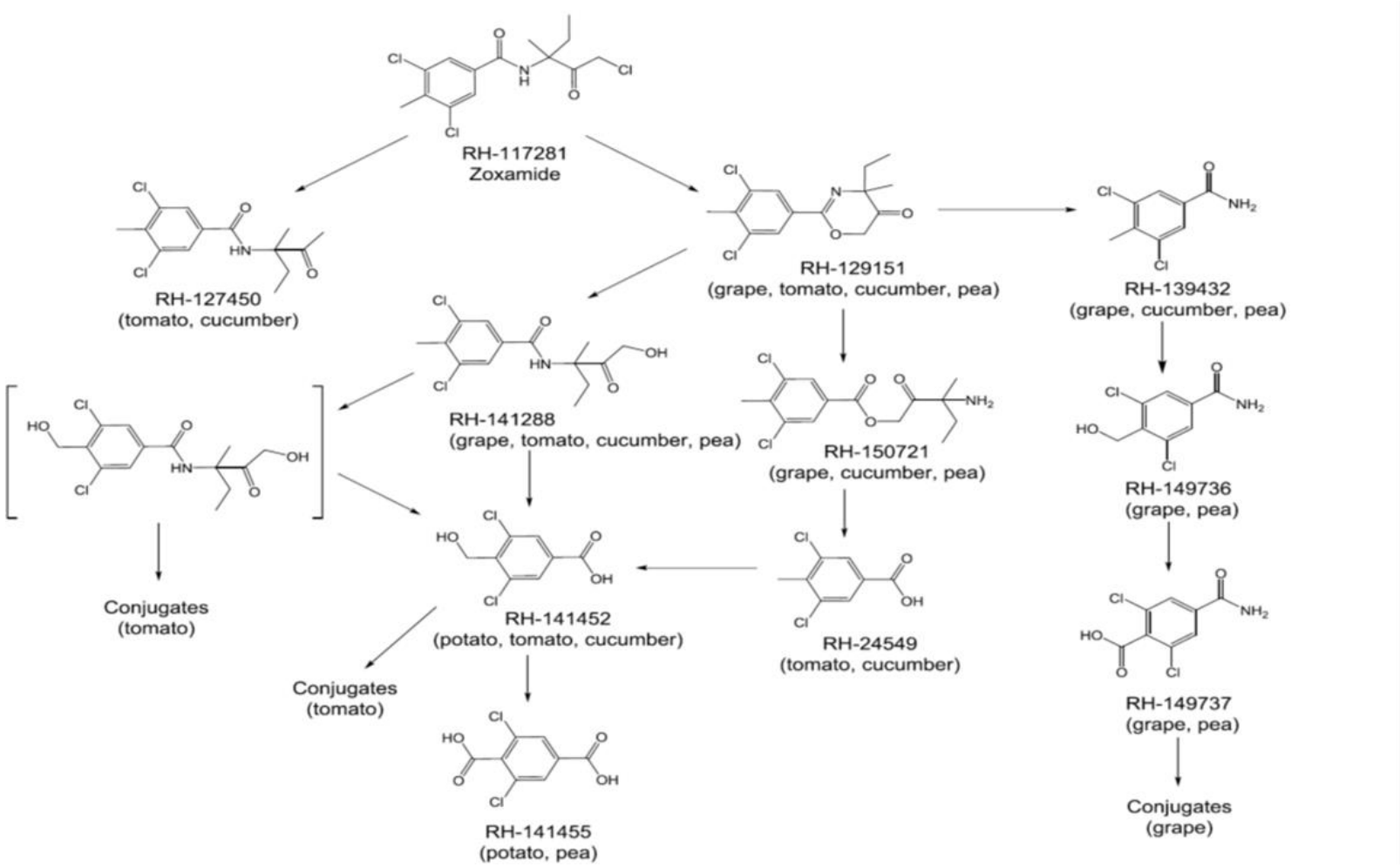


Figure 7.2-1: Plant metabolism of zoxamide

Summary of new plant metabolism studies

No additional / new studies were performed.

Conclusion on metabolism in primary crops

Because of outstanding residues data matching the intended GAP and outstanding questions on the toxicological profile of the zoxamide metabolites RH-141452 and RH-141455, an overall residue definition for plants was not set during AIR (see EFSA Conclusion, 2017).

For risk assessment, provisional residue definitions were proposed by EFSA (2017): For fruit crops the sum of zoxamide and RH-141452, for pulses and oilseeds zoxamide only, and for root crops the sum of metabolites RH-141455 and RH-141452.
For monitoring, the residue definitions were proposed as zoxamide for fruit crops, pulses and oilseed (as marker), and the metabolites RH-141455 and RH-141452 for root crops.

Provisional residue definitions of EFSA (2017):

Plant residue definition for monitoring (RD-Mo) OECD Guidance, series on pesticides No 31	Zoxamide (fruit, pulses and oilseeds) Metabolites RH-141455 and RH-141452 (root crops) pending data gap for RH-141455 and RH-141452
Plant residue definition for risk assessment (RD-RA)	Zoxamide and RH 141452 (fruit) pending data gap on RH-141452 Zoxamide (Pulses and oilseeds) Metabolites RH-141455 and RH-141452 (root crops) pending data gap for RH-141455 and RH-141452
Conversion factor (monitoring to risk assessment)	1

The zoxamide metabolites RH-141452 and RH-141455 were identified as major metabolites (>10% TRR) in the potato metabolism study (Reibach, PH and Spencer, WO, 1998) but are not found in actual supervised field trials at levels ≥ 0.01 mg/kg.

Lower levels of RH-141452 (< 10 % TRR) were also found in the tomato and cucumber metabolism studies, and RH-141455 (< 10 % TRR) in the pea metabolism study. Considering the overdosed supervised residue trials (i.e. 5 x 180 g a.s./ha applied at an interval of 8 ±1 days with a PHI of 28 days), RH-141452 can occur at max. concentrations around the LOQ of 0.01 mg/kg up to 0.0188 mg/kg in grape bunches. However, based on the data available for RH-141455 and RH-141452, both metabolites are regarded as toxicologically not relevant. For further information, please refer to chapter 6.3 in the dRR Part B Section 6 document.

The relevant residue in grape fruits can be concluded as zoxamide.

Following foliar application of ^{14}C-labelled zoxamide to primary crops, most of the applied material generally remains on the plant surface. In the metabolism studies conducted on grapes, tomatoes, cucumbers and peas, the major component of the residue was unchanged zoxamide (RH-7281).

The degradation of zoxamide on/in plants follows a similar metabolism path (see Figure 7.2-1).

A similar degradation path of zoxamide in crops can be concluded, with zoxamide as main / marker residue. However, in contrast to e.g. pulses, vines or tomatoes, different uptake paths via potato tubers and/or their roots of probably the soil metabolites of zoxamide seem to play a role when defining zoxamide residues in bulb and tuber plants (e.g. onions, potatoes).

As zoxamide is a racemate, its metabolites containing the chiral carbon atom may feature two enantiomers. Chiral analysis of residues confirmed the stability of the chiral centre of zoxamide and its metabolites. As a result, the residues are given as the sum of enantiomers for the respective compounds.

7.2.2.2 Nature of residue in rotational crops (KCA 6.6.1)

Available data

No new data are submitted in the framework of this application.
The following table summarises the available studies of zoxamide on rotational crops.

Table 7.2-6: Summary of metabolism studies in rotational crops

Crop group	Crop	Label position	Application and sampling details				Reference
			Method, F or G *	Rate (kg a.s./ha)	PBI (days)	Remarks	
EU data							
Fruits and fruiting vegetables	--	--	--	--	--	--	--
Leafy vegetables	Mustard	^{13}C- and ^{14}C-zoxamide, uniformly ring labelled	F	4 x 0.5 kg a.s./ha	30, 145, 210, 365	After 30 days PBI max. 0.009 mg/kg RH-141452 as only relevant residue in immature mustard leaves	EFSA, 2017
Root and tuber vegetables	Radish, turnip	^{13}C- and ^{14}C-zoxamide, uniformly ring labelled	F	4 x 0.5 kg a.s./ha	30, 137, 210, 365	After 30 days PBI max. 0.006 mg/kg RH-14152 in immature radish tops	EFSA, 2017
Pulses and oilseeds	Soybean	^{13}C- and ^{14}C-zoxamide, uniformly ring labelled	F	4 x 0.5 kg a.s./ha	30, 137, 210, 365	After 30 days PBI max. 0.023 mg/kg RH-14152 in immature soybean forage	EFSA, 2017
Cereals	Sorghum	^{13}C- and ^{14}C-zoxamide, uniformly ring labelled	F	4 x 0.5 kg a.s./ha	30, 137, 210, 365	After 30 days PBI max. 0.001 mg/kg RH-141452 in immature sorghum forage	EFSA, 2017
New data							
Fruits and fruiting vegetable	--	--	--	--	--	--	--
Leafy vegetables	--	--	--	--	--	--	--

Root and tuber vegetables	--	--	--	--	--	--	--
Pulses and oilseeds	--	--	--	--	--	--	--
Cereals	--	--	--	--	--	--	--

* Outdoor/field application (F) or glasshouse/protected/indoor application (G)
PBI = Plant back interval

Summary of plant metabolism studies in rotational crops reported in the EU

Zoxamide degrades quickly - in soil with a DT_{50} of 5.5 days (see chapter 8.3.1.1 in dRR Part B Section 8) and in plants with a foliar half-life of 3.9 days (see Klein & Mendel-Kreusel, 2020; report no. GOW1120-1). In addition, zoxamide is not systemic. Therefore, residues of zoxamide and its metabolites in rotational plants are very unlikely.

In the rotational crop study, ^{14}C-zoxamide was applied directly to bare soil at a rate of 4 x 0.50 kg/ha. Leaf (mustard), root (radish/turnip), small grain (wheat/sorghum), and oilseed (soybean) crops were planted into the soil at 30, 137/145, 210 or 365 days after the last application (DALA), and the crops grown to maturity. The results indicate that following application to bare soil, zoxamide residues are minimally translocated to crop samples and will not result in any detectable levels of parent compound or parent related metabolites in rotational crops. The highest TRR observed was 0.189 mg/kg in 30 DALA soybean straw and no mature crop except soybeans contained ≥ 0.05 mg/kg of TRR in any sample at any plant back interval.

The major portion of the observed residue was not extractable except by hydrolytic processes. Parent zoxamide was not present in any crop sample at any plant back interval. No organic solvent extractable compound was present at ≥ 0.01 ppm in any crop sample at any plant back interval. No component of any extract exceeded 0.023 mg/kg in any sample. Extracts were compared by HPLC to the inventory of known degradates of zoxamide and the only possible match for zoxamide related degradates was for traces of **RH-141452 (at max. of 0.023 mg/kg in in immature soybean forage)**, a highly degraded soil metabolite, also occurring at low levels in potato tubers.

It is most likely that the route of formation of residues found in rotational crops is through metabolism in soil followed by low-level uptake of highly degraded components, including CO_2, which are then incorporated into natural products such as sugars, starch, and cell wall carbohydrates in the plant.

Summary of new plant metabolism studies

No new data are submitted in the framework of this application.

Conclusion on metabolism in rotational crops

Rotational crop studies are not relevant for plant protection products in permanent crops (such as vines).

^{14}C-zoxamide was applied directly to bare soil at a rate of 4 x 0.50 kg/ha. This application rate is equivalent to 4.5-fold the seasonal application rate of zoxamide applied as CYMOXANIL 33% + ZOXAMIDE 33 % WG to tomatoes, grapes or potatoes in the open field.

Leafy (mustard), root (radish/turnip), small grain (wheat/sorghum), and oilseed (soybean) crops were sown or planted into the soil at 30, 137/145, 210 or 365 days after the last application (DALA), and the crops were grown to maturity. The results of the rotational crop study indicate that following application to bare soil, zoxamide residues are minimally available in the soil for uptake by succeeding crops and translocation with the water stream from the roots into the leafy (and edible) parts of these crops. No component of any aqueous extract exceeded 0.023 mg/kg in any sample. Extracts were compared by HPLC to the inventory of known degradates of zoxamide and the only possible match was for traces of **RH-141452 (at max. of**

0.023 mg/kg in immature soybean forage), a highly degraded soil metabolite, also occurring at low levels in potato tubers.

This result was expected since zoxamide degrades quickly - in soil with a DT_{50} of 5.5 days (see chapter 8.3.1.1 in dRR Part B Section 8) and in plants with a foliar half-life of 3.9 days (see Klein & Mendel-Kreusel, 2020; report no. GOW1120-1). In addition, zoxamide is not systemic. Therefore, residues related to moieties of zoxamide and its metabolites in rotational plants are very unlikely. It is most likely that the route of formation of residues found in rotational crops is through metabolism in soil followed by low-level uptake of highly degraded components, including CO_2, which are then incorporated into natural products such as sugars, starch, and cell wall carbohydrates in the plant.

The highest relevant residue of zoxamide related compounds was 0.023 mg/kg RH-141452 in immature soybean forage. The crop was planted 30 days after bare soil treatment 4 times with 500 g zoxamide/ha, amounting to a total of 2000 g zoxamide/ha/season. Related to the lower zoxamide application rates intended with CYMOXANIL 33% + ZOXAMIDE 33% WG on potato and field tomatoes (i.e. max. 3 x 148.5 g/ha = 445.5 g/ha/season – not considering plant interception), this would amount to a max. residue of 0.005 mg a.s./kg in mature radish root. This is below any relevant residue (< 0.01 mg/kg) in food or feed commodity.

7.2.2.3 Nature of residues in processed commodities (KCA 6.5.1)

Available data

New hydrolysis studies have been submitted by the applicant in the framework of this application. These studies are summarised in the table below. The detailed results of the studies are presented in Appendix 2.

The fermentation process with zoxamide has been studied in a radiolabelled vinification study (Mamouni 1998; RAR 2017, B.7.5.1). Red and white grapes were treated in the laboratory with [^{14}C]-zoxamide at 3 mg/kg and processed to juice (must) and 6 different wines. As a result, the concentration of the parent zoxamide decreased slowly in wine during storage, and was mainly degraded to RH-150721 (11.5-19.4% of the applied radioactivity in white wine, 3.1-8.9% of the applied radioactivity in rosé/red wine). Up to 10 additional minor fractions were detected, which included RH-139432 and RH-24549, with no individual fraction exceeding 3.8% of the applied radioactivity. During fermentation very little degradation to carbon dioxide was observed (<0.1 % of the initial dose). Thus, RH-150721 was the only major degradation product.

In a study of Völkl (2000; report no. DERBI 92297) tomatoes were treated with [^{14}C]-zoxamide and the distribution and identification of degradates of zoxamide in products made from tomatoes were investigated. The study was not presented in the DAR or the RAR for zoxamide, but was used for the authorisation of zoxamide products in tomatoes all over Europe. It is therefore included in the submission, regarded as supplementary information, and summarised in Appendix 2 to this document in a short format for completeness. From this study, it appeared that the residue definition for processed tomatoes was the same as for primary crops (zoxamide only). However, evidence was presented in the study that, for certain processed commodities (e.g. tomato puree) the major residue was not zoxamide. In the puree where the whole tomatoes were cooked for 20-30 minutes the parent substance was degraded mainly to RH-129151 and RH-150721. The parent compound amounted to 3.1% (0.092 ppm) of the radioactivity applied. The main degradation products reached levels of 10.9% (0.324 ppm) of the radioactivity applied and 6.7% (0.199 ppm), respectively.

The nature of residues under standard hydrolysis conditions at processing has not been fully addressed for the whole range of processes during AIR. EFSA (2017) therefore requested: *"The nature of residues for zoxamide including the nature of RH-141455 and RH-141452 under standard hydrolysis conditions repre-*

sentative of pasteurisation, baking/brewing/cooking, sterilisation is required (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see Section 3).” New hydrolysis studies with zoxamide and its metabolites RH-141455 and RH-141452 have been performed and are summarised in the following. For details, please refer to Appendix 2.

The results from the new nature of hydrolysis study with the parent compound zoxamide (Grist, 2018) can be summarised as follows:

Table 7.2-7: Summary of results from the nature of residues study under standard processing conditions in buffer solutions treated with [^{14}C]-zoxamide (% TRR)

Metabolites of zoxamide appearing > 10 % are marked in **bold.**

Condition	**pH 4, 90 °C**	**pH 5, 100°C**	**pH 6, 120 °C**
Representative for …	**Pasteurization**	**baking, brewing, boiling**	**Sterilisation**
Zoxamide	35.3	1.2	0.8
RH-24549	9.7	**62.7**	**43.0**
RH-150721	**46.7**	**11.0**	0.7
RH-129151	4.4	**13.1**	**20.8**
RH-141288	1.1	8.1	**27.3**

The hydrolysis of the parent compound zoxamide in water has also been studied in the environmental fate section at lower temperature (i.e. 25°C) with the following results (see EFSA, 2017). Metabolites > 10 % AR are highlighted in yellow:

Hydrolysis of active substance and relevant metabolites > 10% (DT_{50}) (state pH and temperature)	pH 4: Parent: 25°C DT_{50} 16 days (1^{st} order, r^2 1.0) RH-129151 (0.67% AR, day 3) RH-150721 25°C DT_{50} 18.3 days * (37.6% AR, day 21) RH-24549 stable (30.9 %AR, day 30) RH 141288 (0.6% AR, day 30) * kinetics: linear and non-linear compartmental regression analysis (SAS JMP Version 3.2). pH 7: Parent: 25°C DT_{50} 16 days (1^{st} order, r^2 1.0) RH-129151 25°C DT_{50} 9.1 days * (24.5% AR, day 21) RH-150721 (1.5% AR, day 30) RH-24549 stable (20.75% AR, day 30) RH 141288 stable (21.9% AR, day 30) * kinetics: linear and non-linear compartmental regression analysis (SAS JMP Version 3.2). pH 9: Parent: 25°C DT_{50} 8 days (1^{st} order, r^2 1.0) RH-129151 25°C DT_{50} 2.4 days * (16.4% AR, day 7) RH-150721 (0.13% AR, day 30) RH-24549 stable (11.5% AR, day 30) RH 141288 stable (50.2% AR, day 30) * kinetics: linear and non-linear compartmental regression analysis (SAS JMP Version 3.2).

Both studies are showing a similar metabolite profile with the metabolites RH-150721, RH-129151, RH-24549 and RH-141288 appearing at > 10% AR.

Under the processes representative for the processing of food commodities at higher temperatures …

- RH-24549, although not appearing > 10 % during pasteurisation (pH 4, 90 °C) should be considered as major metabolite for all food processing procedures for safety reasons.
- RH-150721 appears > 10 % only at lower pH values, and therefore under the processing conditions representative for pasteurisation (pH 4, 90 °C) and baking, brewing, boiling (pH 5, 100°C).
- RH-129151 appears only > 10 % at higher pH values, and therefore under processing conditions representative for baking, brewing, boiling (pH 5, 100°C) and sterilisation (pH 6, 120 °C).
- RH-141288 appears only > 10 % at high pH values under processing conditions representative for sterilisation (pH 6, 120 °C).

The study performed by Longhi (2019) demonstrates that RH-141452 is stable and stays unchanged under the conditions simulating pasteurisation (pH 4, 90°C for 20 minutes), baking/brewing/boiling (pH 5, 100°C for 60 minutes) and sterilisation (pH 6, 120°C for 20 minutes). No degradation at all was detected for RH-141452.

The study performed by Longhi (2019) demonstrates that RH-141455 is stable and stays unchanged under the conditions simulating pasteurisation (pH 4, 90°C for 20 minutes), baking/brewing/boiling (pH 5, 100°C for 60 minutes) and sterilisation (pH 6, 120°C for 20 minutes). No degradation at all was detected for RH-141455.

Table 7.2-8: Nature of the residues in processed commodities

Conditions (Duration, Temperature, pH)	**Identified compound(s) (%)**	**Reference**
EU data		
Pasteurisation (20 minutes, 90°C, pH 4)		
Baking, boiling, brewing (60 minutes, 100°C, pH 5)		
Sterilisation (20 minutes, 120°C, pH 6)		
Other conditions	**Identified compound(s) (%)**	
Winemaking …	Radiolabelled vinification study showed that the major residue in wine is the metabolite RH-150721.	EFSA, 2017
New data – derived with zoxamide as test item *		
Pasteurisation (20 minutes, 90°C, pH 4)	Parent zoxamide (35.3 %) RH-24549 (9.7 %) RH-150721 (46.7 %)	Grist, 2018 report no. RB66JN
Baking, boiling, brewing (60 minutes, 100°C, pH 5)	Parent zoxamide (1.2 %) RH-24549 (62.7 %) RH-150721 (11.0 %) RH-129151 (13.1 %)	
Sterilisation (20 minutes, 120°C, pH 6)	Parent zoxamide (0.8 %) RH-24549 (43.0 %) RH-129151 (20.8 %) RH-141288 (27.3 %)	
New data – derived with RH-141455 as test item *		
Pasteurisation (20 minutes, 90°C, pH 4)	Parent RH-141455 (99.39 %)	Longhi, 2019 BPL-STUDY-19-000009
Baking, boiling, brewing (60 minutes, 100°C, pH 5)	Parent RH-141455 (98.95 %)	

Conditions (Duration, Temperature, pH)	Identified compound(s) (%)	Reference
Sterilisation (20 minutes, 120°C, pH 6)	Parent RH-141455 (99.75 %)	
New data – derived with RH-141452 as test item *		
Pasteurisation (20 minutes, 90°C, pH 4)	Parent RH-141452 (99.22 %)	Longhi, 2019 BPL-STUDY-18-000092
Baking, boiling, brewing (60 minutes, 100°C, pH 5)	Parent RH-141452 (100.8 %)	
Sterilisation (20 minutes, 120°C, pH 6)	Parent RH-141452 (99.29 %)	
Other conditions	**Identified compound(s) (%)**	
Tomato processing	Radiolabelled processing study showed that the major residue in cooked tomatoes (e.g. tomato puree) was mainly to RH-129151 and RH-150721. The parent compound amounted to 3.1% (0.092 ppm) of the radioactivity applied. The main degradation products reached levels of 10.9% (0.324 ppm) of the radioactivity applied and 6.7% (0.199 ppm), respectively.	Völkl, 2000 DERBI 92297

* considered are the data for the parent and metabolites appearing ≥ 10 % of applied

Conclusion on nature of residues in processed commodities

RH-141455 and RH-141452

The study performed by Longhi (2019) demonstrates that RH-141452 is stable and stays unchanged under the conditions simulating pasteurisation (pH 4, 90°C for 20 minutes), baking/brewing/boiling (pH 5, 100°C for 60 minutes) and sterilisation (pH 6, 120°C for 20 minutes). No degradation at all was detected for RH-141452.

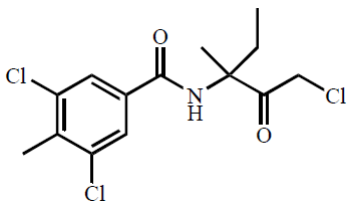
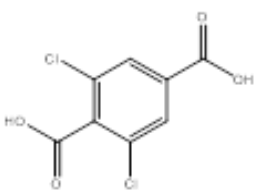
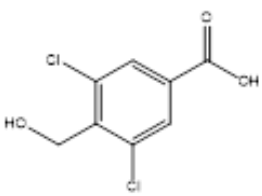
The study performed by Longhi (2019) demonstrates that RH-141455 is stable and stays unchanged under the conditions simulating pasteurisation (pH 4, 90°C for 20 minutes), baking/brewing/boiling (pH 5, 100°C for 60 minutes) and sterilisation (pH 6, 120°C for 20 minutes). No degradation at all was detected for RH-141455.

Considering the overdosed supervised residue trials (i.e. 5 x 180 g a.s./ha applied at an interval of 8 ±1 days with a PHI of 28 days), RH-141452 can occur at max. concentrations around the LOQ of 0.01 mg/kg up to 0.014 mg/kg in grape must, juice, young and bottled wine. A concentration of residues occurs in raisins (with up to 0.0572 mg/kg).

However, based on the data available for RH-141455 and RH-141452 both metabolites are regarded as toxicologically not relevant. For further information, please refer to chapter 6.3 in the dRR Part B Section 6 document.

The metabolites RH-141455 and RH-141452 are structurally related.

Table 7.2-9: Structural formula of zoxamide and metabolites under discussion

		
Zoxamide	RH-141455	RH-141452

RH-141452 and RH-141455 are formed by the hydrolysis of zoxamide to form the intermediate, RH-24549, which is first oxidised to RH-141452 and then to RH-141455 (see rat metabolism in the RAR, 2017 or the dRR Part B Section 6).

Both RH-141452 and RH-141455 are rat metabolites found at low levels in urine (Swenson, R.E., Frederick, C.B., Graves, D.D. 1998a, Report No: 94R-235, ER Ref No: 24.1).

Metabolism of zoxamide to RH-141452 and RH-141455 has the effect of increasing the polarity of the residue and thereby increasing water solubility, which facilitates excretion. From the structures of RH-141452 and RH-141455, which are small molecules containing aromatic carboxylic acids, it would be predicted that these compounds are readily excreted largely unchanged. Rat metabolism studies have been performed with both RH-141452 and RH-141455, which confirm this expectation (xxx, 1998a, Report No: 97RC-154, ER Ref No: 27.1 and Wu, D., Gu, Z. 1998b, Report No: 98RC-017, ER Ref No: 27.2).

Following oral administration of RH-141452 to rats, the majority of the RH-141452 was eliminated unchanged through urine, accounting for >94% of the administered dose. Three minor conjugates, M-2, M-3 (glucuronide conjugates), and M-4 (glycine conjugate) were also found in the urine samples, accounting for ~3% of the administered dose. An additional 1.6% of the administered radioactivity was excreted in the faeces as the parent chemical. Following oral administration of RH-141455 to rats, greater than 96% of radioactivity excreted in faeces (73%) and urine (11%) was identified to be unchanged parent. Some very minor metabolites were also observed in urine samples but were not identified due to their extremely low percentage of dose.

The hydrolysis of zoxamide and the subsequent oxidation steps to form RH-141452 and RH-141455 are regarded as detoxification reactions, and therefore both metabolites are expected to be less toxic than the parent zoxamide. In acute oral toxicity studies, the LD_{50} of RH-141452 and RH-141455 in male and female mice were both > 5000 mg/kg bw.

Complete *in vitro* genotoxicity packages are available for both RH-141455 and RH141452, demonstrating their non-genotoxicity.

In addition, a 14 days range-finder and a 90 days dietary toxicity study with RH-141455 in rats (Satish Kumar, 2020; report no. U-19102), confirming the chronic toxicoogically non-relevance (NOAEL >1000 mg/kg bw/d).

A comparison of the toxicological profile of zoxamide and the two metabolites RH-141452 and RH-141455 based on OECD QSAR Toolbox (Pellizzaro & Da Silva, 2017; see RAR (2017) indicates that both metabolites are expected to have a lower toxicity than the parent zoxamide.

Based on the data available for **RH-141455 and RH-141452,** both metabolites **are** regarded as **toxicologically not relevant**. For further information, please refer to chapter 6.3 in the dRR Part B Section 6 document.

<u>RH-150721</u>

The fermentation process with zoxamide has been studied in a radiolabelled vinification study (Mamouni, 1998; RAR 2017, B.7.5.1). As a result, zoxamide decreased slowly in wine during storage. RH-150721 was the only major degradation product occurring at ≥ 10% AR.

RH-150721 is a proposed intermediate in mammalian metabolism and during other (abiotic) degradation processes. In the "aqueous hydrolysis study" (Reynolds, 1998; report nos. RPT00251, 34-98-39) performed under sterile conditions at 25°C in the dark, zoxamide was unstable and underwent appreciable and rapid hydrolytic degradation in buffered aqueous solutions at pH levels of 4, 7 and 9. The transient metabolite RH-150721 was formed – mainly at lower pH values - by elimination of hydrogen chloride, resulting in the transient cyclic metabolite RH-129151, followed by ring opening and further degradation.

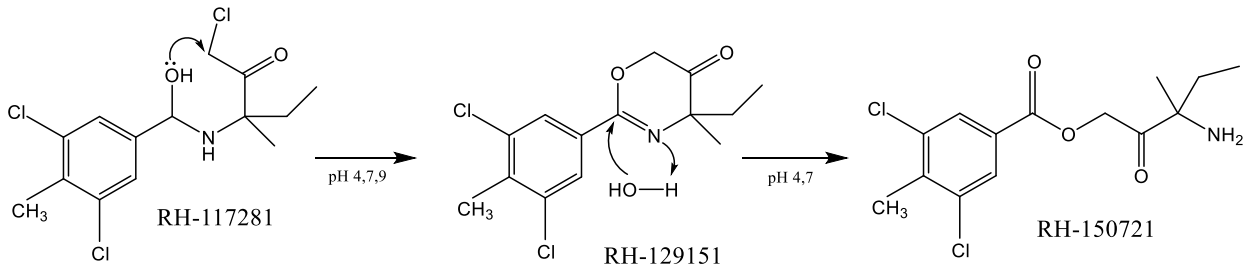


Figure 7.2-2: Formation of RH-150721

Additionally, the stability of zoxamide decreased as both pH and temperature increased. In the aqueous hydrolysis study, at pH 7, levels of RH-150721 increased from 0.45% after 7 days to 1.24% after 21 days.

Considering the overdosed supervised residue trials (i.e. 5 x 180 g a.s./ha applied at an interval of 8 ±1 days with a PHI of 28 days), RH-150721 can occur at max. concentrations around the LOQ of 0.01 mg/kg up to 0.0577 mg/kg in young and bottled wine. A concentration of residues was noted in raisins (with up to 0.056 mg/kg). Besides, it was detected in tomato processed fractions at concentrations around LOQ up to 0.0556 mg/kg in tomato juice after pasteurisation.

The toxicological relevance of RH-150721 has been addressed in a tox data package (see dRR Part B, chapter 6.3). It was demonstrated that the metabolite occurs as an intermediate in the rat metabolism. QSAR analyses performed with both DEREK and the OECD Toolbox did not show structural alerts for any significant toxicological hazards. RH-150721 was shown to be non-genotoxic in a complete *in vitro* genotox data package. As exposure of humans to RH-150721 via food is possible (e.g. wine, juice), further toxicological data was generated, a 14 days dose range finding study and a 90 days dietary study in rats, as a basis to set a chronic reference value (ADI) for consumer risk assessment. In the 14 days oral tox study a NOAEL of 5000 ppm (equivalent to appr. 334 mg/kg bw/day in males and 382 mg/kg bw/day in females) was derived. In the 90 days oral toxicity study, a NOAEL of 2000 ppm (111 mg/kg bw/d in males) was achieved.

The toxicological profile of RH-150721 is mainly driven by body weight and food consumption (avoidance) effects, most other effects are secondary in nature or adaptive. The NOAEL of the 90-day study was set by the study director at 111 mg/kg bw/d. Considering that there were no indications of highly potent toxicological mode of action such as neurotoxicity (FOB negative) or progressive histopathological changes, and the toxicological effect level was very similar between a 14 d treatment regime and a 90 d treatment regime, it is proposed to use an extrapolation factor of 2 from subchronic to chronic to derive an ADI for RH-150721 (see also Strupp, 2020; report no. CS13072020). This proposal is based on the REACH guidance[1].

90 d NOAEL for **RH-150721** : 111 mg/kg bw/d

Safety factor : 100 (standard) x 2 (from 90 d study to chronic[2]) = 200

--> **ADI = 0.56 mg/kg bw/d**

See chapter 6.3 in the dRR Part B Section 6 document.

RH-129151

In a study of Völkl (2000; report no. DERBI 92297) tomatoes were treated with [^{14}C]-zoxamide and the distribution and identification of degradates of zoxamide in products made from tomatoes were investigated. As a result, in puree the parent substance was degraded mainly to RH-129151 and RH-150721

[1,2] Guidance on Information Requirements and Chemical Safety Assessment, Chapter R.8: Characterization of Dose [Concentration]-Response for Human Health, dated Nov. 2012

amounting to 10.9% and 6.7% AR, respectively. In addition, it was demonstrated by Grist (2018) that RH-129151 can appear > 10 % at higher pH values, and therefore under processing conditions representative for boiling (pH 5, 100°C) and sterilisation (pH 6, 120 °C).

Considering the overdosed supervised residue trials (i.e. 5 x 180 g a.s./ha applied at an interval of 8 ±1 days with a PHI of 28 days), RH-129151 can occur at max. concentrations around the LOQ of 0.01 mg/kg up to 0.0164 mg/kg in tomato juice before pasteurisation.

An *in-vitro* genotox package has been performed with this metabolite. The studies presented in the dRR Part B Section 6 demonstrate that RH-129151 is non-genotoxic.

Besides, RH-129151 is a proposed intermediate in mammalian metabolism but was not isolated from a rat metabolism study. However, within the metabolic pathway in rats, reference is made to the "aqueous hydrolysis study" (Reynolds, 1998). The study shows that under sterile conditions at 25°C in the dark zoxamide is unstable and undergoes appreciable and rapid hydrolytic degradation in buffered aqueous solutions at pH levels of 4, 7 and 9. The transient metabolites RH-129151 and RH-150721 are formed by elimination of hydrogen chloride, resulting in the transient cyclic metabolite RH-129151, followed by ring opening and further degradation.

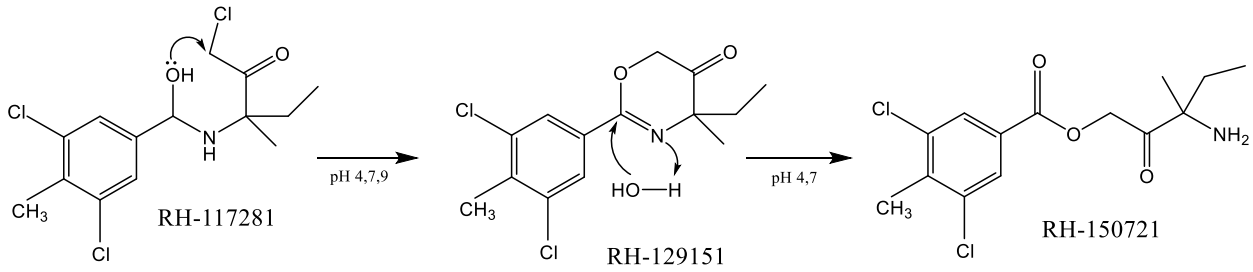


Figure 7.2-3: Formation of RH-150721 via RH-129151

Taking into consideration the physiological pH value (ca. 7.4) and temperature (ca. 37°C) of living mammals, this route of transformation of zoxamide was and is considered to contribute to the metabolic pathway of zoxamide in the rat (and in mammals *per se*).

The metabolite is not relevant for wine grapes, but appears in tomato processed commodities.

RH-24549

RH-24549 occurred at > 10% in a nature of hydrolysis study with the parent compound zoxamide (Grist, 2018) and was therefore considered in the supervised residues trials.

Considering the overdosed supervised residue trials (i.e. 5 x 180 g a.s./ha applied at an interval of 8 ±1 days with a PHI of 28 days), RH-24549 can occur at max. concentrations around the LOQ of 0.01 mg/kg up to 0.02 mg/kg in bottled wine and around the LOQ up to 0.0358 mg/kg in tomato processed fractions.

RH-24549 is a proposed intermediate in mammalian metabolism and during other (abiotic) degradation processes. In the "aqueous hydrolysis study" (Reynolds, 1998; report nos. RPT00251, 34-98-39) performed under sterile conditions at 25°C in the dark, zoxamide was unstable and underwent appreciable and rapid hydrolytic degradation in buffered aqueous solutions at pH levels of 4, 7 and 9. The metabolite RH-24549 was formed by hydrolysis from RH150721. It can be oxidised to RH-141452.

RH-141288

RH-141288 occurred at > 10% in a nature of hydrolysis study with the parent compound zoxamide (Grist, 2018) and was therefore considered in the supervised residues trials.

Considering the range of overdosed supervised residue trials (i.e. 5 x 180 g a.s./ha applied at an interval of 8 ±1 days with a PHI of 28 days), RH-141288 occurred only once in raisins just above the LOQ (at 0.013 mg/kg).

7.2.2.4 Conclusion on the nature of residues in commodities of plant origin (KCA 6.7.1)

Table 7.2-10: Summary of the nature of residues in commodities of plant origin

Endpoints	
Plant groups covered	Fruits and fruiting vegetables (grapes, tomatoes, cucumber) Root and tuber vegetables (potatoes) Pulses and oilseeds (peas)
Rotational crops covered	Yes
Metabolism in rotational crops similar to metabolism in primary crops?	Yes
Processed commodities	a.s. is not stable under standard hydrolysis conditions RH-141455 and RH-141452 are stable under standard hydrolysis conditions
Residue pattern in processed commodities similar to pattern in raw commodities?	Yes other residue definition in processed commodities Yes, including (tentative) for risk assessment: RH-150721 in grape and tomato processed fractions; separate ADI of 0.56 Zoxamide +… RH-129151 in tomato processed fractions RH-24549 in grape and tomato processed fractions RH-141288 in raisins
Plant residue definition for monitoring	Residue definition (Regulation (EU) n° 2017/171) : **Zoxamide**
Plant residue definition for risk assessment	Residue definition EFSA (2017) revised : Zoxamide and RH-141452 (fruits) pending data gap on RH-141452 => RH-141452 toxicologically not relevant Zoxamide (pulses and oilseeds) Metabolites RH-141455 and RH-141452 (root crops) pending data gap for RH-141455 and RH-141452 => RH-141455 and RH-141452 toxicologically not relevant; **Zoxamide proposed as (common) residue definition**
Conversion factor from enforcement to RA	(1 for the provisional residue definition in EFSA 2017)

7.2.2.5 Nature of residues in livestock (KCA 6.2.2-6.2.5)

Available data

No new data are submitted in the framework of this application.

A lactating ruminant study is available on EU level, its characteristics and results are summarised in the

following table.

Table 7.2-11: Summary of animal metabolism studies

Group	Species	Label position	No of animal	Application details		Sample details		Reference
				Rate (mg/kg bw/d)	Duration (days)	Commodity	Time of sampling	
EU data								
Lactating ruminants	Goat	^{14}C-zoxamide labelled in the phenyl ring	2	2.82 mg/kg bw/day (60.7 mg/kg)	8	Milk	twice daily	Latvia, 2017; EFSA, 2017
						Urine and faeces	Daily	
						Tissues	at sacrifice	
Laying poultry	--	--	--	--	--	Eggs	--	--
						Excreta	--	
						Tissues	--	
New data								
Lactating ruminants	--	--	--	--	--	Milk	--	--
						Urine and faeces	--	
						Tissues	--	
Laying poultry	--	--	--	--	--	Eggs	--	--
						Excreta	--	
						Tissues	--	

Zoxamide, labelled uniformly in the aromatic (phenyl) ring with ^{14}C, was administered orally in gelatine 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 60.7 mg/kg (2.82 mg/kg bw/day). 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.

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. The daily total radioactive residue (TRR) in milk reached a maximum on day 2. Individual tissues and cumulative milk samples amounted to <0.3% of the administered dose on day 7. Fractions containing significant TRR levels (>10% of TRR, >0.01 mg/kg) were analysed for their metabolite profiles. A summary of the results is given in the following tables; for details see RAR (2017) Vol. 3, B.7.

Table 7.2-12: Summary of the distribution of radioactivity, expressed as a percent of the administered dose and total terminal residues

Matrices	% administered	Total terminal residues (mg/kg)

	dose	
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%	

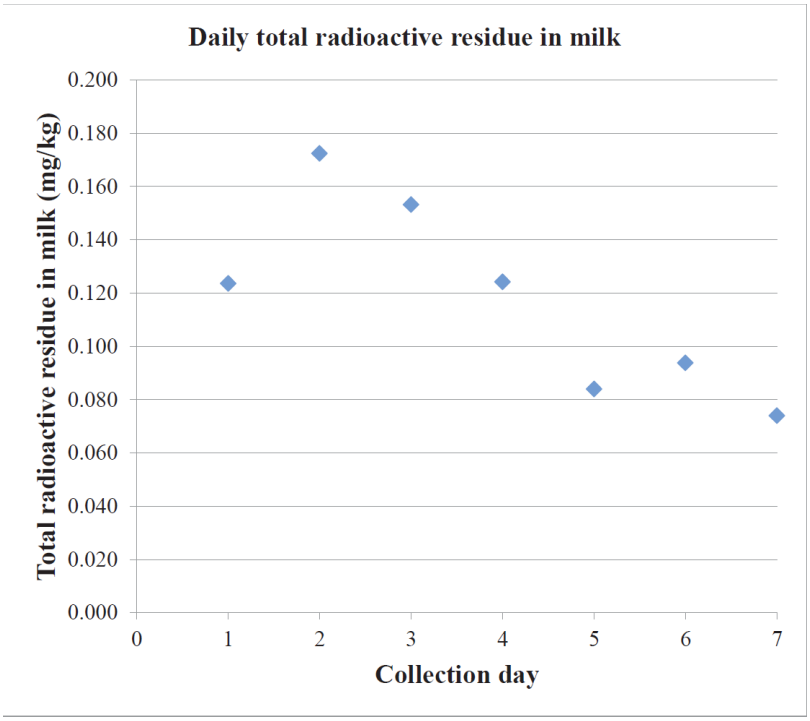


Figure 7.2-3: Total radioactive residues in milk

Table 7.2-13: Major metabolites in extractable fractions form milk and tissues

Metabolite	% TRR	mg/kg (parent equivalents)	Remark
Milk - Day 4			
M-12a, M-12b	37.87	0.090	isomers of a dihydroxylated analogue of RH-127,450
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			
RH-141288	15.75	0.031	
RH-127450	65.18	0.129	
Others	4.07	0.008	
Total	**85.00**	**0.168**	
Liver			
M-1, M-2, M-3, M-4	17.27	0.078	M-2 and M-4 major
M-5	14.53	0.065	
M-5, M6	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**	
Kidney			
M-1, M-2, M-3, M-4	17.822.03	0.065	M-2 and M-4 major
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	0.64	0.002	
M-12a, M-12b	11.72	0.043	isomers of a dihydroxylated analogue of RH-127,450
Others	4.20	0.015	
Total	**88.43**	**0.323**	
Muscle			
M-1, M-2, M-3, M-4	2.96	0.001	
M-5	5.03	0.002	
M-5, M6	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	isomers of a dihydroxylated analogue of RH-127,450
Others	3.49	0.002	
Total	**92.03**	**0.043**	

Summary of new animal metabolism studies

No new data are submitted in the framework of this application.

Conclusion on metabolism in livestock

Zoxamide was regarded as fat soluble on EU level (see DAR, 2017). However, the GAP uses of zoxamide

on potatoes, grapevines and tomatoes do not result in significant residues (above 0.1 mg/kg total diet) occurring in the diet of poultry and ruminants, therefore no data on poultry or ruminant metabolism are required. Nevertheless, a goat metabolism study is available, which showed that zoxamide was extensively metabolised and readily eliminated following oral administration. No parent zoxamide was found in any tissue or milk sample. A number of metabolites were detected, of which RH-127450 was the most abundant metabolite, found at 0.13 mg/kg (65% TRR) in fat. No significant residues are likely to occur in any edible tissue or milk.

7.2.2.6 Conclusion on the nature of residues in commodities of animal origin (KCA 6.7.1)

Table 7.2-14: Summary on the nature of residues in commodities of animal origin

	Endpoints
Animals covered	Lactating goats
	--
Time needed to reach a plateau concentration	2 days in milk
	--
Animal residue definition for monitoring	None/not relevant (Regulation (EU) n° 2017/171)
Animal residue definition for risk assessment	Pending (EFSA 2017)**
Conversion factor	--
Metabolism in rat and ruminant similar	Yes statement about metabolism in pig***
Fat soluble residue	Yes (potentially), log Pow 3.76 for zoxamide. However, as zoxamide is extensively metabolised and total residue levels in fat were low and not significantly different from other edible tissues, it is proposed that zoxamide should be listed as not fat soluble. Data gap identified by EFSA (2017) for RH-141288 and RH-127450. However, these data are available: Log Pow RH-141288 : 2.892 (Cashmore, 2019; report no. 3202371) Log Pow RH-127450 : 3.5 (Tognucci, 1988; see RAR 2017)
Fish	Zoxamide has a log P_{OW} of 3.76, and therefore has potential to bioaccumulate. However, residues of zoxamide in fish feed arising from the representative uses on potatoes, grapevines and tomatoes are <0.01 mg/kg diet, and therefore a fish metabolism study is not required. A metabolism study in Bluegill sunfish (*Lepomis macrochirus*) is available which shows that metabolism was extensive and no parent zoxamide was found in fillet or viscera. RH-127450 was the major metabolite detected. (see RAR, 2017)
Bee product	Residue data in honey are provided. Proposed residue definition: Zoxamide

*** If metabolism in rat and ruminant are not similar

7.2.3 Magnitude of residues in plants (KCA 6.3)

7.2.3.1 Summary of European data and new data supporting the intended uses

New studies on the magnitude of residue have been submitted by the applicant in the framework of this application. These studies are summarised in the table below. The detailed assessment of these studies is presented in Appendix 2.

Table 7.2-15: Summary of EU reported and new data supporting the intended uses of CYMOXANIL 33% + ZOXAMIDE 33 % WG and conformity to existing MRL - grapes

Commodity	Source	Residue zone	Evaluation E = according to enforcement residue definition RA = according to risk assessment residue definition	STMR (mg/kg)	HR (mg/kg)	R_{ber} (mg/kg)	R_{max} (mg/kg)	OECD calculator MRL (mg/kg)	Current EU MRL (mg/kg)*	MRL compliance
Residue definition for monitoring and enforcement (E) according to Reg. (EU) 2017/171 : Zoxamide **Residue definition for risk assessment (RA) : Zoxamide**										
Table and wine Grapes	EFSA, 2017	N-EU	Not relevant (not GAP compliant; overdosed; higher LOQ)	N/A						
	EFSA, 2017	S-EU	Not relevant (not GAP compliant; overdosed; higher LOQ)							
	Spain + UK 2013 Authorisation dossier	N-EU (n=5)	Trials GAP: 5 x 0.15 kg as/ha, int. 7 d, PHI 28 d E / RA : 0.01*, 0.2656, 0.2980, 0.4586, 0.8598	0.298	0.86	1.32	1.70	2.0	5	Yes
		S-EU (n=5)	Trials GAP: 5 x 0.15 kg as/ha, int. 7 d, PHI 28 d E / RA : 0.0186, 0.0733, 0.0883, 0.2594, 0.2748	0.088	0.275	0.53	0.63	0.7	5	Yes
	New trials	N-EU (n=7)	Trials GAP: 5 x 0.18 kg as/ha, int. 7-8 d, PHI 28 d E / RA : 0.231, 0.541, 0.768, 0.94, 1.19, 1.22, 1.503	0.94	1.50	2.44	2.40	3.0	5	Yes
	New trials	N-EU (n=6)	Trials GAP: 3 x 0.18 kg as/ha, int. 7-8 d, PHI 28 d E / RA : 0.163, 0.289, 0.302, 0.46, 0.48, 0.91	0.38	0.91	1.17	1.40	1.5	5	Yes
	New trials	S-EU (n=8)	Trials GAP: 5 x 0.18 kg as/ha, int. 7-8 d, PHI 28 d E / RA : 0.22, 0.26, 0.31, 0.39, 0.452, 0.52, 0.573, 1.406	0.42	1.41	1.12	1.73	2.0	5	Yes
	New trials**	S-EU (n=3)	Trials GAP: 3 x 0.18 kg as/ha, int. 7-8 d, PHI 28 d, outdoor E / RA : 0.270, 0.430, 1.026	0.43	1.03	No test (<3)	3.63	4.0	5	Yes

Commod-ity	Source	Resi-due zone	Evaluation E = according to enforcement residue definition RA = according to risk assessment residue definition	STMR (mg/kg)	HR (mg/kg)	R_{ber} (mg/kg)	R_{max} (mg/kg)	OECD calculator MRL (mg/kg)	Current EU MRL (mg/kg)*	MRL com-pliance
Residue definition for monitoring and enforcement (E) according to Reg. (EU) 2017/171 : Zoxamide **Residue definition for risk assessment (RA) : Zoxamide**										
	Overall supporting data for cGAP	EU (n=15)	Trials GAP: 5 x 0.18 kg as/ha, int. 7-8 d, PHI 28d, outdoor E/RA : 0.22, 0.231, 0.26, 0.31, 0.39, 0.452, 0.52, 0.541, 0.573, 0.768, 0.94, 1.19, 1.22, 1.406, 1.503	0.54	1.50	2.38	1.84	3.0	5	Yes
	Overall supporting data for cGAP	EU (n= 25)	Trials GAP: 5x 0.15 + 5x 0.18 kg as/ha, int. 7-8 d, PHI 28 d, outdoor E/RA : 0.01*, 0.0186, 0.0733, 0.0883, 0.22, 0.2594, 0.231, 0.26, 0.2656, 0.2748, 0.2980, 0.31, 0.39, 0.452, 0.4586, 0.52, 0.541, 0.573, 0.768, 0.8598, 0.94, 1.19, 1.22, 1.406, 1.503	0.39	1.50	1.63	1.52	2.0	5	Yes

* Source of EU MRL: Reg. (EU) 2017/17

Table 7.2-16: Summary of EU reported and new data supporting the intended uses of CYMOXANIL 33% + ZOXAMIDE 33 % WG and conformity to existing MRL – tomatoes

Commodity	Source	Residue zone	Evaluation E = according to enforcement residue definition RA = according to risk assessment residue definition	STMR (mg/kg)	HR (mg/kg)	R_{ber} (mg/kg)	R_{max} (mg/kg)	OECD calculator MRL (mg/kg)	Current EU MRL (mg/kg)*	MRL compliance
Residue definition for monitoring and enforcement (E) according to Reg. (EU) 2017/171 : Zoxamide **Residue definition for risk assessment (RA) : Zoxamide**										
Tomatoes open field	EFSA, 2017	EU	Not assessed.	N/A						
	Spain + UK 2013 Authorisation dossier	S-EU (n=21)	Trials GAP: 5 x 0.150 kg as/ha, int. 7 ± 1 d, PHI 3 d, outdoor E / RA : 0.03, 2x 0.04, 2x 0.05, 0.12, 0.13, 0.14, 0.15, 0.16, 0.1613, 2x 0.18, 0.20, 0.22, 2x 0.24, 0.2746, 0.30, 0.3031, 0.3073	0.16	0.31	0.48	0.38	0.5	5	Yes
	New trials	S-EU (n=4)	Trials GAP: 5 x 0.18 kg as/ha, int. 7-8 d, PHI 3 d, outdoor E / RA : 0.0825, 0.113, 0.163, 0.198	0.14	0.198	--	--	--	5	Yes
	New trials**	S-EU (n=1)	Trials GAP: 3 x 0.18 kg as/ha, int. 7-8 d, PHI 3 d, outdoor E / RA : 0.11	--	0.11	--	--	--	5	Yes
	Overall supporting data for cGAP	S-EU (n=25)	Trials GAP: 5 x 0.18 kg as/ha, int. 7-8 d, PHI 3 d, outdoor E / RA : 0.03, 2x 0.04, 2x 0.05, 0.0825, 0.113, 0.12, 0.13, 0.14, 0.15, 0.16, 0.1613, 0.163, 2x0.18, 0.198, 0.20, 0.22, 2x 0.24, 0.2746, 0.30, 0.3031, 0.3073	0.16	0.31	0:46	0:36	0:5	5	Yes

* Source of EU MRL: Reg. (EU) 2017/17

** Intended GAP has been reduced due to resistance reasons.

Table 7.2-17: Summary of EU reported and new data supporting the intended uses of CYMOXANIL 33% + ZOXAMIDE 33 % WG and conformity to existing MRL – potatoes

Commodity	Source	Residue zone	Evaluation E = according to enforcement residue definition RA = according to risk assessment residue definition	STMR (mg/kg)	HR (mg/kg)	R_{ber} (mg/kg)	R_{max} (mg/kg)	OECD calculator MRL (mg/kg)	Current EU MRL (mg/kg)*	MRL compliance
Residue definition for monitoring and enforcement (E) according to Reg. (EU) 2017/171 : Zoxamide **Residue definition for risk assessment (RA) : Zoxamide**										
Potatoes	EFSA, 2017	EU	Not relevant (not GAP compliant; overdosed; higher LOQ; only considered where relevant (see Spain, 2013))	N/A						
	Spain + UK 2013 Authorisation dossier	N-EU (n=2)	Trials GAP: 6*0.15 kg as/ha, int. 7 ± 1 d, PHI 7 d, outdoor E/RA: 2x <0.01	--	0.01	--	--	--	0.02	Yes
		S-EU (n=2)	Trials GAP: 5*0.15 kg as/ha, int. 7 ± 1 d, PHI 7 d, outdoor E/RA: 2x <0.01	--	0.01	--	--	--	0.02	Yes
	New trials	N-EU (n=2)	Trials GAP: 5 x 0.18 kg as/ha, int. 7 ± 1 d, PHI 7 d, outdoor E/RA : 2x ND (<0.003)	--	ND (<0.003)	--	--	--	0.02	Yes
		S-EU (n=2)	Trials GAP: 5 x 0.18 kg as/ha, int. 7 ± 1 d, PHI 7 d, outdoor E/RA : 2x ND (<0.003)	--	ND (<0.003)	--	--	--	0.02	Yes
	Overall supporting data for cGAP	S-EU (n=8)	Trials GAP: 5 x 0.18 kg as/ha, int. 7 ± 1 d, PHI 7 d, outdoor E/RA : 4x ND (<0.003), 4x <0.01	0.01	0.01	0.02	0.01	0.02	0.02	Yes

* Source of EU MRL: Reg. (EU) 2017/171

7.2.3.2 Conclusion on the magnitude of residues in plants

The present application is intended for the renewal of the authorisation of CYMOXANIL 33% + ZOXAMIDE 33 % WG as a fungicide against downy mildew in grapes, tomato, eggplant and potato.
Data were already available supporting the intended uses on grapes, tomato and potato but, following the renewal of the active ingredient, new studies were submitted to comply with the requests of the authorities in order to verify if some of the identified metabolites (no one regarded as toxicologically relevant) could affect the level of residues in the different crops.

The available residues trials, performed according to OECD 509 guidelines, were done for different zoxamide formulations inclusive the here intended WG formulation CYMOXANIL 33% + ZOXAMIDE 33 % WG. However, according to the EC (2019) guidance document SANTE/2019/12752 it is possible to extrapolate since experience shows that emulsifiable concentrates (EC), wettable powders (WP), dispersible granules (WG), and suspension concentrates (SC) formulations usually produce comparable residues.

Crop-by-crop evaluation

Grapes: According to the available data, the intended uses on wine and table grapes are considered acceptable, for both Southern EU and Northern EU/France – even if considering supervised residues trials for GAP uses overestimating the risk from the here intended GAP uses (i.e. performed for an application pattern with higher single application rates and/or numbers but the same PHI). Please note that the intended GAP uses were restricted to a maximum of 3 per crop per year in order to avoid resistance.

The residue values in grape berries from study no. BPL-STUDY-19-000041, trial no. B7284 BW1, which are only available for a PHI of 20 days (this is outside the ± 25% range) have not been taken into account for the consumer risk assessment.

A comparison of the available new data sets from Northern and Southern EU with each 5 applications of 180 g a.s./ha at an interval of 8 ± 1 days with a PHI of 28 days according to Mann-Whitney U-Test (FAO Manual 197, p. 87-88) confirms that there are no statistically relevant differences ($\alpha = 0.05$) between the data sets. Therefore, it is applicable to combine them.
Both datasets separately and combined demonstrate lower calculated MRLs compared to the MRL of 5 set on EU level, although the underlying trials have been performed with worse application pattern compared to the here intended GAP use (i.e. higher number of applications and higher single application rates at a PHI of 28 days).

Table 7.2-18: Comparison of residue datasets for grapes (5 x 0.18 kg as/ha, int. 7-8 d, PHI 28 d) from N-EU and S-EU via Mann-Whitney U-Test (FAO Manual 197, p. 87-88)

		N-EU dataset	S-EU dataset
	Number of values:	7	8
	Sum Rank:	71	49
	U_1 and U_2 values:	13	43
	Critical value:	10	(α= 0.05)
n_a =	7	n_b =	8
Result:	**Populations similar**		

There are formally enough supervised residue trials available (n=15) to support the uses on wine and table grapes in S-EU and N-EU (also relevant for France) if taking into account the (worst-case) application pattern of 5x 150 g a.s./ha or 5x 180 g a.s./ha with sprayed at an interval of 7-8 days with a PHI of 28 days.

However, both datasets can also be combined since they differ only in the single application rates, but not more than ±25%. No exceedance of the current EU MRL for zoxamide is expected. According to EU guidelines, extrapolation from table grapes to wine grapes is possible.

Tomato: According to the available data, the intended use on tomatoes in the open field (Southern EU) is considered acceptable. The majority of the trials can be considered as a worst case, as they were conducted with an over-dosed GAP due to a higher application and/or a higher number of applications. Please note that the intended GAP uses were restricted to a maximum of 3 per crop per year in order to avoid resistance. No exceedance of the current EU MRL for zoxamide is expected.

According to EU guidelines, extrapolation from tomato to eggplant is possible. Although actually a MRL for eggplants is not fixed, an application for its setting will be submitted in parallel with this application.

Potato: Data are available from the authorisation dossier (4 trials – 2 Northern EU, 2 Southern EU) together with new data performed under Northern (2 trials) and Southern (2 trials) EU growing conditions. All the data are derived from trials with an over-dosed GAP, but residues were either not detectable (i.e. <0.003 mg/kg) or below the LOQ (< 0.01). Even under these assumptions, and when taking on only the highest available residues (i.e. calculating with LOQ values), no exceedance of the current EU MRL is to expected.

Conclusions

The data submitted show that no exceedance of the EU MRLs will occur for grapes, tomatoes open field (extrapolation to eggplant proposed) and potato. Thus, the intended uses are considered acceptable.
A MRL for eggplants is not fixed, an application for its setting will be submitted in parallel with this application.

7.2.4 Magnitude of residues in livestock

7.2.4.1 Dietary burden calculation

Grape and tomato pomace are not fed to livestock. New trials are available to support the potato use that demonstrate no residues of zoxamide in potato tubers. The trials have been performed with a method at a LOQ of 0.01 mg/kg for zoxamide, RH-141455 and RH-141452. Additional data are available to support the toxicologically non relevance of both RH-141452 and RH-141455. This point is under re-evaluation on EU level in an interzonal procedure. Therefore, dietary burden calculation is not needed here.

7.2.4.2 Livestock feeding studies (KCA 6.4.1-6.4.3)

Livestock feeding studies are not relevant since animals are not exposed to residues via feed above the trigger value established in Reg. (EC) No 1107/2009.

7.2.5 Magnitude of residues in processed commodities (Industrial Processing and/or Household Preparation) (KCA 6.5.2-6.5.3)

Data on processing studies were reviewed during the renewal of zoxamide and were considered acceptable. These data of the RAR (2017) are summarised in the following.
In addition, new data on the magnitude of residues in processed commodities have been generated during the course of supervised residues trials (see KCA 6.3). These data are presented in detail in Appendix 2 under data point A 2.1.3 and are summarised in the following chapter. As far as results at about 10-fold above the analytical method Limit of Quantification (LOQ) were available in the commodity relevant for

processing and were determined (as distinct value) in the processed commodity, they have been taken forward for the calculation of processing factors.

7.2.5.1 Available data for all crops under consideration

New processing studies have been submitted by the applicant in the framework of this application. These studies are summarized in the table below. The detailed results are presented in Appendix 2.

Table 7.2-19: Overview of the available processing studies

Processed commodity	Number of studies	Median PF *	Median CF **	Comments	Reference
EU data					
Enforcement residue definition: Zoxamide					
Grapes, juice (unclarified)	2	0.13	--		Latvia, 2017
Grapes, juice (clarified)	2	0.05	--		Latvia, 2017
Grapes, died (raisins)	2	2.85			Latvia, 2017
Grapes, pomace	2	0.09	--		Latvia, 2017
Enforcement residue definition 2 (if applicable)					
New data					
Enforcement residue definition: Zoxamide					
Grapes, dried (raisins)	2	1.4205	--		BPL-STUDY-19-000058 18097-03R
Grapes, juice before pasteurisation	2	0.037	--		AB2-18-35355
Grapes, juice after pasteurisation	6	0.041	--		AB2-18-35355 BPL-STUDY-19-000041 BPL-STUDY-19-000051 19200-01R
Must	5	0.846	--		AB2-18-35355 19200-01R RAU-049-15
Young wine	7	0.034	--		AB2-18-35355 BPL-STUDY-19-000041 BPL-STUDY-19-000051 19200-01R
Bottled wine	5	0.048	--		AB2-18-35355 BPL-STUDY-19-000041 19200-01R
Enforcement residue definition 2 (if applicable)					
				-	

* The median processing factor is obtained by calculating the median of the individual processing factors of each processing study.
** The median conversion factor for enforcement to risk assessment is obtained by calculating the median of the individual conversion factors of each processing study.

Conversion Factors (CFs) - the molecular weight ratios of a metabolite/zoxamide – are presented in the RAR (2017), Vol. 3 B.7. These values can be used to convert residue values expressed as mg analyte/kg to mg parent equivalents/kg.

7.2.5.2 Conclusion on processing studies

New processing studies have been submitted by the applicant in the framework of this application. These are summarised in the table above. The detailed results are presented in Appendix 2.

7.2.6 Magnitude of residues in representative succeeding crops

Considering the available data dealing with nature of residues (see 7.2.2.2), no study dealing with magnitude of residues in succeeding crops is needed.

7.2.7 Other / special studies (KCA 6.10, 6.10.1)

New magnitude of residues data according to SANTE/11956/2016 rev. 9 (2018) has been assessed in the field in order to allow the setting of a Maximum Residue Level (MRL) for zoxamide in honey (Poraczki, K. 2020; report no. 19 48 BTR 0003). However, since the procedure of the MRL setting for honey is not yet clear, these data are presented here for completeness but without proposing an MRL. Since the evaluation of MRLs according to article 12 of Regulation (EC) No 396/2005 is outstanding, it is proposed to evaluate the zoxamide MRL on honey (and other bee products) within this procedure.

In total four trials (2 under northern and 2 under southern EU growing conditions) have been performed by Poraczki (2020; report no. 19 48 BTR 0003) to determine the residues of zoxamide in Phacelia (*Phacelia tanacetifolia* BENTH.) honey after application of GWN-9790EU (a 240 g/L zoxamide SC formulation) at a worst-case use pattern of 3 x 180 g a.s./ha with a minimum interval of 7(+1) days under semi-field conditions (in field tunnels). Applications took place as far as possible to the flowering crop. The analysis of specimens (honey) was conducted by an extraction according to QuEChERS method using high performance liquid chromatography (HPLC) with a chiral column and mass-spectrometric (MS-MS) detection at a limit of quantification of 0.01 mg/kg for Zoxamide. The method was fully validated according to SANCO/3029/99 rev. 4 (2000) and SANCO/825/00 (2010).

The trials to determine the residues of zoxamide in honey are compliant for the here assessed GAP uses. The residues in *Phacelia* honey analysed over all trials were 0.0784 mg/kg, <LOQ (< 0.01 mg/kg), <LOQ (< 0.01 mg/kg) and <LOD (< 0.003 mg/kg). They show comparable residues under Northern and Southern European conditions. The resulting median residue is 0.01 mg/kg (n=4). This is below the currently set MRL of 0.05 mg/kg according to Reg. (EU) 2017/171.

7.2.8 Estimation of exposure through diet and other means (KCA 6.9)

Toxicological reference values relevant for dietary risk assessment are reported in the summary of the evaluation (see 7.1.2).
As an ARfD was not deemed necessary, an acute risk assessment is not relevant.

7.2.8.1 Input values for the consumer risk assessment

Table 7.2-20: Input values for the consumer risk assessment

Commodity	Chronic risk assessment		Acute risk assessment	
	Input value (mg/kg)	Comment	Input value (mg/kg)	Comment
Risk assessment – Enforcement residue definition : Zoxamide				
Wine and table grapes	0.541*	Median residue (Table 7.2-15)	--	--
Tomato fruits	0.16*	Median residue (Table 7.2-16)	--	--
Potato tubers	< 0.01	Median residue (Table 7.2-17)		
All other commodities		EU MRLs Reg. (EU) 2017/171	--	--
Risk assessment residue definition 2 (if applicable)				

* For trials with an application pattern of 5x 0.18 kg a.s./ha.

7.2.8.2 Conclusion on consumer risk assessment

Extensive calculation sheets are presented in Appendix 3.

An ARfD for zoxamide was not deemed necessary, an acute consumer risk assessment has therefore not been performed.

Table 7.2-21: Consumer risk assessment

TMDI (% ADI) according to EFSA PRIMo *	7 % (NL toddler)
Highest contribution: 4% via spinach	

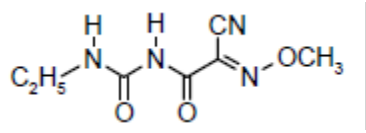
* EFSA PRIMo revision 3. EFSA Journal 2018;16(1):5147, 43 pp

The proposed uses of zoxamide in the formulation Cymoxanil 33% + Zoxamide 33% WG do not represent unacceptable acute and chronic risks for the consumer.

7.3 Cymoxanil

General data on cymoxanil are summarised in the table below (last updated 2020/09/30)

Table 7.3-1: General information on cymoxanil

Active substance (ISO Common Name)	Cymoxanil
IUPAC	*1-[(E/Z)-2-cyano-2-methoxyiminoacetyl]-3-ethylurea*
Chemical structure	
Molecular formula	$C_7H_{10}N_4O_3$
Molar mass	198.2 g/mol
Chemical group	Cyanoacetamideoxime
Mode of action (if available)	FRAC code 27, unknown mode of action
Systemic	Yes
Company (ies)	DuPont de Nemours SAS Oxon Italia SpA
Rapporteur Member State (RMS)	Austria
Approval status	Approved Date of (01/09/2009) and reference to decision (COMMISSION DIRECTIVE 2008/125/EC - REGULATION (EU) No 2017/195)
Restriction (e.g. is restricted to use as "...")	Only uses as fungicide may be authorised
Review Report	SANCO/179/08 – final rev. 1 09/07/2010
Current MRL regulation	Regulation (EC) No 832/2018
Peer review of MRLs according to Article 12 of Reg No 396/2005 EC performed	Yes
EFSA Journal: Conclusion on the peer review	Yes – EFSA, 2008
EFSA Journal: conclusion on article 12	Yes – EFSA, 2015
Current MRL applications on intended uses	No

7.3.1 Stability of Residues (KCA 6.1)

7.3.1.1 Stability of residues during storage of samples

Available data

Please refer to DAR (Austria, 2007) and Final Addendum to DAR (Austria, 2008).
Cymoxanil residues in homogenised potato tuber were stable for 12.5 months at least while as indicated in the DAR for whole lettuce plant stability reported is 12 months.
The applicant has submitted a storage stability study in grape (KCA 6.1/01 Lucini L., 2006a) and in whole processed tomatoes (KCA 6.1/02, Lucini L., 2006b).
Following storage at approximately -20°C, residues of cymoxanil in whole grape bunches were stable for 24 months, whilst residues of cymoxanil in homogeneised grape bunches were stable for 30 days.
Regarding tomato, Cymoxanil residues were stable in tomato homogenized berry for the whole period tested (76.7% of the initial after 30 days of storage) while residues in whole tomato were stable up to 18 months of storage.

No new data are submitted in the framework of this application.

Conclusion on stability of residues during storage

According to the available data Cymoxanil residues are stable in the matrix of interest.

7.3.1.2 Stability of residues in sample extracts (KCA 6.1)

Storage stability in sample extracts can be assessed by the recovery samples. Recovery samples gave suitable recoveries within the acceptable range of 70-100 %, even after storage >24 hours. Because recoveries were good and samples of the corresponding field trials were treated alike and stored under exactly the same conditions, the validity of the analysed sample extracts is proven. Moreover, according to the standard operation procedures followed for residues analysis, extracts generated from field samples were always stored till the analysis at 4°C under dryness condition after removing the aqueous component of the matrix which might have led to degradation of residues. Stability of whole extracts (i.e. aqueous phase) after frozen storage was proven during the metabolism studies performance by comparison of chromatographic behaviour of fresh and stored samples.

7.3.2 Nature of residues in plants, livestock and processed commodities

7.3.2.1 Nature of residue in primary crops (KCA 6.2.1)

Available data

For potato, please refer to the PRAPER conclusions on Cymoxanil list of end points (September 2008) – Metabolism studies were conducted in a range of crops including potatoes, lettuce, rotational crops and lactating goats. The residue definition was Cymoxanil parent only for risk assessment and monitoring.
A report on metabolism of Cymoxanil in tomato is available and submitted by the applicant (KCA 6.2.1/01, Melkebeke, B. van Noorloos, 2003). According to SANCO 7028/VI/95 rev. 3 tomato is considered as a fruit and can also cover the requirement for grapes.

During the peer review, the metabolism of cymoxanil has been investigated for foliar treatment in two different crop groups: root crops and leafy crops. The reported studies indicated a rapid and extensive

degradation of the parent compound. Cymoxanil was rapidly degraded over intermediates (metabolites IN-W35958, IN-KQ9609 or IN-KP53310) to glycine, which was further conjugated or incorporated in natural substances (carbohydrates, peptides or proteins). None of these metabolites was considered as toxicologically relevant (EFSA, 2008).
In the framework of the present MRL review, the RMS submitted four additional studies investigating the metabolism of cymoxanil in fruit crops (tomatoes and grapes), hereby covering a third crop group (Austria, 2013). According to the RMS, these studies were also considered acceptable during the zonal assessment (central EU) of plant protection products containing cymoxanil. Although only one of these studies is GLP compliant, all results corroborate the metabolic pattern depicted in root and leafy crops. The parent compound is also extensively degraded in fruits. It was quantified at levels of 0.01 mg/kg in tomatoes (PHI 3 days) and 0.05 mg/kg in grapes (PHI 18 days). Apart from glycine, significant metabolites were not identified. Major part of the non-extracted radioactivity was characterised as polar metabolites, conjugates or incorporated into plant constituents as well.

The issue of cymoxanil's Z-isomer was addressed in the EFSA conclusion:
"First, the meeting of experts discussed if the Z-isomer of cymoxanil was also identified as a residue in plant and animal matrices, taking into account that the active substance is assumed to be mainly the E-isomer with small amount of Z-isomer. This point was considered in the plant and animal metabolism studies provided by DuPont, since it was mentioned that the samples were analysed using an HPLC method separating both isomers, but no statement was made in the DAR on the possible presence of the Z-isomer. After the meeting, and checking the chromatograms that were originally provided by the applicant, the RMS confirmed that no Z-isomer could be detected in the different plant and animal matrices analysed."
No further consideration of the Z-isomer is required.

Summary of new plant metabolism studies

No new study has been submitted by the applicant.

Conclusion on metabolism in primary crops

The metabolism of cymoxanil has been investigated for foliar treatment in three different crop groups. Based on the available data, EFSA was able to derive a general residue definition for monitoring and risk assessment being cymoxanil.

UK: A report on metabolism of Cymoxanil in tomato (fruits and fruiting vegetables) is available. Grapes are in the same metabolism group as tomato and therefore the study on tomato covers grapes. Since studies on three metabolism groups are now available, and the same residue definition is proposed on the basis of all of them, a general residue definition can be proposed.

7.3.2.2 Nature of residue in rotational crops (KCA 6.6.1)

Available data

Data were available and reviewed at EU level (Austria, 2007).

No new data submitted in the framework of this application.

A rotational crop study investigating residues uptake in lettuce, sugar beet and wheat for two different plants back intervals (30-days PBI and 120-days PBI) was evaluated during the peer review. The application rate used in this study (1.2 kg a.s./ha on bare soil) covers the maximal application rates authorised for non-perennial crops within the EU (see Appendix A). At final harvest, total radioactivity was not significant (<0.01 mg eq/kg) in lettuce heads and was only of 0.01 mg eq/kg in sugar beet roots from the 30-days plant

back interval. Significant amounts of total radioactive residue (TRR) were only detected in wheat grain (0.04-0.05 mg eq/kg) and in wheat straw (0.12-0.14 mg eq/kg) for both PBI. In cereals where TRR was more than 0.01 mg eq/kg, the radioactivity was further analysed. Cymoxanil or structurally related metabolites were not identified and individual components accounting for more than 0.02 mg eq/ha were not detected. Based on this study it was concluded that significant residues of cymoxanil are not expected in practice in rotational crops (EFSA, 2008). This conclusion is still relevant in the framework of the present review.

Summary of new plant metabolism studies

No new data submitted in the framework of this application.

Conclusion on metabolism in rotational crops

Studies on the nature of the residues in succeeding crops show that significant residues of cymoxanil are not expected in rotational crops.

7.3.2.3 Nature of residues in processed commodities (KCA 6.5.1)

Available data

Residues of cymoxanil in the assessed crops were found to be below the threshold of 0.1 mg/kg (all residue values are below the LOQ at harvest date). Therefore, processing studies with this active substance are not required. Moreover, TMDI for consumption of tomato and grapes is well below 10% for all diets. Supplementary data are not required.
No new data submitted in the framework of this application.

Conclusion on nature of residues in processed commodities

No new data are submitted in the framework of this application and are not required.

7.3.2.4 Conclusion on the nature of residues in commodities of plant origin (KCA 6.7.1)

Table 7.3-2: Summary of the nature of residues in commodities of plant origin

Endpoints	
Plant groups covered	Fruit crops (Tomatoes, grapes) Root crops (Potatoes) Leafy crops (Lettuce)
Rotational crops covered	Yes
Metabolism in rotational crops similar to metabolism in primary crops?	Yes
Processed commodities	Not available
Residue pattern in processed commodities similar to pattern in raw commodities?	Yes (tentative)
Plant residue definition for monitoring	Cymoxanil
Plant residue definition for risk assessment	Cymoxanil
Conversion factor from enforcement to RA	Not applicable

7.3.2.5 Nature of residues in livestock (KCA 6.2.2-6.2.5)

Available data

Data were available and were reviewed at EU level (EFSA, 2008).
The Cymoxanil data provided in Annex IIA Section 4 and evaluated during inclusion of cymoxanil in Annex I of guideline 91/414/EC are sufficient to describe the metabolism in animal livestock (please refer to the respective DAR).

Summary of plant metabolism studies reported in the EU

Cymoxanil is authorised for use on potatoes, dry pulses, sunflower seed and soya bean that might be fed to livestock. Although the calculated dietary burdens (max 0.045 mg/kg DM) may have been slightly under-estimated (missing data for oilseeds), the calculated intake was sufficiently low (compared to the trigger value of 0.1 mg/kg DM) to conclude that MRLs for cymoxanil in animal commodities are not required. Nevertheless, if a residue definition for ruminant and pig commodities would be needed in the future, the available metabolism study would be sufficient to propose parent cymoxanil as a residue definition for monitoring and risk assessment in ruminants and pigs

Summary of new animal metabolism studies
No new data submitted in the framework of this application.

Conclusion on metabolism in livestock

The available metabolism study would be sufficient to propose parent cymoxanil as a residue definition for monitoring and risk assessment.

7.3.2.6 Conclusion on the nature of residues in commodities of animal origin (KCA 6.7.1)

Table 7.3-3: Summary on the nature of residues in commodities of animal origin

	Endpoints
Animals covered	Lactating goats
Time needed to reach a plateau concentration	1 day
Animal residue definition for monitoring	Residue definition in animal commodities is not needed but could be set as cymoxanil (for ruminant and pigs) if needed in the future (EFSA, 2015)
Animal residue definition for risk assessment	Residue definition in animal commodities is not needed but could be set as cymoxanil (for ruminant and pigs) if needed in the future (EFSA, 2015)
Conversion factor	Not applicable
Metabolism in rat and ruminant similar	Yes
Fat soluble residue	No

7.3.3 Magnitude of residues in plants (KCA 6.3)

7.3.3.1 Summary of European data and new data supporting the intended uses

No new data are submitted in the framework of this application.
The studies already evaluated during the first registration and which were considered acceptable, were below summarised and highlighted in grey.
Tier I summaries of these studies are presented in the Appendix 2.

Table 7.3-4: Summary of EU reported and new data supporting the intended uses of cymoxanil and conformity to existing MRL

Commodity	Source	Residue zone (N-EU, S-EU, EU, outside EU)	Evaluation GAP Residue levels (mg/kg) E = according to enforcement residue definition RA = according to risk assessment residue definition	STMR (mg/kg)	HR (mg/kg)	Unrounded OECD calculator MRL (mg/kg)	Current EU MRL (mg/kg) *	MRL compliance
Residue definition for monitoring and enforcement (E) and risk assessment (RA): cymoxanil								
Grape	Old trials	N-EU	GAP: 5x 150 g as/ha, interval between application 7/10 days, PHI 28d E: 4x <0.05 RA: 4x <0.05	N/A				
	Old trials	S-EU	GAP: 5x 150 g as/ha, interval between application 7/10 days, PHI 28d E: 4x <0.05 RA: 4x <0.05					
	Overall supporting data for cGAP	N-EU + S-EU	8x <0.05	0.05	0.05	0.05	0.3	yes
Potato	Old trials	N-EU	GAP: 6x 150 g as/ha, interval between application 7/10 days, PHI 7d E: 2x <0.01 RA: 2x <0.01					

Commodity	Source	Residue zone (N-EU, S-EU, EU, outside EU)	Evaluation GAP Residue levels (mg/kg) E = according to enforcement residue definition RA = according to risk assessment residue definition	STMR (mg/kg)	HR (mg/kg)	Unrounded OECD calculator MRL (mg/kg)	Current EU MRL (mg/kg) *	MRL compliance
	Old trials	S-EU	GAP: 6x 150 g as/ha, interval between application 7/10 days, PHI 7d E: 2x <0.01 RA: 2x <0.01					
	Overall supporting data for cGAP	S-EU + N-EU	4x <0.01	0.01	0.01	0.01	0.01	Yes

* Source of EU MRL: Regulation (EC) No 2018/832

7.3.3.2 Conclusion on the magnitude of residues in plants

Sufficient residue data on grape and potato are available.

The data submitted show that no exceedance of the MRL will occur. The uses are considered acceptable.

UK comments:

Commodity	Residue region [a]	Outdoor/Indoor	Individual trial results (mg/kg)		Median residue (mg/kg) [b]	Highest residue (mg/kg) [c]	MRL proposal (mg/kg)	Median CF [d]	Comments
			Enforcement (cymoxanil)	Risk assessment (cymoxanil)					
Cymoxanil									
Potatoes	**NEU (UK, SK, IR, PL, DE, AT, RO)**	**Outdoor**	**Cymoxanil** **2 × < 0.01**	**Cymoxanil** **2 × < 0.01**	**Cym** **0.01**	**Cym** **0.01**	**Cym** **0.01**	**Cym** **1**	Sufficient trials to support proposed central zone GAP.
	SEU	**Outdoor**	**Cymoxanil** **2 × < 0.01**	**Cymoxanil** **2 × < 0.01**	**Cym** **0.01**	**Cym** **0.01**	**Cym** **0.01**	**Cym** **1**	**Prior to March 2011 RO was considered to be in the southern climatic region.**

Commodity	Residue region [a]	Outdoor/Indoor	Individual trial results (mg/kg)		Median residue (mg/kg) [b]	Highest residue (mg/kg) [c]	MRL proposal (mg/kg)	Median CF [d]	Comments
			Enforcement (cymoxanil)	Risk assessment (cymoxanil)					
Grapes (wine)	**NEU**	**Outdoor**	**Cymoxanil** **4 × < 0.05**	**Cymoxanil** **4 × < 0.05**	**Cym** **0.05**	**Cym** **0.05**	**Cym** **0.05**	**Cym** **1**	**Cymoxanil** **Sufficient trials to support proposed central zone GAP.**
	SEU	**Outdoor**	**Cymoxanil** **4 × < 0.05**	**Cymoxanil** **4 × < 0.05**	**-**	**-**	**-**	**Cym** **1**	Authorisation in SEU not requested. **n.b. prior to March 2011 RO was considered to be in the southern climatic region.**

7.3.4 Magnitude of residues in livestock

7.3.4.1 Dietary burden calculation

The crop under consideration, potato, is intended to be fed to livestock however, the use of the formulated product resulted in a zero-residue situation then no residue is expected in livestock.

Feeding intake models for livestock according to EU commission working document 7031/VI/95 rev. 4 (dated 22/7/96) demonstrate for chicken, pig, dairy cattle and beef cattle the following estimated maximum intakes for Cymoxanil, taking into consideration potato and fruit (tomato, grapes) pomace. The calculation was done using the UK's 'Dietary burden calculator 2.8'; results were checked against EFSA's dietary burden model ('PROFile(2.1)_DB_calculator') – the results of the two models (in this case) are in agreement.

The trigger value of ≥0.1 mg residue/kg diet (dry weight) is slightly above the threshold for meat ruminants. Nevertheless, feeding studies are not considered to be necessary because a 'no residue situation' (ND, therefore <0.05 mg cymoxanil/kg, LOQ and <0.02, LOD) on these crops (relevant feedingstuff) is established for the feedingstuff here considered. Additionally, according to the goat metabolism study (see Cymoxanil DAR and PRAPER) no Cymoxanil (< 0.05 mg/kg) or structurally related metabolites were detected in any goat tissue (e.g. milk, liver, kidney, muscles or fat) when the animal was fed with 15 mg Cymoxanil/day, which amounts to at least 10 times the maximum intake.

Table 7.3-5 Input values for the dietary burden calculation (cymoxanil)

Commodity	Median dietary burden		Maximum dietary burden	
	Input value (mg/kg)	Comment	Input value (mg/kg)	Comment
Cymoxanil (applicable to ruminants only)				
Potatoes	**0.01**	**Median residue**	**0.01**	**Highest residue**
Grapes (fruit pomace)	**0.05**	**Median residue**	**-**	**Not used – pomace is a processed feed and therefore the HR is not applicable.**
Tomatoes (fruit pomace)	**0.05[(a)]**	**Median residue**	**-**	**Not used – pomace is a processed feed and therefore the HR is not applicable.**

(a): based on trials done in SEU

Table 7.3-6 Results of the dietary burden calculation (cymoxanil)

	Median dietary burden (mg/kg bw per d)	Maximum dietary burden (mg/kg bw per d)	Highest contributing commodity	Max dietary burden (mg/kg DM)	Trigger exceeded (Y/N)
Cymoxanil (applicable to ruminants only)					
Dairy ruminants	**0.0015**	**0.0015**	**Fruit pomace**	**0.042**	**N**
Meat ruminants	**0.0045**	**0.0045**	**Fruit pomace**	**0.105**	**Y**
Pigs	**0.0016**	**0.0016**	**Potatoes**	**0.040**	**N**
Poultry	**0.0008**	**0.0008**	**Potatoes**	**0.013**	**N**

According to EFSA: *Cymoxanil is authorised for use on potatoes, dry pulses, sunflower seed and soya bean that might be fed to livestock. Although the calculated dietary burdens (max 0.045 mg/kg DM) may have been slightly underestimated (missing data for oilseeds), the calculated intake was sufficiently low (compared to the trigger value of 0.1 mg/kg DM) to conclude that MRLs for cymoxanil in animal commodities are not required.*

7.3.4.2 Livestock feeding studies (KCA 6.4.1-6.4.3)

Data are not available and considered as not required.

No new data were submitted in the framework of this application.

7.3.5 Magnitude of residues in processed commodities (industrial processing and/or household preparation) (KCA 6.5.2-6.5.3)

7.3.5.1 Available data for all crops under consideration

Residues of Cymoxanil in the assessed crops were found to be below the threshold of 0.1 mg/kg (all residue values are below the LOQ at harvest date). Therefore, processing studies with this active substance are not required. Moreover, TMDI for consumption of tomato and grapes is well below 10% for all diets. Supplementary data are not required.

However, three trials (two on grapes, North and South Europe, and one on tomato, South Europe) have been conducted, which include the analysis for both Cymoxanil and Zoxamide on some processed commodities. Please see study summaries reported in Appendix 2.

7.3.5.2 Conclusion on processing studies

Residues of Cymoxanil in the assessed crops were found to be below the threshold of 0.1 mg/kg (all residue values are below the LOQ at harvest date). Therefore, processing studies with this active substance are not required.

7.3.6 Magnitude of residues in representative succeeding crops

The crops under consideration can be grown in rotation.

Considering available data dealing with nature of residues (see 7.2.2.2), no study dealing with magnitude of residues in succeeding crops is needed.

Information on possible residues in succeeding crops are presented in the DAR of Cymoxanil. Note: No cymoxanil or structurally related metabolites were identified in any analysed lettuce, sugar beet or spring wheat sample. No evidence of relevant residues in succeeding crops is identifiable. Supplementary data are not required.

A waiting period before sowing or planting of succeeding crops is not required.

7.3.7 Other / special studies (KCA6.10, 6.10.1)

The available data for the active substance sufficiently address aspects of the residue situation that might arise from the use of PPP. Therefore, other special studies are not needed.

7.3.8 Estimation of exposure through diet and other means (KCA 6.9)

Toxicological reference values relevant for dietary risk assessment are reported in the summary of the evaluation (see 7.1.2).

7.3.8.1 Input values for the consumer risk assessment

The EU MS national NESTIs and EU MS national NEDIs for cymoxanil and commodities listed in the above tabels have been calculated using PRIMo – Pesticide Residues Intake Model (revision 2).
The following assumptions have been made:

1. All produce eaten which may have been treated, has been treated and contains residues at the MRL, as given in Table 7.3-7
2. There is no loss of residue during transport or storage, or processing of foods prior to consumption.

Table 7.3-7: EU MRLs Cymoxanil

Plant or livestock commodity	**Cymoxanil** MRL (mg/kg)
Potatoes	0.01*
Grapes (table and wine)	0.2
Tomatoes	0.2

7.3.8.2 Conclusion on consumer risk assessment

Extensive calculation sheets are presented in Appendix 3.
Chronic and acute intakes for all consumer groups are below the relevant end point therefore no health effects are expected.

No unacceptable risk was identified for all diets.

Table 7.3-5: Consumer risk assessment

TMDI (% ADI) according to EFSA PRIMo	9.1% WHO Cluster diet B (Tomato)
IEDI (% ADI) according to EFSA PRIMo	Not necessary
IESTI (% ARfD) according to EFSA PRIMo	Primary commodities: Table grape 16.4% Tomatoes 14.5% Potatoes 9.6% Processed commodities: Grape juice 8.2% Tomato juice 4.4%
NTMDI (% ADI)	Not necessary
NEDI (% ADI)	Not necessary
NESTI (% ARfD)	Not necessary

The proposed uses of cymoxanil in the formulation do not represent unacceptable chronic risks for the consumer.

UK comment: For both actives the total UK NEDIs and EU MS national NEDIs for all consumer groups are below the ADI therefore no health effects due to chronic exposure are expected.

For cymoxanil (zoxamide is not acutely toxic) the UK NESTI and EU MS national NESTIs for all consumer groups are below the ARfD therefore no health effects due to acute exposure are expected.

7.4 Combined exposure and risk assessment

From a scientific point of view, it is regarded necessary to take into account potential combination effects. However, the evaluation of cumulative or synergistic effects as requested by Art. 4 (3b) of Regulation (EC) No. 1107/2009 should only be performed when harmonised "scientific methods accepted by the Authority to assess such effects are available."
Currently, no EU-harmonised guidance is available on the risk assessment of combined exposure to multiple active substances; this approach is not mandatory at EU level.
reference dose been allocated.

7.4.1 Acute consumer risk assessment from combined exposure

Not relevant.
The product is a mixture of two active substances; however, for just one active substance (cymoxanil) an acute reference dose has been set.

7.4.2 Chronic consumer risk assessment from combined exposure

The uses under consideration provide only a minor contribution to the overall chronic exposure of consumers to pesticide residues. The issue requires a more universal consideration and possibly the generic usage of monitoring data. A harmonised approach is not yet available, and currently no specific consideration is warranted in the scope of this evaluation.

7.5 References

Zoxamide

Latvia 2017. Renewal Assessment Report (RAR) Zoxamide.

EFSA (European Food Safety Authority) 2016. Reasoned opinion on the modification of the existing maximum residue levels for zoxamide in various leafy crops. EFSA Journal 2016;14(7):4527, 13 pp.

EFSA (European Food Safety Authority) 2017. Conclusion on the peer review of the pesticide risk assessment of the active substance zoxamide. EFSA Journal 2017; 15 (9): 4980, 25 pp.

EC (European Commission) 2018. Final Renewal report for the active substance zoxamide. SANTE/10052/2018 Rev 2, dated 23 March 2018.

EC (European Commission) 2017. Commission Regulation (EU) 2017/171, dated 30 January 2017.

Cymoxanil

Austria, 2007. Draft assessment report on the active substance cymoxanil prepared by the rapporteur Member State Austria in the framework of Council Directive 91/414/EEC, June 2007. Available online: www.efsa.europa.eu

Austria, 2008. Final addendum to the draft assessment report on the active substance cymoxanil prepared by the rapporteur Member State Austria in the framework of Council Directive 91/414/EEC, September 2008

Austria, 2013. Evaluation report prepared under Article 12.1 of Regulation (EC) No 396/2005. Review of the existing MRLs for cymoxanil, July 2013. Available online: www.efsa.europa.eu

EFSA (European Food Safety Authority), 2008. Conclusion on the peer review of the pesticide risk assessment of the active substance cymoxanil. EFSA Scientific Report (2008) 167, 1-116, doi:10.2903/j.efsa.2008.167r

EFSA (European Food Safety Authority), 2015. Reasoned opinion on the review of the existing maximum residue levels for cymoxanil according to Article 12 of Regulation (EC) No 396/2005. EFSA Journal 2015;13(12):4355, 47 pp. doi:10.2903/j.efsa.2015.4355

Appendix 1 Lists of data considered in support of the evaluation

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
KCA 6.1	Sala A.	2021	Interim Report – Storage stability of Zoxamide residues under frozen conditions (-18°C) in potato tubers, potato flakes and fried potatoes Gown Crop Protection Ltd., UK LabAnalysis, Italy, Report No. BPL-STUDY-18-000047 GLP Not published	N	GWI
KCA 6.1	Longhi D.	2021	Evaluation of the stability of the analyte RH-129151 in the final extract of the following commodities and processed: grape, grape juice, wine, raisin, potato, fried potato, potato fries, tomato, peeled tomato, cucumber Gown Crop Protection Ltd., UK LabAnalysis, Italy, Report No. GLP-STUDY-20-77 GLP Not published	N	GWI
KCA 6.1	Lucini L.	2006	Freezer storage stability of cymoxanil residue in grape bunches. Sipcam SpA, Italy Report no. SIP 1380 GLP, Unpublished	N	Sipcam Oxon S.p.A.
KCA 6.1	Lucini L.	2006	Freezer storage stability of cymoxanil in whole and processed tomatoes. Sipcam SpA, Italy Report no. SIP 1381 GLP, Unpublished	N	Sipcam Oxon S.p.A.

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
KCA 6.2.1	Melkebeke, T., van Noorloos, B.	2003	Metabolism, distribution, and expression of Cymoxanil residues in tomato. NOTOX B.V., 's-Hertogenbosch, The Netherlands Report no 257783 GLP, Unpublished	N	Sipcam Oxon S.p.A.
KCA 6.3 KCA 6.5.1	Romanini M.	2011	Determination of cymoxanil and zoxamide residues at harvest in raw and processed agricultural commodity grape (bunch, must, young and bottled wine) following five applications of Harpon WG (cymoxanil 33% + zoxamide 33% WG) - four trials, Italy 2010. CREG, Research Centre "E. Gagliardini", SIPCAM S.p.A., 26857 Salerano sul Lambro (LO), ITALY Report no CREG2117 GLP, Unpublished	N	Sipcam Oxon S.p.A. Gowan
KCA 6.3	Romanini M.	2011	Determination of cymoxanil and zoxamide residues at harvest in raw and processed agricultural commodity grape (bunch, must, young and bottled wine) following five applications of Harpon WG (cymoxanil 33% + zoxamide 33% WG) - four trials, Northern Europe, 2010. CREG, Research Centre "E. Gagliardini", SIPCAM S.p.A., 26857 Salerano sul Lambro (LO), ITALY Report no CREG2120 GLP, Unpublished	N	Sipcam Oxon S.p.A. Gowan
KCA 6.3 KCA 6.5.3	Peterek, S.	2020	Magnitude of the residues of zoxamide and its metabolites in grapevine (RAC bunches) and processed fractions, following applications of Zoxium 240 SC, Northern Europe – 2018 Gowan Crop Protection Ltd., UK Staphyt GmbH, Germany, Report No. AB2-18-35355; 18097-01R GLP Not published	N	GWI
KCA 6.3 KCA 6.5.3	Sala, A.	2020	Determination of zoxamide and its metabolites in raw agricultural commodity wine grape (berries) and processed fractions (juice, wine) following five applications of Zoxium 240 SC (GWN-9790 EU) in open field condition, 2 harvest trials, Northern Europe, year 2017 – final report amendment no. 1 Gowan Crop Protection Ltd., UK LabAnalysis, Italy, Report No. BPL-STUDY-19-000041 GLP Not published	N	GWI

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
KCA 6.3 KCA 6.5.3	Thomas-Delille, E.	2020	RH-150721 Residues in wine grape and processed fractions following five foliar applications with Zoxium 240 SC under field conditions in Northern Europe in 2017 – amended final report Gowan Crop Protection Ltd., UK Anadiag, France, Report No. B7284 GLP Not published	N	GWI
KCA 6.3 KCA 6.5.3	Sala, A.	2020	Determination of Zoxamide and its metabolites in raw agricultural commodity wine grape (berries) and processed fractions (juice, wine) following five applications of Zoxium 240 SC (GWN-9790 EU) in open field condition 2 harvest trials, Southern Europe, year 2017 - final report amendment no. 1 Gowan Crop Protection Ltd., UK LabAnalysis, Italy, Report No. BPL-STUDY-19-000051 GLP Not published	N	GWI
KCA 6.3 KCA 6.5.3	Casalinuovo, L.	2020	Determination of zoxamide and his metabolite RH-150721 residues in raw agricultural commodity red grapes and processed fraction following five applications of Zoxium 240 SC (Zoxamide 240 g/L) (South Europe - 2 trials year 2017) plus amendment no. 1 to final report Gowan Crop Protection Ltd., UK Biotecnologie B.T., Italy, Report No. BIU-005-17 GLP Not published	N	GWI
KCA 6.3	Longhi, D.	2020	Determination of zoxamide and its metabolites in raw agricultural commodity of grape wine in open field following five and three applications of the formulated product GWN 9790 EU (North Europe - 4 trials year 2019) Gowan Crop Protection Ltd., UK LabAnalysis, Italy, Report No. BPL-STUDY-19-000057 GLP Not published	N	GWI
KCA 6.3	Longhi, D.	2020	Determination of zoxamide and its metabolites in raw agricultural commodity of table grape and processed (raisin) in open field following five and three applications of the formulated product GWN 9790 EU (South Europe – 1 trial year 2019)	N	GWI

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
KCA 6.5.3			Gowan Crop Protection Ltd., UK LabAnalysis, Italy, Report No. BPL-STUDY-19-000058 GLP Not published		
KCA 6.3 KCA 6.5.3	Maccaferri, L.	2020	Magnitude of the residues of zoxamide in table grape bunches and in raisins processed fraction, following applications of Zoxium 240 SC. One harvest trial, Southern Europe – 2018 Gowan Crop Protection Ltd., UK Renolab S.r.l., Italy, Report No. 18097-03R GLP Not published	N	GWI
KCA 6.3 KCA 6.5.3	Maccaferri, L.	2019	Determination of the residues of zoxamide and/or phosphorous acid in table grape raw agricultural commodity following five applications of GOW F 716, Zoxium 240 SC, GOW F 316 in open field conditions (one harvest trial, Italy 2017) Gowan Crop Protection Ltd., UK Renolab S.r.l., Italy, Report No. 17120-01R GLP Not published	N	GWI
KCA 6.3 KCA 6.5.3	Maccaferri, L.	2019	Determination of the residues of zoxamide and/or phosphorous acid in raw agricultural commodity of grapevine and processed commodities (juice, must, young wine and bottled wine) following five applications of GOW F 716, Zoxium 240 SC, GOW F 316 in open field conditions (one harvest trial, Italy 2017) Gowan Crop Protection Ltd. UK Renolab S.r.l., Italy, Report No. 17120-02R GLP Not published	N	GWI
KCA 6.3 KCA 6.5.3	Maccaferri, L.	2020	Magnitude of residues of zoxamide enantiomers and metabolites in grapes and processed commodities (juice, must, young wine and bottled wine) following five applications of GOW F 716 and Zoxium 240 SC in open field condition (Italy 2017) Gowan Crop Protection Ltd., UK Renolab S.r.l, Italy, Report No. 19200-01R	N	GWI

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
			GLP Not published		
KCA 6.3 KCA 6.5.3	Luciani, G.P.	2016	Determination of zoxamide and benalaxyl-m residues after three applications of GWN-10392 on wine grapes under field conditions – Italian trial, year 2015 Gowan Comercio Internacional et Servicos Lda., Portugal Tentamus AgriParadigma S.r.l., Italy, Report No. AGRI 009/15 GLP HAR GLP Not published	N	GWI
KCA 6.3 KCA 6.5.3	Perboni, A.	2017	Determination of benalaxyl-m and zoxamide residues in raw agricultural commodity grapes (wine and table) and processed commodity (must, fermenting must, wine and aged wine) following three applications of GWN-10392 (benalaxyl-m 150 g/L + zoxamide 225 g/L) in open field condition (3 harvest trials, Northern and Southern Europe, year 2015) Gowan Crop Protection Ltd., UK Biotecnologie BT, Italy, Report No. RAU-049-15 GLP Not published	N	GWI
KCA 6.3 KCA 6.5.1	Romanini M.	2011	Determination of cymoxanil and zoxamide residues at harvest in raw and processed commodity tomato (fruit, juice, puree and canned) following five applications of Harpon WG (cymoxanil 33% + zoxamide 33% WG) - four trials, Italy 2010.CREG, Research Centre “E. Gagliardini”, SIPCAM S.p.A., 26857 Salerano sul Lambro (LO), ITALY Report no: CREG2118 GLP, Unpublished	N	Sipcam Oxon S.p.A. Gowan
KCA 6.3	Devine, H.C.	2008	Residues of mancozeb and zoxamide in field and protected tomatoes at intervals and at harvest following multiple applications of Electis, Northern France and The United Kingdom – 2006 Dow Agrosciences Ltd., UK CEM Analytical Services Ltd. (CEMAS), UK, Report No. CEMS-2967 GLP Not published	N	GWI
KCA 6.3	Longhi, D.	2020	Determination of (R) and (S) zoxamide residues and its metabolites RH-150721, RH-129151, RH-	N	GWI

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
KCA 6.5.3			141452, RH-141288, RH-24549 in raw agricultural commodity of industrial tomato and its processed products (juice, puree and peeled tomatoes) following five applications of formulated product Zoxium 240 SC (sponsor code GWN-9790 EU) in open field (South Europe – 4 trials years 2018) Gowan Crop Protection Ltd., UK LabAnalysis, Italy, Report No. BPL-STUDY-18-000014 GLP Not published		
KCA 6.3	Longhi, D.	2020	Determination of (R) and (S) zoxamide residues and its metabolites RH-150721, RH-129151, RH-141452, RH-141288, RH-24549 in fresh market tomato raw agricultural commodity following five applications of the formulated product Zoxium 240 SC (sponsor code GWN-9790 EU) in greenhouse (South Europe – 4 trials years 2018) - final report amendment no. 1 Gowan Crop Protection Ltd., UK LabAnalysis, Italy, Report No. BPL-STUDY-18-000015 GLP Not published	N	GWI
KCA 6.3	Longhi, D.	2020	Determination of zoxamide and its metabolites in raw agricultural commodity of industrial tomato in open field following five applications of the formulated product GWN 9790 EU (South Europe - 4 trials year 2019) Gowan Crop Protection Ltd., UK LabAnalysis, Italy, Report No. BPL-STUDY-19-000059 GLP Not published	N	GWI
KCA 6.3	Pandolfi, A.	2020	Determination of zoxamide residues and its metabolites in raw agricultural commodity tomato (fruits) following five applications of Zoxium 240 SC (sponsor code GWN-9790 EU) in protected condition (Italy - Southern Europe - 4 trials year 2019) Gowan Crop Protection Ltd., UK RES AGRARIA, Italy, Report No. RA 19 043 BPL GW GLP Not published	N	GWI
KCA 6.3	Tetuan, B.	2016	Determination of residues at harvest of zoxamide, benalaxyl-m and cymoxanil in tomato, following three	N	GWI

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
KCA 6.5.3			broadcast applications of GWN-10392, GWN-9823 and IR6141-copper oxychloride-copper hydroxide 5-15-15 WG under greenhouse conditions and determination of residues at harvest of zoxamide and benalaxyl-m in industry tomato and its processed products (canned tomatoes, puree and juice), following three broadcast applications of GWN-10392 under open field conditions - South Europe - season 2015 Gowan Comercio Interncional et Servicos Ltd., Portugal Promovert Crop Services SL, Spain, Report No. 15 F CL GW P/A GLP Not published		
KCA 6.3	Tetuan B.	2011	Determination of residues at harvest in potatoes following six broadcast applications of Harpon WG under field conditions-Northern Europe, season 2010.Promovert Report no.10 F PT GW P/A GLP Unpublished	N	Sipcam Oxon S.p.A. Gowan
KCA 6.3	Tetuan B.	2011	Determination of residues at harvest in potatoes following six broadcast applications of Harpon WG under field conditions-Southern Europe, season 2010. Promovert, Report no.10 F PT GW P/B GLP Unpublished	N	Sipcam Oxon S.p.A. Gowan
KCA 6.3 KCA 6.5.3	Terranegra A.	2020	Magnitude of residue of zoxamide and metabolite RH-1452 and RH-1455 in potatoes (RAC tubers) and processed fractions, following 5 applications of GWN 9790 EU in two trials (2 HS), Northern Europe (France and Poland) – 2017 – amended Final Report Staphyt Italia S.r.l., Italy, Report No. ATA-18-30694 GLP Not published	N	GWI
KCA 6.3 KCA 6.5.3	Pandolfi A.	2020	Determination of the residues of zoxamide (R), (S) and sum and its metabolites in raw agricultural commodity potato (tubers) and its processed fractions (chips, baked/cooked, fried and flakes) following five applications of Zoxium 240 SC (sponsor code GWN-9790 EU) in open field condition (Italy - Southern Europe - 2 trials year 2018) Res Agraria S.r.l., Italy, Report No. RA 18 051 BPL GW GLP	N	GWI

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
			Not published		
KCA 6.3	Luciani, G.P.	2012	Determination of zoxamide and dimethomorph residues after two applications of Zoxium 240 SC and GWN-9963 on lettuce, rocket salad and endive under field conditions - Italian trial year 2012 Gowan Italia Spa, Italy AgriParadigma Srl, Italy, Report No. AGRI 013/12 GLP DEC GLP Not published	N	GWI
KCA 6.3	Luciani, G.P.	2012	Determination of zoxamide and dimethomorph residues after two applications of Zoxium 240 SC and GWN-9963 on lettuce and rocket - Italian trial, year 2012 Gowan Italia Spa, Italy AgriParadigma Srl, Italy, Report No. AGRI 014/12 GLP DEC GLP Not published	N	GWI
KCA 6.3	Lucini L.	2008	Determination of cymoxanil residues in raw agricultural commodity tomato (fruit) following application of SIP40936 (CYMOXANIL 33% + ZOXAMIDE 33% WG) SIPCAM S.p.A., Italy Report SIP 1551 GLP, Unpublished AIIA study on the active substance Cymoxanil	N	Sipcam Oxon S.p.A.
KCA 6.5.1	Grist, A.	2018	Zoxamide: Hydrolysis under simulated processing conditions Gown Crop Protection Ltd., UK Envigo CRS Ltd., UK, Report No. RB66JN GLP Not published	N	GWI
KCA 6.5.1	Longhi, D.	2019	RH-141452: Hydrolysis under simulated processing conditions Gown Crop Protection Ltd., UK LabAnalysis s.r.l., Italy, Report No. BPL-STUDY-18-000092 GLP	N	GWI

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
			Not published		
KCA 6.5.1	Longhi, D.	2019	RH-141455: Hydrolysis under simulated processing conditions Gown Crop Protection Ltd., UK LabAnalysis s.r.l., Italy, Report No. BPL-STUDY-19-000009 GLP Not published	N	GWI
KCA 6.5.1	Völkl S.	2000	^{14}C-RH-117281: Processing of tomatoes Rohm and Haas Comp., USA, Report No. 34-01-25, DERBI 92297 RCC Ltd, Switzerland, Report No. 34P-99-25 GLP Not published	N	GWI
KCA 6.7.1	Cashmore, A.	2019	RH-141288: Partition coefficient (n-octanol/water) Shake flask method Gowan Crop Protection Ltd., Uk Smithers ERS Ltd, Uk, Report No. 3202371 GLP Not published	N	GWI
KCA 6.10	Poráczki, K.	2020	Magnitude of residues of zoxamide in *Phacelia* (*Phacelia tanacetifolia* BENTH.) honey after three applications of GWN-9790EU under semi-field conditions in Northern and Southern Europe Gowan Crop Protection Ltd, UK BioChem agrar, Germany, Report No. 19 48 BTR 0003 GLP Not published	N	GWI
KCP 9.2.5	Appeltauer, A.	2020	Determination of Residues of Zoxamide on/in Typical Feed Items of Herbivorous Birds and Mammals after Two Applications of Zoxium 240 SC on Sugar Beet and Wheat in Germany 2017 Gowan Crop Protection Ltd., UK Eurofins GmbH, Germany, Report No. S16-05375 GLP Not published	N	GWI

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
KCP 9.2.5	Appeltauer, A.	2020	Determination of Residues of Zoxamide on/in Typical Feed Items of Herbivorous Birds and Mammals after Two Applications of the test item on Sugar Beet and Wheat in The Netherlands in 2019 Gowan Crop Protection Ltd., UK Eurofins GmbH, Germany, Report No. S19-01450 GLP Not published	N	GWI
KCP 9.2.5	Appeltauer, A.	2020	Determination of Residues of Zoxamide on/in Typical Feed Items of Herbivorous Birds and Mammals after Two Applications of Zoxium 240 SC on Sugar Beet and Wheat in Southern Europe 2017 Gowan Crop Protection Ltd., UK Eurofins GmbH, Germany, Report No. S16-05376 GLP Not published	N	GWI
KCP 9.2.5	Appeltauer, A.	2020	Determination of Residues of Zoxamide on/in Typical Feed Items of Herbivorous Birds and Mammals after Two Applications of the test item on Sugar Beet and Wheat in Italy in 2020 Gowan Crop Protection Ltd., UK Eurofins GmbH, Germany, Report No. S19-23773 GLP Not published	N	GWI

SIPCAM Oxon S.p.A. is the legal successor of Oxon Italia S.p.A.; Gowan Crop Protection (GWI) is the legal eternity of the company Gowan in Europe.

GWI – Gowan Crop Protection Ltd.

Grey shaded = data / reference already provided and assessed during product authorisation.

List of data submitted or referred to by the applicant and relied on, but already evaluated at EU peer review - Zoxamide

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCA 6.1	Ross, J.R.	1998	Storage stability of RH-117281 residues in grapes, grape juice, raisins and potatoes under conditions of frozen storage Rohm and Haas, McKenzie Laboratories, Enviro Test Laboratories, Report No. 34-98-161, December 15,1998, ER R61.1 GLP Not published	N	GWI
KCA 6.1	Ross, J.R.	1998	Storage stability of RH-141,455 and RH-141,452 residues in potatoes, potato chips and potato flakes under conditions of frozen storage Rohm and Haas, McKenzie Laboratories, Enviro Test Laboratories, Report No. 34-98-162, December 15, 1998, ER R61.2 GLP Not published	N	GWI
KCA 6.1	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, Report No. 34-00-80, ER R77.11 GLP Not published	N	GWI
KCA 6.1	Weber, H., Hissmann, H.	2014	Storage stability of residues of Zoxamide, RH-150721, RH-1452 and RH-1455 in grape and processed products and potato Eurofins AgroScience Services Chem GmbH, Report No. S12-03952, February 15, 2016 GLP Not published	N	GWI
KCA 6.2.1	Reibach, P.H., Spencer W.O.	1998	^{14}C-RH-117,281: Nature of the residue in fruiting grape plants Rohm and Haas, American Agricultural Services, Inc. (AASI), Report No. 34-98-49, ER R14.5 GLP Not published	N	GWI

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCA 6.2.1	Reibach, P.H., Spencer W.O.	1998	^{14}C-RH-117,281: Nature of the residue in potato Rohm and Haas, American Agricultural Services, Inc. (AASI), Report No. 34-98-50, September 17, 1998, ER R14.3 GLP Not published	N	GWI
KCA 6.2.1	Graves, D.D., Reibach, P.H.	2000	Consideration of the difference in the magnitude of the residues of RH-7281 in grapes from supervised field residue trials compared to the ^{14}C grape metabolism Study ER 14.5 Rohm and Haas, Report No. 34-00-83, ER R76.6 GLP Not published	N	GWI
KCA 6.2.1	Staffa, C., Möndel, M.	2014	^{14}C-phenyl UL Zoxamide: Plant metabolism in grape RLP AgroScience GmbH, Report No. AS209, July 15, 2014 GLP Not published	N	GWI
KCA 6.2.1	Sharma, A.K.	1999	RH-117,281: Nature of residue in fruiting tomato plants Rohm and Haas, Grayson Research, LLC, Report No. 34-99-159, December 14, 1999 GLP Not published	N	GWI
KCA 6.2.1	Sharma, A.K.	1999	RH-117,281: Nature of residue in cucurbits (cucumber) Rohm and Haas, Grayson Research, LLC, Report No. 34-99-57, November 30, 1999 GLP Not published	N	GWI
KCA 6.2.1	Hein, W.	2014	[Phenyl-UL-^{14}C] Zoxamide: Plant metabolism in pea RLP AgroScience GmbH, Report No. AS290, June 12, 2014 GLP Not published	N	GWI
KCA 6.2.1	Hein, W.	2014	Extraction efficiency of [phenyl-UL-^{14}C] Zoxamide from plant metabolism samples (pea) RLP AgroScience GmbH, Report No. AS362, June 25, 2014	N	GWI

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			GLP Not published		
KCA 6.2.1	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, RCC Ltd., Report No. 799773 March 16, 2001, ER ref. no. R 79.1 GLP Not published	N	GWI
KCA 6.2.3	xxx	1998	Metabolism of 14C-RH-117,281 in lactating goats xxx, Report No. 34-97-166, September 10, 1998, ER R16.1 xxx, Report No. RPT00299 GLP Not published	Y	GWI
KCA 6.3.1	Wais, A.	1999	Determination of residues of RH-117,281 and mancozeb in/on potatoes (RAC tubers) following treatment with RH-7281 2F and dithane/RH-117,281 75DG blend from field trials in Germany, 1996 Rohm and Haas, Report No. 649776, April 12, 1999, ER R66.4 RCC Ltd., Report No. 553002 GLP Not published	N	GWI
KCA 6.3.1	Wais, A.	1999	Determination of residues of RH-1l7281 and mancozeb in/on potatoes (RAC tubers) following treatment with RH-72812F and dithane/RH-117,281 75 DG blend from field trials in the United Kingdom, 1996 Rohm and Haas, Report No. 649811, April 16, 1999, ER R70.3 RCC Ltd., Report No. 553300 GLP Not published	N	GWI
KCA 6.3.1	Grolleau, G.	1999	Magnitude of the Residue of RH-7281 and its metabolites RH-1452 and RH-1455 in potato raw agricultural commodity Rohm and Haas, Report No. EA960112, April 6, 1999, ER R63.3	N	GWI

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			RCC Ltd, Report No. 714925 GLP Not published		
KCA 6.3.1	Wais, A.	1999	Determination of residues of RH-117,281 and mancozeb in/on potatoes (RAC tubers) following treatment with RH-7281 2F and dithane/RH-117,281 75DG blend from field trials in Italy, 1996 Rohm and Haas, Report No. 649800, April 13, 1999, ER R67.5/67.6 RCC Ltd., Switzerland, Report No. 553103 GLP Not published	N	GWI
KCA 6.3.1	Wais, A.	1999	Determination of residues of RH-117,281 and mancozeb in/on potatoes (RAC tubers) following treatment with RH-7281 2F and dithane/RH-117,281 75DG blend from field trials in Germany, 1997 Rohm and Haas, RCC Ltd., Report No. 652252, March 18, 1999, ER R64.4/65.5 GLP Not published	N	GWI
KCA 6.3.1	Wais, A.	1999	Determination of residues of RH-117,281 and mancozeb in/on potatoes (RAC tubers) following treatment with RH- 7281 2F and dithane/RH-117,281 75DG blend from field trials in the UK, 1997 Rohm and Haas, RCC Ltd., Report No. 652263, March 23, 1999, ER R65.5/65.6 GLP Not published	N	GWI
KCA 6.3.1	Grolleau, G.	1999	Magnitude of the residue of RH-7181 and its metabolites RH-1452 and RH-1455 in potato raw agricultural commodity - Northern and Southern France – 1997 Rohm and Haas, Report No. EA970131, April 6, 1999, ER R64.1 RCC Ltd., Report No. 714936 GLP Not published	N	GWI
KCA 6.3.1	Wais, A.	1999	Determination of residues of RH-117,281 and mancozeb in/on potatoes (RAC tubers) following treatment with RH- 7281 2F and dithane/RH-117,281 75DG blend from field trials in Italy, 1997 Rohm and Haas, RCC Ltd., Report No. 652285, March 25, 1999, ER R65.3/65.4	N	GWI

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			GLP Not published		
KCA 6.3.1	Wais, A.	1999	Determination of residues of RH-117,281 and mancozeb in/on potatoes (RAC tubers) following treatment with RH-7281 2F and dithane/RH-117,281 75DG blend from field trials in Greece, 1997 Rohm and Haas, RCC Ltd., Report No. 652307, March 17, 1999, ER R64.2/64.3 GLP Not published	N	GWI
KCA 6.3.1	Wais, A.	1999	Determination of residues of RH-117,281 and mancozeb in/on potato (RAC tubers) following treatment with dithanelRH-117,281 75DG (blend (8:1) and DithanelRH-117,281 75WP blend (8:1) from two field trials (semi residue decline studies) in Germany, 1998 Rohm and Haas, RCC Ltd., Report No. 688904, April 13, 1999, ER R68.1/68.2 GLP Not published	N	GWI
KCA 6.3.1	Wais, A.	1999	Determination of residues of RH-117,281 and mancozeb in/on potato (RAC tubers) following treatment with dithane/RH-117,281 75DG blend (8:1) and dithane/RH-117,281 75WP blend (8:1) from two field trials (semi residue decline studies) in UK, 1998 Rohm and Haas, RCC Ltd., Report No. 688937, April 13, 1999, ER R68.3/68.4 GLP Not published	N	GWI
KCA 6.3.1	Wais, A.	1999	Determination of residues of RH-117,281 in/on potato (RAC tubers) following treatment with dithane/RH-117,281 75DG blend (8:1) and dithane/RH-117,281 75WP blend (8: 1) from four field trials (semi residue decline studies) in Spain, 1998 Rohm and Haas, RCC Ltd., Report No. 688926, April 13, 1999, ER R66.6/66.7 GLP Not published	N	GWI
KCA 6.3.1	Wais, A.	2000	Determination of residues of RH-117,281 and its metabolites RH-141,452 and RH-141,455 in/on potato (RAC tubers) following treatment with RH-7281/mancozeb 75WG from a field trial (semi residue decline studies) in the Netherlands, 1999	N	GWI

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			Rohm and Haas, RCC Ltd., Report No. 734567, January 31, 2000, ER R72.5 GLP Not published		
KCA 6.3.1	Wais, A.	2000	Determination of residues of RH-117,281 and its metabolites RH-141,452 and RH-141,455 in/on potato (RAC tubers and processing products) following treatment with RH-7281/mancozeb 75WG from a field trial (semi residue decline studies) in Northern France, 1999 Rohm and Haas, RCC Ltd., Report No. 734556, February 21, 2000, ER R72.9 GLP Not published	N	GWI
KCA 6.3.1	Wais, A.	2000	Determination of residues of RH-117,281 and its metabolites RH-141,452 and RH-141,455 in/on potato (RAC tubers) following treatment with RH-7281/mancozeb 75WG from a field trial (semi residue decline studies) in Northern France, 1999 Rohm and Haas, RCC Ltd., Report No. 739001, March 15, 2000 ER R72.4 GLP Not published	N	GWI
KCA 6.3.1	Wais, A.	2000	Determination of residues of RH-117,281 and its metabolites RH-141,452 and RH-141,455 in/on potato (RAC tubers and processing products) following treatment with RH-7281/mancozeb 75WG from a field trial (semi residue decline studies) in Italy, 1999 Rohm and Haas, RCC Ltd., Report No. 734545, March 15, 2000, ER R73.2 GLP Not published	N	GWI
KCA 6.3.1	Luciani, G.P.	2010	Determination of Zoxamide residues after five application of Electis MZ and Zoxium 240 SC on potato - Italian trial, year 2010 Res Agraria s.r.l, AgriParadigma s.r.l., Report No. AGRI 012/010 GLP DEC, October 29, 2010 GLP Not published	N	GWI
KCA 6.3.2	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 75DG blend from field trials in Germany, 1996	N	GWI

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			Rohm and Haas, Report No. 649765, April 16, 1999, ER R69.4/69.5 RCC Ltd., Report No. 553001 GLP Not published		
KCA 6.3.2	Grolleau, G.	1999	Magnitude of the Residue of RH-7281 and mancozeb in grape raw agricultural commodity and of RH-7281 in wine and processed fractions Rohm and Haas, Report No. EA960110, March 15, 1999, ER R60.1 ANADIAG, Report No R6055 GLP Not published	N	GWI
KCA 6.3.2	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 Italy, 1996 Rohm and Haas, Report No. 649787, April 16, 1999, ER R70.1/70.2 RCC Ltd, Report No. 553101 GLP Not published	N	GWI
KCA 6.3.2	Wais, A.	1999	Determination of residues of RH-117,281 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 Rohm and Haas, Report No. 649798, April 16, 1999, ER R71.3/71.4 RCC Ltd, Report No. 553102 GLP Not published	N	GWI
KCA 6.3.2	Wais, A.	1999	Determination of residues ofRH-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 Rohm and Haas, Report No. 620875, April 16, 1999, ER R70.5/70.6 RCC Ltd, Report No. 553200 GLP Not published	N	GWI

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCA 6.3.2	Wais, A.	1999	Determination of residues of RH-1l7281 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, 1997 Rohm and Haas, RCC Ltd, Report No. 652241, April 16, 1999, ER R71.1/71.2 GLP Not published	N	GWI
KCA 6.3.2	Grolleau, G.	1999	Magnitude of the residue of RH-7281 and mancozeb in grape raw agricultural commodity and of RH-7281 in wine Rohm and Haas, Report No. EA970130, March 15, 1999, ER R62.3 ANADIAG, Report No. R7097 GLP Not published	N	GWI
KCA 6.3.2	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 Italy, 1997 Rohm and Haas, RCC Ltd, Report No. 652274, April 14, 1999, ER R68.5/68.6 GLP Not published	N	GWI
KCA 6.3.2	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 Greece, 1997 Rohm and Haas, RCC Ltd, Report No. 652296, April 14, 1999, ER R69.2/69.3 GLP Not published	N	GWI
KCA 6.3.2	Wais, A.	1999	Determination of residues of RH-117281 and mancozeb in/on table grapes (RAC grapes) following treatment with RH-117281 2F and dithane/RH-117281 75 DG blend from field trials in Italy, 1997 Rohm and Haas, RCC Ltd, Report No. 660688, March 19, 1999, ER R65.1/65.2 GLP Not published	N	GWI
KCA 6.3.2	Wais, A.	1999	Determination of residues of RH-117281 and mancozeb in/on vine grapes (RAC grapes) following treatment with dithane/RH-117281 75 DG blend (8:1), dithane/RH-117281 75 WP blend (8:1) and RH-	N	GWI

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			117281 2F experimental fungicide from four field trials (semi residue decline studies) in Germany, 1998 Rohm and Haas, RCC Ltd, Report No. 688893, April 13, 1999, ER R67.2/67.3 GLP Not published		
KCA 6.3.2	Grolleau, G.	1999	Magnitude of the Residue of RH-7281 and mancozeb (as CS_2) in grape raw agricultural commodity Rohm and Haas, Report No. EA980117, March 15, 1999, ER R63.1 ANADIAG, Report No. R8153 GLP Not published	N	GWI
KCA 6.3.2	Wais, A.	1999	Determination of residues of RH-117281 and mancozeb in/on vine grapes (RAC grapes) following treatment with dithane/RH-117281 75 DG blend (8:1) and dithane/RH-117281 75 WP blend (8:1) from two field trials (semi residue decline studies) in Italy, 1998 Rohm and Haas, RCC Ltd, Report No. 688961, April 12, 1999, ER R66.2/66.3 GLP Not published	N	GWI
KCA 6.3.2	Wais, A.	1999	Determination of residues of RH-117281 and mancozeb in/on vine grapes (RAC grapes) following treatment with dithane/RH-117,281 75 DG blend (8:1) from two field trials (semi residue decline studies) in Spain, 1998 Rohm and Haas, RCC Ltd, Report No. 688915, April 14, 1999, ER R67.4 GLP Not published	N	GWI
KCA 6.3.2	Wais, A.	1999	Determination of residues of RH-117281 and mancozeb in/on table grapes (RAC grapes) following treatment with dithane/RH-117281 75 DG blend (8:1) from two field trials (semi residue decline studies) in Spain, 1998 Rohm and Haas, RCC Ltd, Report No. 693674, April 12, 1999, ER R66.1 GLP Not published	N	GWI

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCA 6.3.2	Wais, A.	1999	Determination of residues of RH-117281 and mancozeb in/on table grapes (RAC grapes) following treatment with dithane/RH-117281 75 DG blend (8:1) from two field trials (semi residue decline studies) in Portugal, 1998 Rohm and Haas, RCC Ltd, Report No. 688948, April 14, 1999, ER R69.1 GLP Not published	N	GWI
KCA 6.3.2	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 Rohm and Haas, RCC Ltd, Report No. 734578, February 2, 2000, ER R72.8 GLP Not published	N	GWI
KCA 6.3.2	Grolleau, G.	2000	Magnitude of the residue of RH-2781/mancozeb 76.25 WG in grapes raw agricultural commodity. Northern France 1999 Rohm and Haas, European Agricultural Services, Report No. EA990175, March 28, 2000, ER R73.3 C.I.T., Report No. 19239 ADR GLP Not published	R	GWI
KCA 6.3.2	Grolleau, G.	2000	Magnitude of the residue of RH-2781/mancozeb 76.25 WG in grapes raw agricultural commodity. Southern France 1999 Rohm and Haas, European Agricultural Services, Report No. EA990176, March 28, 2000, ER R73.4 C.I.T., Report No. 19240 ADR GLP Not published	N	GWI
KCA 6.3.2	Luciani, G.P.	2010	Determination of zoxamide residues after five application of ELECTIS MZ and ZOXIUM 240 SC on wine grape and table grape – Italian trial, year 2010. Res Agraria s.r.l., AgriParadigma s.r.l., Report no. AGRI 010/10 GLP DEC, October 29, 2010 GLP Not published	N	GWI

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCA 6.5.1	Mamouni, A.	1998	^{14}C-RH-117281: Vinification study Rohm and Haas, RCC Ltd, Report No. 34-98-151, ER R30.17 GLP Not published	N	GWI
KCA 6.5.3	Graves, D.D.	1998	RH-117281 80W and 2F residue studies in grapes and grape process fractions 1996 and 1997 trials Rohm and Haas, Agri Business Group, Inc., Enviro-Test Laboratories, McKenzie Laboratories, California State University at Fresno, Report No. 34-98-154, November 24, 1998, ER R62.1 GLP Not published	N	GWI
KCA 6.5.3	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 Rohm and Haas, RCC Ltd, Report No. 734580, April 10, 2001, ER R77.10 GLP Not published	N	GWI
KCA 6.6.1	Kim-Kang, H.	1998	^{14}C-RH-117281: Confined rotational crop study Rohm and Haas, Report No. 34-98-144, December 4, 1998, ER R60.2 XenoBiotic Laboratories Inc., Report No. RPT00387 GLP Not published	N	GWI

GWI – Gowan Crop Protection Ltd.

SIPCAM Oxon S.p.A. is the legal successor of Oxon Italia S.p.A.; Gowan Crop Protection (GWI) is the legal eternity of the company Gowan in Europe

List of data submitted or referred to by the applicant and relied on, but already evaluated at EU peer review - Cymoxanil

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCA 6.3	Röser K.	2003	Determination of residues of Cymoxanil after application of Cymoxanil 50% WP in potatoes at 4 sites in Europe, 2002 Arbeitsgemeinschaft GAB Biotechnologie GmbH & IFU Umweltanalytik GmbH, Germany Report no. 20021200/E1-FPPO GLP, Unpublish AIIA study on the active substance Cymoxanil	N	Sipcam Oxon S.p.A.*
KCA 6.3	Freschi G.	2003	Determination of Cymoxanil residues in potato specimens (tuber) after application of Cymoxanil 50% WP SIPCAM S.p.A., Italy Report no. SIP 1353 GLP, Unpublished AIIA study on the active substance Cymoxanil	N	Sipcam Oxon S.p.A.* .
KCA 6.3	Domenichini P.	1997	Generation of crop samples suitable for residue analysis following applications of Cymoxanil+man-cozeb, Cymoxanil+Cu-oxychloride, Cymoxanil+chlorthalonil SIPCAM S.p.A., Italy Report no. COF/1 GLP, Unpublished AIIA study on the active substance Cymoxanil	N	Sipcam Oxon S.p.A.* .
KCA 6.3	Wasser C.	1997	Determination of the residues of Cymoxanil in grapes, potatoes and tomatoes in Italy, 1996 ANADIAG S.A., France Report no. R 6124 GLP, Unpublished AIIA study on the active substance Cymoxanil	N	Sipcam Oxon S.p.A.* .
KCA 6.3	Domenichini P.	1998	Generation of crop samples suitable for residue analysis following applications of Cymoxanil+man-cozeb, Cymoxanil+Cu-oxychloride, Cymoxanil+chlorthalonil (2nd year of trial) SIPCAM S.p.A., Italy Report no. COF/2	N	Sipcam Oxon S.p.A.*

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			GLP, Unpublished AIIA study on the active substance Cymoxanil		.
KCA 6.3	Wasser C.	1999	Magnitude of the residues of Cymoxanil in samples of grapes, tomatoes and potatoes ANADIAG S.A., France Report no. R 7180 GLP, Unpublished AIIA study on the active substance Cymoxanil	N	Sipcam Oxon S.p.A.* .
KCA 6.3	Domenichini P.	2003b	Generation of potato tuber samples, suitable for residue analysis (decline curve) following applications of (Cymoxanil 6%+mancozeb 70%) WP SIPCAM S.p.A. Italy, Report no. CY2/I/02PA GLP, Unpublished AIIA study on the active substance Cymoxanil	N	Sipcam Oxon S.p.A.* .
KCA 6.3	Freschi G.	2001c	Residue analysis of Cymoxanil in potato samples (tuber) SIPCAM S.p.A., Italy Report no. SIP 1298 GLP, Unpublished AIIA study on the active substance Cymoxanil	N	Sipcam Oxon S.p.A.*
KCA 6.3	Soler J.R.	2002	Generation of potato specimens, suitable for residue analysis of Cymoxanil following applications of MICENE PLUS (Cymoxanil 4%+Mancozeb 40% WP) SIPCAM Inagra S.A., Spain Report no. CY2/PO GLP, Unpublished AIIA study on the active substance Cymoxanil	N	Sipcam Oxon S.p.A.* .
KCA 6.3	Freschi G.	2001d	Residue analysis of Cymoxanil in potato samples (tuber) SIPCAM S.p.A., Italy Report no. SIP 1265 GLP, Unpublished	N	Sipcam Oxon S.p.A.*

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			AIIA study on the active substance Cymoxanil		.
KCA 6.3	Partington K.	1998	Study to generate samples of tubers for residue analysis from potato plants treated with sequential applications of SIP 4109 and SIP 40876 Agrisearch, UK Report no. AP/3389/SP GLP, Unpublished AIIA study on the active substance Cymoxanil	N	Sipcam Oxon S.p.A.*
KCA 6.3	Wasser C.	1997	Determination of the residues of Cymoxanil in potatoes in England, 1996 Anadiag, France Report no. 6125 GLP, Unpublished AIIA study on the active substance Cymoxanil	N	Sipcam Oxon S.p.A.*
KCA 6.3	Wasser C.	1998	Magnitude of residues of CYMOXANIL in potatoes Anadiag, France Report no. 7182 GLP, Unpublished AIIA study on the active substance Cymoxanil	N	Sipcam Oxon S.p.A.* .
KCA 6.3	Puy E.	1996	Determination of residues of cymoxanil in grapes after treatment with preparations “1.4108.00 and 1.40109.00” under field conditions in France in 1996 Anadiag, France Report no. R 6047 GLP, Unpublished AIIA study on the active substance Cymoxanil	N	Sipcam Oxon S.p.A.* .
KCA 6.3	Puy E.	1996	Residues of cymoxanil in grapes after treatment with preparation “1.4108.00”. Residues of Cymoxanil and Mancozeb in grapes after treatment with the preparation “ 1.4109.00” Anadiag, France Report no. DE 6046 GLP, Unpublished	N	Sipcam Oxon S.p.A.* .

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			AIIA study on the active substance Cymoxanil		
KCA 6.3	Prevotat M.	1996	Vigne mildiou: Projet Cymoxanil essais residues. SIPCAM Phyteurop Report no. PRE 96025 GLP, Unpublished AIIA study on the active substance Cymoxanil	N	Sipcam Oxon S.p.A.*
KCA 6.3	Pollmann B.	2001	Determination of residues of cymoxanil after application of (Cymoxanil 6%&+Mancozeb WP 70%)WP in grape – 2 sites in France and 2 sites in Germany, 2000 GAB, Germany Report no. 20001157/E1/FPVI GLP, Unpublished AIIA study on the active substance Cymoxanil	N	Sipcam Oxon S.p.A.*
KCA 6.3	Freschi G	2001	Residue analysis of Cymoxanil in grapes samples (bunches) SIPCAM S.p.A., Italy Report no. SIP 1253 GLP, Unpublished AIIA study on the active substance Cymoxanil	N	Sipcam Oxon S.p.A.* .
KCA 6.3	Pollmann B.	2002	Determination of residues of cymoxanil after application of (Cymoxanil 6%&+Mancozeb WP 70%)WP in grape – 2 sites in France and 2 sites in Germany, 2001 GAB, Germany Report no. 20011074/E1-FPVI GLP, Unpublished AIIA study on the active substance Cymoxanil	N	Sipcam Oxon S.p.A.* .
KCA 6.3	Freschi G	2001	Residue analysis of Cymoxanil in grapes samples (bunches) SIPCAM S.p.A., Italy Report no. SIP 1302 GLP, Unpublished AIIA study on the active substance Cymoxanil	N	Sipcam Oxon S.p.A.* .

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCA 6.3	Soler J.R.	2001	Generation of wine grapes samples suitable for residue analysis following applications of MICENE PLUS (Cymoxanil 4%+Mancozeb 40 % WP) SIPCAM Inagra S.A., Spain Report no. CY1-VI GLP, Unpublished AIIA study on the active substance Cymoxanil	N	Sipcam Oxon S.p.A. .
KCA 6.3	Domenichini P.	2001	Generation of wine grapes samples suitable for residue analysis (decline curve) following applications of (Cymoxanil 6%+Mancozeb 70 % WP) SIPCAM S.p.A., Italy, Report no. CY1/I/01VI GLP, Unpublished	N	Sipcam Oxon S.p.A.*
KCA 6.3	Soler J.R.	2001	Generation of tomato samples suitable for residue analysis following applications of MICENE PLUS (Cymoxanil 4%+Mancozeb 40 % WP) SIPCAM Inagra S.A., Spain Report CY2-TO GLP, Unpublished AIIA study on the active substance Cymoxanil	N	Sipcam Oxon S.p.A.*
KCA 6.3	Freschi G	2001	Residue analysis of Cymoxanil in tomato (whole fruit) SIPCAM S.p.A. Italy, Report SIP 1266 GLP, Unpublished AIIA study on the active substance Cymoxanil	N	Sipcam Oxon S.p.A.*
KCA 6.3	Domenichini P.	2003	Generation of tomato fruit samples, suitable for residue analysis (decline curve) following applications of (Cymoxanil 6%+ mancozeb 70%) WP SIPCAM S.p.A., Italy Report CY2/I/01PO GLP, Unpublished AIIA study on the active substance Cymoxanil	N	Sipcam Oxon S.p.A.*

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCA 6.3	Freschi G	2001	Residue analysis of Cymoxanil in tomato (whole fruit) SIPCAM S.p.A., Italy Report SIP 1299 GLP, Unpublished AIIA study on the active substance Cymoxanil	N	Sipcam Oxon S.p.A.*

Appendix 2 Detailed evaluation of the additional studies relied upon

A 2.1 Zoxamide

A 2.1.1 Stability of residues

A 2.1.1.1 Stability of residues during storage of samples

A 2.1.1.1.1 Storage stability of residues in plant products

A 2.1.1.1.1.1 Study 1

Comments of zRMS:	The study is acceptable. The validation parameters were within the required range. In HPLC-MS/MS method transitions for (R) and (S) zoxamide were monitored. The sufficient stability was demonstrated. The method employed was validated according to the SANCO/825/00 rev.8.1 and SANCO/3029/99 rev.4 guidelines in the BPL-STUDY-18-000085.
Reference:	**KCA 6.1/01**
Report:	Sala A., 2021: Interim report – storage stability of zoxamide residues under frozen conditions (-18°C) in potato tubers, potato flakes and fried potatoes[3] Gown Crop Protection Ltd., UK Lab Analysis s.r.l., Italy, Report no. BPL-STUDY-18-000047, GLP, Not published
Guideline(s):	OECD 506 (2007)
Deviations:	The recovery check results for RH-150721 (R) in the samples 18/47/FP/89R and 18/47/FP/90R (67.4% and 62.2%) were below the acceptability range (70% - 110%). The recovery check results for RH-141452 and RH-141455 in the samples 18/47/PF/56R and 18/47/PF/57R (61.6% and 54.4%) were below the acceptability range (70% - 110%). However, retain samples were extracted along with recovery checks prepared with leftover starting material (BPL-SMPL-18-000421). The recovery check results for RH-141455 in the samples 18/47/PF/63R and 18/47/PF/64R (52.5% and 53.6%) were below to the acceptability range (70-110%). However, the results obtained from the samples 18/47/PF/61T and 18/47/PF/62T were close to the recovery check ones and, if corrected, the results obtained are close to 100%. Due to unexpected reason, the calibration point at level 4 (Tuber L4) for the analyte RH-141455 in the calibration curve, had a response higher than expected. However, the calibration range was not altered and 4 points were enough for a correct interpolation. This deviation was solved with no impact on the study. The recovery check results for RH-141455 in the samples 18/47/PO/30R and 18/47/PO/31R (128.1% and 125.5%) were above the acceptability range (70% - 110%). However, the above deviations were regarded as not relevant for the integrity of

[3] Confirmatory study for zoxamide currently being evaluated by RMS (Latvia).

	the study.
GLP:	Yes
Acceptability:	Yes

Materials and methods

The freezer storage stability (at ≤-18°C) of zoxamide and its metabolites (RH-141455 and RH-141452) was studied in recovery experiments according to OECD 506 (2007). This interim report was issued to summarise the results after 18 months (22 months for zoxamide) of storage. It will be extended to a storage period of 24 months.

Whole samples used for the study were bought in a local market and immediately stored at -18°C (December 12, 2018). The whole samples were homogenized by a mechanical vegetable grinder in frozen condition on December 18 2018. Thereafter, analytical aliquots (12.5 g each) of the sample were weighed in a 50 mL screw capped plastic test tube and spiked at 0.1 µg/g spiking level by addition of 125 µL of a spiking solution (containing 12.5 mg/L of each analyte in acetonitrile). The spiked samples were manually shaken and immediately stored at -18°C. The "0 Day samples" were spiked on March 19, 2019 (concurrently with the "3 months" stored samples and analysed the same day (2 h after spiking). At different timepoints thereafter, further samples stored in the freezer were analysed for their analyte content.

Aliquots of samples were weighed and extracted twice with an extraction mixture of water/acetonitrile/methanol (20/40/40 v/v/v) containing 0.2% of formic acid. The 2 extracted fractions were pooled and brought up to a final volume of 40 mL using the same solvent mixture. After centrifugation, the extract was transferred in a 2 mL glass HPLC vial for final determination with HPLC-MS techniques.
The analytical method was validated under GLP compliance according to the SANCO/825/00 rev. 8.1 and SANCO/3029/99 rev.4 guidelines in study BPL-STUDY-18-000085. Procedural recoveries – performed during the course of this study - confirmed the applicability of the method.

Results and discussions

The storage stability data are reported in the following tables. The results are reported as % of the initial spiked concentration (0.1 µg/g) for each analyte. The results were not corrected by the procedural recovery results.

Table A 1: Storage stability of zoxamide residues in frozen (-20 °C) potato samples - summary of recovery data (%)

Sample code	Initial spiking level (µg/g)	Recovery (% of initial spiking concentration			
		(R)-Zoxamide	(S)-Zoxamide	RH-141452	RH-141455
		Check point: 0 Days (2h after spiking)			
18/47/PO/2T	0.1	95.4	100.0	96.3	108.6
18/47/PO/3T	0.1	96.5	101.6	94.9	108.7
	Mean (%)	**96.0**	**100.8**	**95.6**	**108.7**
		Check point: 3 Months			
18/47/PO/7T	0.1	105.3	108.5	95.2	122.1
18/47/PO/8T	0.1	105.7	108.6	95.4	120.6
	Mean (%)	**105.5**	**108.5**	**95.3**	**121.4**
		Check point: 6 Months			
18/47/PO/14T	0.1	99.7	100.8	90.5	92.2
18/47/PO/15T	0.1	107.6	106.8	98.7	101.9
	Mean (%)	**103.6**	**103.8**	**94.6**	**97.1**
		Check point: 12 months			

18/47/PO/21T	0.1	106.0	107.4	97.9	106.9
18/47/PO/22T	0.1	106.0	106.9	97.9	106.9
	Mean (%)	**106.0**	**107.1**	**97.9**	**106.9**
		Check point: 18 months			
18/47/PO/28T	0.1	95.2	100.6	95.7	139.1
18/47/PO/29T	0.1	96.7	101.4	99.2	129.4
	Mean (%)	**95.9**	**101.0**	**97.5**	**134.1**
		Check point: 22 months			
18/47/PO/32T	0.1	102.4	104.8	- [1]	-
18/47/PO/33T	0.1	102.4	103.9	-	-
	Mean (%)	**102.4**	**104.4**	-	-

-: Not Analysed

Conclusion

The freezer storage stability (at ≤-18°C) of zoxamide and its metabolites (RH-141455 and RH-141452) in tomato tuber samples was studied in recovery experiments according to OECD 506 (2007). An interim report demonstrated the stability of the analytes for at least 18 months (22 months for zoxamide). The study will be extended to a storage period of 24 months.

(Sala A. 2021)

A 2.1.1.1.2 Storage stability of residues in animal products

Not relevant. No new data submitted.

A 2.1.1.1.3 Storage stability of residues in sample extracts

The stability of residues of zoxamide and its metabolites in sample extracts was checked as part of individual studies in case necessary (i.e. stored for > 24 hours in the refrigerator). The results do not indicate any residue decrease within the necessary storage periods.

In addition, a study to determine the storage stability of RH-129151 in final sample extracts has been performed.

A 2.1.1.1.1.1 Study 1

Comments of zRMS:	The study is acceptable. The method was validated in the same facility (BPL-STUDY-18-000085). No significative differences can be noticed between the results obtained at the end of the storage at 4°C (± 2°C) in the dark for at least 8 and 24 hours and the "time 0" values for all tested commodities. The % residue analyte after 8 and 24 hours is within the range of 70-120%, that is SANCO/825/00 rev. 8.1 guideline requirement to consider stable a chemical. Therefore, both the enantiomers of the analyte RH-129151 can be considered stable for 24 hours in the final extracts of the tested commodities and processed under the storage conditions of 4°C (± 2°C) in dark.

Reference:	**KCA 6.1/02**
Report:	Longhi, D., 2021: Evaluation of the stability of the analyte RH-129151 in the final extract of the following commodities and processed: grape, grape juice,

	wine, raisin, potato, fried potato, potato fries, tomato, peeled tomato, cucumber[4] Gown Crop Protection Ltd., UK Lab Analysis s.r.l., Italy, Report no. GLP-STUDY-20-77, GLP, Not published
Guideline(s):	SANCO/825/00 rev. 8.1 (2010)
Deviations:	None
GLP:	Yes
Acceptability:	Yes

Materials and methods

The stability of the stability of the zoxamide metabolite RH-129151 (A, B and sum) in the final sample extract of grape (grape, grape juice, wine, raisin), potato (potato tuber, fried potato, potato flakes), tomato (tomato, peeled tomato), and cucumber commodities when stored for 8 and 24 hours under dark and refrigerated conditions at 4°C (± 2°C) has been studied by recovery experiments and analysed concurrently to freshly spiked samples. For the spiking, final extracts of each sample were divided into aliquots of 1 mL. Some aliquots were treated with about 30 µg/L of racemic RH-129151 (15 µg/L per enantiomer). Other control aliquots were kept untreated. Some samples were stored for 8 and 24 hours before analysis with method BPL-STUDY-18-000085, which has been validated according to SANCO/825/00 rev. 8.1 (2010).

Results and discussions

The obtained results of the spiking experiments are summarised in the following tables.
Please note: Since the enantiomers of RH-129151 could not be assigned as the R- and R-form (individual reference standards missing), they were assigned as “A” and “B”.

Table A 2: Recovery results of RH-129151 (A)

Commodity/processed	Commodity type	% Recovery analyte	
		T 8h	T 24 h
Grape	High acid content	102.1	101.1
Grape Juice	Processed	98.8	95.8
Wine	Processed	98.3	94.7
Raisin	Processed	98.6	97.1
Potato tuber	High water content	97.4	87.4
Fried potato	Processed	94.3	107.2
Potato flakes	Processed	106.9	95.4
Tomato	High water content	107.0	104.6
Peeled tomato	Processed	99.9	96.7
Cucumber	High water content	100.9	106.3

Table A 3: Recovery results of RH-129151 (B)

Commodity/processed	Commodity type	% Recovery analyte	
		T 8h	T 24 h
Grape	High acid content	96.4	93.8
Grape Juice	Processed	99.3	99.5
Wine	Processed	96.8	92.4
Raisin	Processed	100.2	101.0
Potato tuber	High water content	101.1	101.0

[4] Confirmatory study for zoxamide currently being evaluated by RMS (Latvia).

Commodity/processed	Commodity type	% Recovery analyte	
		T 8h	T 24 h
Fried potato	Processed	97.3	106.9
Potato flakes	Processed	99.7	93.8
Tomato	High water content	105.2	103.3
Peeled tomato	Processed	98.4	97.1
Cucumber	High water content	105.5	109.0

No significative differences in the recovery values were noticed after storage of the sample extracts at 4°C (± 2°C) in the dark for at least 8 and 24 hours compared to the initial "time 0" values for all the tested commodities: the % residue analyte after 8 and 24 hours is within the range of 70-120%, that is within the SANCO/825/00 rev. 8.1 guideline requirement to consider a chemical as stable. Therefore, RH-129151 (sum) as well as its enantiomer's ratio was considered stable for 24 hours in the final sample extracts of the tested commodities when stored at 4°C (± 2°C) in dark.

Conclusion

The study demonstrates the stability of RH-129151 (sum) as well as its enantiomer's ratio in sample extracts of relevant crop commodities for 24 hours when stored at 4°C (± 2°C) in dark.

(Longhi D. 2021)

A 2.1.2 Nature of residues in plants, livestock and processed commodities

A 2.1.2.1 Nature of residue in plants

A 2.1.2.1.1 Nature of residue in primary crops

No further data needed / no new data submitted.

A 2.1.2.1.2 Nature of residue in rotational crops

No further data needed / no new data submitted.

A 2.1.2.1.3 Nature of residues in processed commodities

EFSA (2017) requested *"studies on the nature of residues for zoxamide including the nature of RH-141455 and RH-141452 under standard hydrolysis conditions representative of pasteurisation, baking/brewing/cooking, sterilisation is required (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see Section 3)".* These studies with zoxamide, RH-141455 and RH-141452 were submitted to Latvia as RMS for zoxamide and cMSs for an interzonal evaluation mid of 2020 and are presented in the following.

A 2.1.2.1.1.1 Study 1

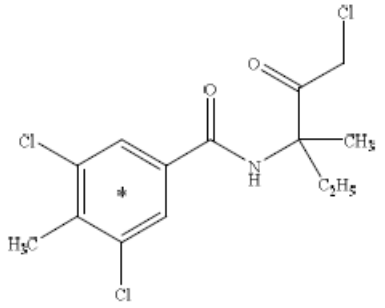
Comments of zRMS:	The study is accepted. The determination was done by LC-MS/MS. The validation values were within the required range.

	The degradation pathway for zoxamide under processing conditions was proposed.

Reference:	**KCA 6.5.1/01**
Report	Grist, A., 2018: Zoxamide: Hydrolysis under simulated processing conditions[5] Gowan Crop Protection Ltd, UK Envigo CRS Limited, UK, Report No. RB66JN, GLP, Not published
Guideline(s):	OECD 507 (2007)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Radiolabelled Test item (Lot/Batch No.)



* [phenyl-U-^{14}C]-zoxamide (QW55QS/OOE01/03)

Radiochemical purity	99.0%
Expiry date	12 July 2019

This study was conducted to provide information on the effects of hydrolysis on zoxamide when exposed to conditions representative of relevant processing operations.

The water used for buffer solution preparation was obtained from an Elga reverse osmosis system. Buffer at concentrations of 0.01 M were prepared with potassium hydrogen phthalate (pH 4), glacial acetic acid adjusted with sodium hydroxide (pH 5.0) and sodium dihydrogen orthophosphate dihydrate adjusted with sodium hydroxide (pH 6.0). The buffer solutions were filled in sterilised test vessels (borosilicate glass tubes) under aseptic conditions. A fortification solution of [phenyl-U-^{14}C]-zoxamide in acetonitrile was prepared at a nominal concentration of 50 mg/L. The actual concentration of zoxamide in the fortification solutions was determined in triplicate just before test item application. For test item application, separate portions (140 mL) of the buffer solutions were treated with each an aliquot (1.4 mL) of the test item stock solution to give nominal concentrations of 0.5 mg/L zoxamide in the test systems. The proportion of co-solvent was 1% (v/v). The conditions of incubation were as follows:

pH	Temperature (ºC)	Time (minutes)	Process represented
4	90	20	Pasteurisation
5	100	60	Baking, brewing, boiling
6	120*	20	Sterilisation

[5] Confirmatory study for zoxamide currently being evaluated by RMS (Latvia).

The application conditions (pH and temperature) were recorded. Immediately after set up of the test systems and at the end of the incubation in the cooled down samples, the radioactivity in the buffer was determined in solution with Ultima Gold (Perkin Elmer) by liquid scintillation counting with automatic quench correction. The portion of unchanged zoxamide and its radiolabelled degradation products were analysed by reversed phase HPLC with UV and radio detection concurrently to external standard solutions. Identification was confirmed by HPLC-MS/MS. Samples were stored in the refrigerator and deep frozen (≤-15°C) until analysis.

The following conditions were used for radiochemical purity analysis:

Column:	Inertsil ODS-3 (25 cm x 4.6 mm internal diameter)
Column temp.:	25°C
Mobile phase:	A: Water B: Acetonitrile

Gradient

Time (minutes)	% A	% B
0	60	40
25	35	65
30	0	100
35	60	40
40	60	40

Flow rate:	1 mL/min
Radioactivity detector:	β-ram radioactivity detector with 500 µL liquid cell, 1:3 split with Pro-flow G+
UV detection wavelength:	210 nm
Software:	Laura, version 1.4a (LabLogic Systems Ltd, Sheffield, UK)

The following conditions were used for sample analysis:

Column:	Inertsil ODS-3 (25 cm x 4.6 mm internal diameter)
Column temp.:	25°C
Mobile phase:	A: 10 mM ammonium formate + 0.1% formic acid in water B: 0.1% Formic acid in acetonitrile

Gradient:

Time (minutes)	% A	% B
0	90	10
5	90	10
35	0	100
45	0	100
45.1	90	10
50	90	10

Flow rate:	1 mL/min
Radioactivity detector:	β-ram radioactivity detector with 500 µL liquid cell, 1:3 split with Pro-flow G+
UV detection at:	240 nm
Software:	Laura, versions 1.4a and 4.1 (LabLogic Systems Ltd, Sheffield, UK)

The mass spectrometry analysis was conducted on pH 4, 5 and 6 buffer samples for confirmation of zoxamide and the known degradation products (RH-141288, RH-150721 and RH-24945). The presence of the RH-129151 degradation product in the incubated buffer samples was elucidated by LC-MS/MS. The analytical conditions used were es follows:

Mass spectrum conditions

Ionisation mode:	Heated Electrospray Ionisation (HESI)
Ionisation polarity:	Positive/negative switching
Spray voltage:	3.5 kV
Capillary temperature:	320°C
Auxiliary gas heater temperature:	400°C
Sheath gas flow:	40 units Nitrogen
Auxiliary gas flow:	10 units Nitrogen
Collision gas:	Nitrogen
Scan range:	105-800 amu
Collision energy:	10 eV RH-141455 and RH-24549 30 eV RH-129151 15, 20 and 25 eV RH-142188, RH-150721 and zoxamide (presented data labelled with the median value)
Resolution (full scan):	70,000
Resolution (MS/MS):	17,500

LC conditions

Column:	Phenomenex Intersil ODS-3,5 µm (250 x 4.6 mm)
Guard column:	Phenomenex Security Guard cartridge C18,4 x 3 mm
Flow rate:	1.0 mL/min (split ratio ca 8:2 with the majority to the fraction collector and remainder to mass spectrometer)
Mobile phase:	A: 10mM aqueous ammonium formate with 0.1% formic acid (v/v) B: 0.1 % formic acid in acetonitrile (v/v)

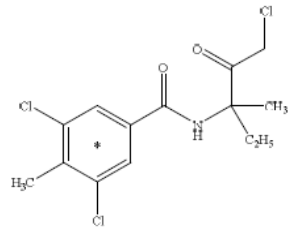
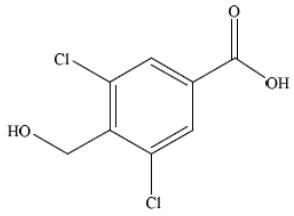
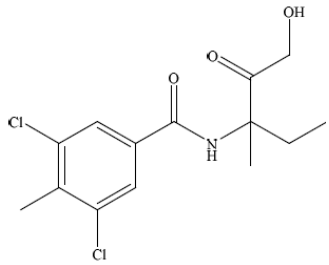
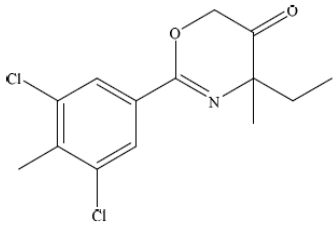
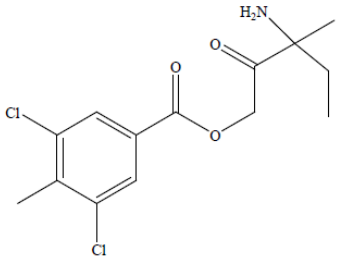
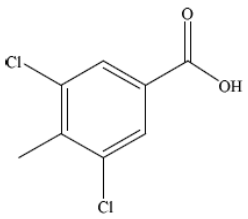
Gradient:

Time (minutes)	% A	% B
0	90	10
5	90	10
35	0	100
45	0	100
45.1	90	10
50	90	10

Column compartment temperature:	25°C
UV detector wavelength:	240 nm

Example chromatograms can be found in the report. For identification of the metabolites the following reference substances were used:

Table A 4: Identification of compounds from high temperature hydrolysis study

Common name/code ID No.	Chemical name	Chemical structure
Zoxamide	3,5-dichloro-N-(3-chloro-1-ethyl-1-methyl-2-oxopropyl)-4-methylbenzamide	
RH-141452	3,5-dichloro-4-(hydroxymethyl)benzoic acid	
RH-141288	3,5-dichloro-N-(1-hydroxy-3-methyl-2-oxopentan-3-yl)-4-methylbenzamide	
RH-129151	2-(3,5-dichloro-4-methylphenyl)-4-ethyl-4-methyl-4H-1,3-oxazin-5(6H)-one	
RH-1507212	3-amino-3-methyl-2-oxopentyl-3,5-dichloro-4-methylbenzoate hydrochloride	
RH-24549	3,5-dichloro-4-methylbenzoic acid	

Results and discussion

Incubation temperatures were within ± 5°C of the target value, the pH remained within ±0.1 units of the target pH. The correct dosing of the test item at study start was confirmed. The recovery of radioactivity from the buffer solutions was in the range 93.1 – 105.9% of applied radioactivity (AR).

Samples incubated at pH 4 and pH 5 were stored for at max. 33 days (for HPCL analysis) and 183 days (for LC-MS/MS analysis) in the freezer, samples incubated at pH 6 were stored for at max. 33 days (for HPCL analysis) and 134 days (for LC-MS/MS analysis). Overall, there was no significant degradation of the samples after frozen storage. Minor differences in the profiles of the HPLC and LC-MS/MS radiochromatograms were observed, specifically for RH-129151 in the pH 4 and pH 5 incubated buffers.

Degradation of zoxamide was detected in all three buffers. In the treated and incubated pH 4 buffer samples zoxamide was hydrolysed to one major degradation product, RH-150721 (mean of 46.7% TRR, 0.234 mg/L), and three minor degradation products, RH-24549 (mean of 9.7% TRR, 0.049 mg/L), RH-129151 (mean of 4.4% TRR, 0.022 mg/L) and RH-141288 (mean of 1.1% TRR, 0.006 mg/L).

In the treated and incubated pH 5 buffer samples, zoxamide was hydrolysed to three major degradation products, RH-150721 (mean of 11.0% TRR, 0.056 mg/L), RH-24549 (mean of 62.7% TRR, 0.314 mg/L) and RH-129151 (mean of 13.1% TRR, 0.066 mg/L), and to one minor metabolite, RH-141288 (mean of 8.1% TRR, 0.041 mg/L).

In the treated and incubated pH 6 buffer samples, zoxamide was hydrolysed to RH-150721 (mean of 0.7% TRR, 0.004 mg/L), RH-141288 (mean of 27.3% TRR, 0.146 mg/L), RH-24549 (mean of 43.0% TRR, 0.23 mg/L) and RH-129151 (mean of 20.8% TRR, 0.111 mg/L).

Table A 5: Radioactivity in buffer solutions treated with [^{14}C]-zoxamide (% TRR)

Time zero sampling

Components	Rt. (min)	pH 4			pH 5			pH 6		
		A	B	Mean	A	B	Mean	A	B	Mean
Zoxamide	34.1	97.9	96.9	97.4	97.0	96.8	96.9	99.1	98.4	98.8
RH-150721	21	1.0	1.1	1.1	0.5	-	0.3	-	-	-
Unknown 3	29.4	-	0.9	0.5	-	-	-	-	-	-
RH-129151	40.5	0.9	0.7	0.8	1.5	1.9	1.7	-	-	-
Others[a]	~	0.2	0.4	0.3	1.0	1.3	1.2	0.8	0.7	0.8

Following incubation

Components	Rt. (min)	pH 4 90°C for 20 minutes			pH 5 100°C for 60 minutes			pH 6 120°C for 20 minutes		
		Representative for pasteurisation			Representative for baking, brewing, boiling			Representative for sterilisation		
		A	B	Mean	A	B	Mean	A	B	Mean
Zoxamide	34.1	38.5	32.1	35.3	2.4	-	1.2	1.3	0.3	0.8
RH-150721	21	42.5	50.9	46.7	11.3	10.7	11.0	0.8	0.6	0.7
RH-141288	28.4	1.1	1.1	1.1	8.8	7.4	8.1	27.6	26.9	27.3
RH-24549	29.6	10.1	9.3	9.7	64.7	60.6	62.7	43.3	42.7	43.0
RH-129151	40.5	5.1	3.6	4.4	11.2	14.9	13.1	20.3	21.2	20.8
Unknown 1	20.7	0.8	0.4	0.6	-	-	-	-	-	-
Unknown 2	25.6	-	-	-	0.5	0.6	0.6	0.5	0.3	0.4
Unknown 3	29.4	-	-	-	-	1.0	0.5	-	-	-

Unknown 4	35	-	-	-	0.6	-	0.3	-	-	-
Unknown 5	37.2	1.3	1.7	1.5	-	0.7	0.4	-	0.6	0.3
Unknown 6	38.9	-	-	-	-	0.8	0.4	0.3	0.5	0.4
Others[a]	~	0.6	0.9	0.8	0.5	3.3	1.9	0.3	0.1	0.2

Results are expressed as % Total Reactive Residues (% TRR)
Rt. Retention time in minutes of component
- Not detected
[a] Radioactivity not associated with specific components

It seems that zoxamide was hydrolysed to form RH-150721, RH-141288, RH-24549 and RH-129151. The degradation pathway is proposed as follows:

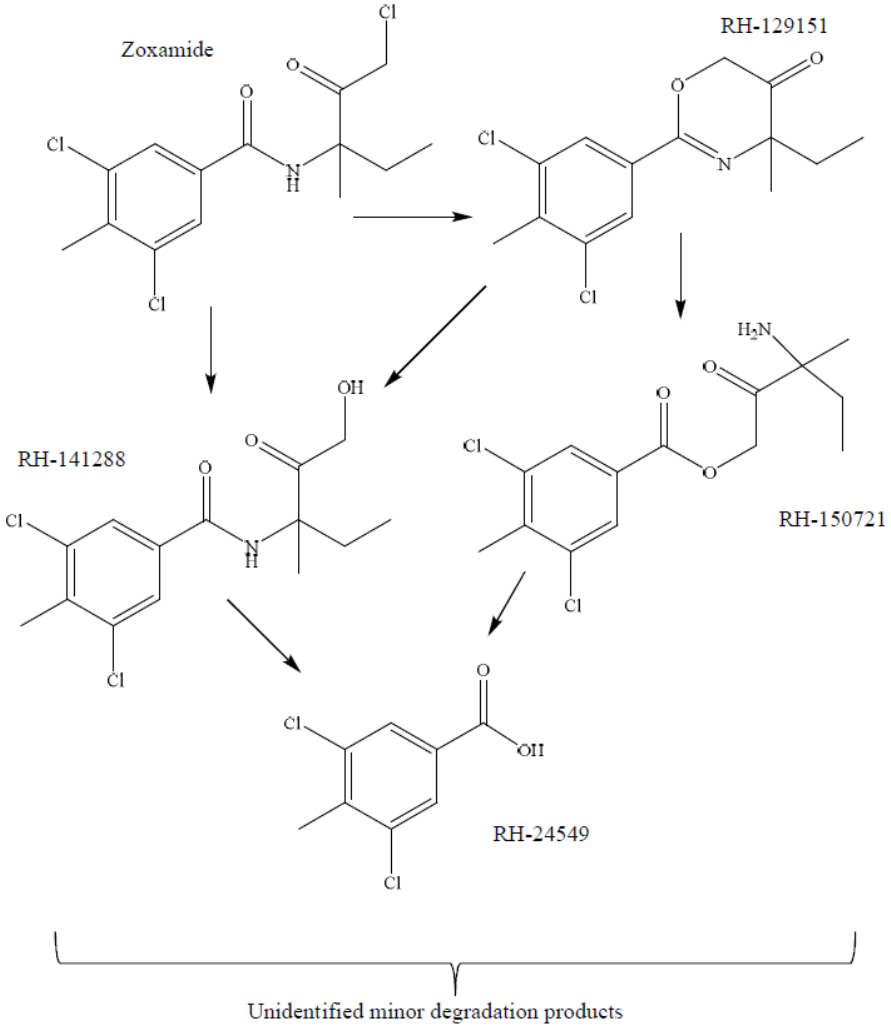


Figure A 1: Proposed pathway of zoxamide under simulated processing conditions

Conclusions

The study was conducted to provide information on the effects of hydrolysis on zoxamide under conditions representative for pasteurisation, baking/brewing/boiling, and sterilisation. The study was performed with radiolabelled test item.

Zoxamide was found to account for 101.1 – 102.8% applied radioactivity (AR) when incubated under conditions representative of pasteurisation (pH 4 at 90ºC for 20 minutes), 102.1 – 102.7% AR when incubated

under conditions representative of baking, brewing and boiling (pH 5 at 100ºC for 60 minutes) and 93.1 – 94.4% AR when incubated under conditions representative of sterilisation (pH 6 at 120°C for 20 minutes).

Degradation of zoxamide was observed in all three incubated buffer systems. Zoxamide hydrolysed to form RH-150721 (up to a mean maximum of 46.7% TRR, 0.234 mg/L), RH-141288 (up to a mean maximum of 27.3% TRR, 0.146 mg/L), RH-24549 (up to a mean maximum of 62.7% TRR, 0.314 mg/L), RH-129151 (up to a mean maximum of 20.8% TRR, 0.111 mg/L) and up to 6 unknown degradation products (≤1.7% TRR, ≤0.009 mg/L).

Table A 6: Summary of results from the nature of residues study under standard processing conditions in buffer solutions treated with [^{14}C]-zoxamide (% TRR)

Metabolites of zoxamide appearing > 10 % are marked in **bold.**

Condition	**pH 4, 90 °C**	**pH 5, 100°C**	**pH 6, 120 °C**
Representative for …	**pasteurisation**	**baking, brewing, boiling**	**sterilisation**
Zoxamide	35.3	1.2	0.8
RH-24549	9.7	**62.7**	**43.0**
RH-150721	**46.7**	**11.0**	0.7
RH-129151	4.4	**13.1**	**20.8**
RH-141288	1.1	8.1	**27.3**

(Grist A. 2018)

A 2.1.2.1.1.2 Study 2

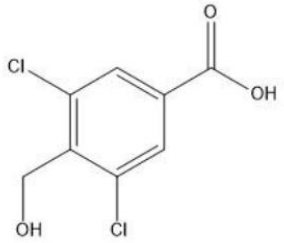
Comments of zRMS:	The study is acceptable. The aim of this study was the determination of the effect of simulated processing conditions on the stability of the Zoxamide's metabolite RH-141452. The analytical method was based on HPLC-DAD system and it was validated according to the SANCO/825/00 rev.8.1. The validation parameters were within the required range. In the study no degradation products were found.

Reference:	**KCA 6.5.1/02**
Report	Longhi, D., 2019: RH-141452: Hydrolysis under simulated processing conditions[6] Gowan Crop Protection Ltd, UK LabAnalysis s.r.l., Italy, Report No. BPL-STUDY-18-000092, GLP, Not published
Guideline(s):	OECD 507 (2007)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Test material (Lot/Batch No.) RH-141452 (21109)

[6] Confirmatory study for zoxamide currently being evaluated by RMS (Latvia).



Purity 99.82 % (w/w)

The study was conducted to provide information on the effects of hydrolysis on the zoxamide metabolite RH-141452. Therefore, it was exposed to conditions representative for pasteurisation, baking/brewing/ boiling, and sterilisation.

The water used for buffer solutions was UPLC grade water. Buffer were prepared as follows:
- **pH 4.0**: 90 mL of a solution of 0.1 N NaOH (402 mg of NaOH pellets in 100 mL purified water) and 500 mL of a solution of 0.1 M monopotassium citrate (11.521 g of monopotassium citrate in 500 mL purified water) were diluted with purified water to a volume of 1 L.
- **pH 5.0:** 8 mL of a solution of 0.667 M disodium phosphate (2.377 g of Na_2HPO_4 in 250 mL purified water) and 992 mL of a solution of 0.667 M monopotassium phosphate (18.156 g of KH_2PO_4 in 2 L of purified water) were pulled.
- **pH 6.0**: 111 mL of a solution of 0.667 M disodium phosphate (2.377 g of Na_2HPO_4 in 250 mL of purified water) and 889 mL of a solution of 0.667 M monopotassium phosphate (18.156 g of KH_2PO_4 in 2 L of purified water) were pulled.

The buffer solutions were filled in sterilised glass vials and tubes under a laminar flow hood. Sterility was reached carrying out sterilisation of buffers, glassware and all the necessary equipment in an autoclave at 121°C for 15 minutes. Sterility was checked on samples prepared ad-hoc before and after the incubation, using a colony counting method.

Fortification solutions in acetonitrile were prepared and applied to the test vessels. The actual concentration of RH-141452 in matrix-matched fortification solutions were determined 5-fold just before test item application. For test item application, portions of the buffer solutions were treated with an aliquot of the test item stock solution to give nominal concentrations of 10 mg/L RH-141452 in the test systems. Triplicate test samples per buffer were prepared and incubated in an oven at 90 °C and 100 °C and in an autoclave at 120 °C. The incubation conditions were as follows:

pH	Temperature (ºC)	Time (minutes)	Process represented
4	90	20	Pasteurisation
5	100	60	Baking, brewing, boiling
6	120*	20	Sterilisation

Freshly prepared buffers were analysed concurrently. Blank buffers served as a control.

The application conditions (pH and temperature) were recorded.

The stability of the analyte was checked by HPLC-DAD comparing the response of the analyte before and after the incubation at the different conditions.

Immediately after set up of the test systems and at the end of the incubation in the cooled down samples, aliquots of the buffer samples were directly injected in a HPLC-DAD system. Concentrations of the test item were determined using matrix-matched calibration solutions. The analytical method with an LOQ of 1 mg/L in all buffers was validated according to the SANCO/825/00 rev. 8.1 guideline. The confirmation

of the analyte identification was performed using a high-resolution mass spectrometer detector and a DAD detector recording the UV spectra between 200 and 400 nm. Both the MS and the UV spectra of the standard and of the sample before and after each incubation were compared. The same profile confirmed the identification of the analyte detected in the samples.

Results and discussion

Sterility checked on samples prepared ad-hoc before and after the incubation showed a number of colony-forming unit less than 1 (CFU< 1) for each analysed sample, demonstrating that the tests were carried out in sterile conditions. Incubation temperatures were within ± 5°C of the target value, the pH remained within ±0.1 units of the target pH. The correct dosing of the test item at study start was confirmed. Blank buffers did not show any analyte or contaminants.

The effects of the different hydrolysis conditions on the analyte RH-141452 were evaluated for buffer concentrations of 10 mg/L RH-141452. In the following table the means and the relative standard deviations (RSDs) of the buffer samples analysed before and after the incubation are reported:

Table A 7: Results summary

Condition	Before the incubation		After the incubation		Residual Amount (%)
	Mean (mg/L)	RSD (%)	Mean (mg/L)	RSD (%)	
Pasteurisation (pH 4, 90°C, 20 minutes)	9.812	0.890	9.735	0.181	99.22
Baking, brewing, boiling (pH 5, 100°C, 60 minutes)	9.686	0.281	9.762	0.987	100.8
Sterilisation (pH 6, 120°C, 20 minutes)	9.692	0.651	9.623	0.196	99.29

The residual amount is the percentage ratio between the mean of the results obtained from the analyses after the incubation and those before. It is a number that indicates the ratio of non-hydrolysed analyte. These values were obtained in amounts higher than 99% for each buffer solution. This is higher than the 90% required to assess that the test substance is stable under the applied conditions. No degradation products were found. The identity of the analyte was verified with high-resolution HPLC-MS (HPLC-HRMS), both before and after the incubation.

Conclusions

The amount of unchanged recovered RH-141452 was high (99.2-100.8 %). No degradation of RH-141452 was detected in all three buffers. The zoxamide metabolite RH-141452 is therefore regarded stable under the conditions simulating pasteurisation (pH 4, 90°C for 20 minutes), baking/brewing/boiling (pH 5, 100°C for 60 minutes) and sterilisation (pH 6, 120°C for 20 minutes).

(Longhi D. 2019)

A 2.1.2.1.1.3 Study 3

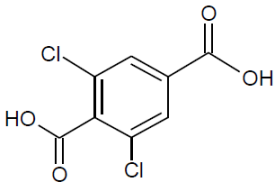
Comments of zRMS:	The study is acceptable. The aim of this study was the determination of the effect of simulated processing conditions on the stability of the Zoxamide's metabolite RH-141455. The analytical method was based on HPLC-DAD system and it was validated according to the SANCO/825/00 rev.8.1. The validation parameters were within the

	required range. In the study no degradation products were found.

Reference:	**KCA 6.5.1/03**
Report	Longhi, D., 2019: RH-141455: Hydrolysis under simulated processing conditions[7] Gowan Crop Protection Ltd, UK LabAnalysis s.r.l., Italy, Report No. BPL-STUDY-19-000009, GLP, Not published
Guideline(s):	OECD 507 (2007)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Test material (Lot/Batch No.) RH-141455 (14-SBT-100-1)



Purity 94.53 % (w/w)

The study was conducted to provide information on the effects of hydrolysis on the zoxamide metabolite RH-141455. Therefore, it was exposed to conditions representative for pasteurisation, baking/brewing/ boiling, and sterilisation.

The water used for buffer solutions was UPLC grade water. Buffer were prepared as follows:
- **pH 4.0**: 90 mL of a solution of 0.1 N NaOH (402 mg of NaOH pellets in 100 mL purified water) and 500 mL of a solution of 0.1 M monopotassium citrate (11.521 g of monopotassium citrate in 500 mL purified water) were diluted with purified water to a volume of 1 L.
- **pH 5.0:** 8 mL of a solution of 0.667 M disodium phosphate (2.377 g of Na_2HPO_4 in 250 mL purified water) and 992 mL of a solution of 0.667 M monopotassium phosphate (18.156 g of KH_2PO_4 in 2 L of purified water) were pulled.
- **pH 6.0**: 111 mL of a solution of 0.667 M disodium phosphate (2.377 g of Na_2HPO_4 in 250 mL of purified water) and 889 mL of a solution of 0.667 M monopotassium phosphate (18.156 g of KH_2PO_4 in 2 L of purified water) were pulled.

The buffer solutions were filled in sterilised glass vials and tubes under a laminar flow hood. Sterility was reached carrying out sterilisation of buffers, glassware and all the necessary equipment in an autoclave at 121°C for 15 minutes. Sterility was checked on samples prepared ad-hoc before and after the incubation, using a colony counting method.

Fortification solutions in acetonitrile were prepared and applied to the test vessels. The actual concentration of RH-141455 in fortification solutions were determined 5-fold just before test item application. For test item application, portions of the buffer solutions were treated with an aliquot of the test item stock solution

[7] Confirmatory study for zoxamide currently being evaluated by RMS (Latvia).

to give nominal concentrations of 10 mg/L RH-141455 in the test systems. Triplicate test samples per buffer were prepared and incubated in an oven at 90 °C and 100 °C and in an autoclave at 120 °C. The incubation conditions were as follows:

pH	Temperature (°C)	Time (minutes)	Process represented
4	90	20	Pasteurisation
5	100	60	Baking, brewing, boiling
6	120*	20	Sterilisation

Freshly prepared buffers were analysed concurrently. Blank buffers served as a control.

The application conditions (pH and temperature) were recorded.

The stability of the analyte was checked by HPLC-DAD comparing the response of the analyte before and after the incubation at the different conditions.

Immediately after set up of the test systems and at the end of the incubation in the cooled down samples, aliquots of the buffer samples were directly injected in a HPLC-DAD system. Concentrations of the test item were determined using external calibration solutions. The analytical method with an LOQ of 1 mg/L in all buffers was validated according to the SANCO/825/00 rev. 8.1 guideline. The confirmation of the analyte identification was performed using a high-resolution mass spectrometer detector and a DAD detector recording the UV spectra between 200 and 400 nm. Both the MS and The UV spectra of the standard and of the sample before and after each incubation were compared. The same profile confirmed the identification of the analyte detected in the samples.

Results and discussion

Sterility checked on samples prepared ad-hoc before and after the incubation showed a number of colony-forming unit less than 1 (CFU< 1) for each analysed sample, demonstrating that the tests were carried out in sterile conditions. Incubation temperatures were within ± 5°C of the target value, the pH remained within ±0.1 units of the target pH. The correct dosing of the test item at study start was confirmed. Blank buffers did not show any analyte or contaminants.

The effects of the different hydrolysis conditions on the analyte RH-141455 were evaluated for buffer concentrations of 10 mg/L RH-141455. In the following table the means and the relative standard deviations (RSDs) of the buffer samples analysed before and after the incubation are reported:

Table A 8: Results summary

Condition	Before the incubation		After the incubation		Residual Amount (%)
	Mean (mg/L)	RSD (%)	Mean (mg/L)	RSD (%)	
Pasteurisation (pH 4, 90°C, 20 minutes)	9.806	0.046	9.746	1.021	99.39
Baking, brewing, boiling (pH 5, 100°C, 60 minutes)	9.944	0.561	9.840	0.528	98.95
Sterilisation (pH 6, 120°C, 20 minutes)	9.791	0.120	9.767	0.186	99.75

The residual amount is the percentage ratio between the mean of the results obtained from the analyses after the incubation and those before. It is a number that indicates the ratio of non-hydrolysed analyte. These values were obtained in amounts ≥ 99% for each buffer solution. This is higher than the 90% required to assess that the test substance is stable under the applied conditions. No degradation products were found.

The identity of the analyte was verified with high-resolution HPLC-MS (HPLC-HRMS), both before and after the incubation.

Conclusions

The amount of unchanged recovered RH-141455 was high (99.4-99.95 %). No degradation of RH-141455 was detected in all three buffers. The zoxamide metabolite RH-141455 is therefore regarded stable under the conditions simulating pasteurisation (pH 4, 90°C for 20 minutes), baking/brewing/boiling (pH 5, 100°C for 60 minutes) and sterilisation (pH 6, 120°C for 20 minutes).

(Longhi D. 2019)

A 2.1.2.1.1.4 Study 4

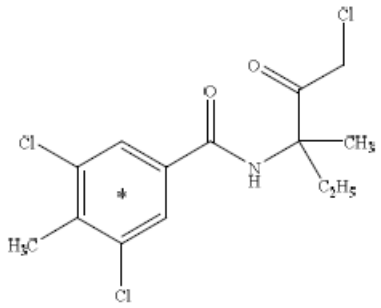
In a study of Völkl (2000; report no. DERBI 92297) tomatoes were treated with [^{14}C]-zoxamide and the distribution and identification of degradates of zoxamide in products made from tomatoes were investigated. The study was not presented in the DAR or the RAR for zoxamide, but was used for the authorisation of zoxamide products in tomatoes all over Europe. It is therefore included in the submission, regarded as supplementary information, for completeness. The study has also been submitted to Latvia as RMS for zoxamide and cMSs for an interzonal evaluation mid of 2020.

Comments of zRMS:	Not relevant. No tomatoes in the intended CEU GAP.

Reference:	**KCA 6.5.1/04**
Report	Völkl S., 2000: ^{14}C-RH-117281: Processing of tomatoes[8] Rohm and Haas Comp., USA, Report No. 34-01-25, DERBI 92297 RCC Ltd, Itingen, Switzerland, Report No. 34P-99-25, GLP, Not published
Guideline(s):	OPPTS 860.152 (1996) Directive 96/68/EC, Section 6.5 (1996)
Deviations:	No
GLP:	Yes
Acceptability:	Supportive information

Materials and methods

Radiolabelled Test item (Lot/Batch No.)



* [phenyl-U-^{14}C]-zoxamide (942.01)

Radiochemical purity 96.3 % (1.312 MBq/mg)

[8] Confirmatory study for zoxamide currently being evaluated by RMS (Latvia).

Expiry date	Not stated. The purity was checked prior to use.

This study was conducted to provide a radioactive mass balance and information on the degradates of zoxamide in processed tomatoes.

Aliquots from the application solution in acetone were spotted onto each tomato separately and distributed on the surface using a syringe. The tomato fruits were treated at a rate of 3 mg [^{14}C]-zoxamide per kg tomato. After evaporation of the acetone the tomatoes were processed to either juice, puree or preserved tomatoes.

Juice: The treated and cut tomatoes were squeezed in a juice press and the juice separated from the remaining skins and pulp. The juice was then centrifuged and separated from a pulp fraction.

Puree: The treated and cut tomatoes were cooked for 20-30 minutes. Thereafter, the tomatoes and cooking water were separated. The residual tomatoes were pressed through a sieve into a separate pot by means of a ladle. About 1 g of sodium chloride was added to the resulting sieved puree and this was cooked again for 10 to 15 minutes.

Preserves: Whole tomatoes were boiled for two minutes and thereafter their skins were pulled-off. The skinless tomatoes were cut into halves and transferred into 200-mL bottling jars. 13.3 mL of a 1% sodium chloride solution was then added to each jar. The closed jars were transferred to a preserving pan filled with water up to the top of the bottling jars. The samples were heated to 85°C and kept at this temperature for about 15 minutes.

The amount of radioactive residues after processing was determined directly by liquid scintillation counting in all liquid samples and in solid fractions after combustion. Additionally, the samples were analysed by HPLC/RAM for parent substance and degradates.

Results and discussion

Juice: Based on the total radioactivity applied, the level of residues in the juice amounted to 13.0% of the initial dose or 0.393 mg parent equivalents (p.e.)/kg tomato. In the centrifuged tomato juice 4.7% (0.142 ppm) of the radioactivity applied were recovered. In the pulp which had been separated from the juice, the residues represented 8.3% of the radioactivity applied or 0.250 mg p.e./kg tomato. Most of the radioactivity from the residue could be extracted from the pulp with acetone (i.e., 7.7% of the radioactivity applied, 0.232 ppm).

Puree: In the tomato puree, the radioactivity amounted to 26.2% of the initial dose or 0.778 mg p.e./kg tomato. The remaining radioactivity (in total 57.8% or 1.715 ppm) was detected in skins, seeds and cooking water that were separated from the puree. Most of the radioactivity from puree was extracted with acetone (i.e., 23% of the radioactivity applied, 0.683 ppm). Only 3.2% of the radioactivity applied (0.095 ppm) remained non-extracted.

Preserves: Based on the total radioactivity applied, the level of residues in the preserved tomatoes amounted to only 1.9% of the initial dose or 0.028 mg p.e./kg tomato. Most of the radioactivity was in the separated skins representing 87.0% of the radioactivity applied (2.635 ppm) and in the cooking water amounting to 9.2% (0.279 ppm). Most of the radioactivity applied (0.026 ppm). Only 0.2% of the radioactivity applied (0.002 ppm) remained non-extracted.

Pattern of degradates:

For juice parent zoxamide was the main radioactive component recovered amounting to 12.2% (0.369 ppm) of the radioactivity applied. No HPLC was performed for the preserves due to the low amount of radioactivity recovered in the extract of the preserves (1.8%). Similarly, parent substance was the main radioactive

fraction in the extracted skin of the preserved tomatoes and was recovered without important degradation due to the fact that in the early stages of processing the skin was separated from the remaining tomatoes.

However, in the puree where the whole tomatoes were cooked for 20-30 minutes, then sieving the mass and additionally cooked for 10-15 additional minutes, the parent substance was degraded mainly to RH-129151 and RH-150721. It is assumed that the pH is governed by the intended commodity (tomato) and the term "cooked" implies at least 100ºC. The parent compound amounted to 3.1% (0.092 ppm) of the radioactivity applied. The main degradation products reached levels of 10.9% (0.324 ppm) of the radioactivity applied and 6.7% (0.199 ppm), respectively. In addition, four minor fractions were detected not exceeding 1.4% (0.040 ppm) of the radioactivity applied.

Table A 9: Radioactivity in tomatoes treated with [^{14}C]-zoxamide processed to preserves – total amounts (% TRR) and (mg parent equivalents/kg)

Tomatoes processed to preserves	Weight (g or mL)	Amount (mg parent equivalents)	% of applied	mg parent equivalents / kg tomato (=ppm)
Preserved tomatoes	1060.4	0.03	2.0	0.029
Total preserves:		0.03	1.8	0.027
- extractable		0.00	0.2	0.002
- non-extractable				
Skins after processing	76.6	2.61	85.8	2.600
Total skins		2.50	82.4	2.498
- extractable		0.10	3.4	0.102
- non-extractable				
Cooking water	1288	0.28	9.2	0.279
Washing of tools	244	0.02	0.6	0.019
Total preserve processing		2.93	97.6	2.926
100% applied	1002.6	3.04	100.0	3.030

Table A 10: Radioactivity in tomatoes treated with [^{14}C]-zoxamide processed to preserves – metabolite pattern (% TRR) and (mg parent equivalents/kg)

Tomatoes processed to preserves	Preserved Tomatoes		Skins after processing		Cooking water		Total	
Radioactive fraction	%-applied	mg/kg	%-applied	mg/kg	%-applied	mg/kg	%-applied	mg/kg
Parent	n.a.	n.a.	77.1	2.335	n.a.	n.a.	77.1	2.335
RH-139432	n.a.	n.a.	*	*	n.a.	n.a.	*	*
RH-127450	n.a.	n.a.	0.7	0.022	n.a.	n.a.	0.7	0.022
M1 (unknown)	n.a.	n.a.	0.6	0.019	n.a.	n.a.	0.6	0.019
RH-129151	n.a.	n.a.	0.5	0.014	n.a.	n.a.	0.5	0.014
Total extractables	n.a.	n.a.	78.9	2.390	-	-	78.9	2.390
Non-extractables	0.2	0.002	8.1	0.245	-	-	8.2	0.247
Not analysed	1.8	0.026	-	-	9.2	0.279	11.0	0.305
Washing of tools	-	-	-	-	-	-	0.6	0.019
Total in tomatoes	1.9	0.028	87.0	2.635	9.2	0.279	98.7	2.961

*: Not detected
mg/kg (ppm): mg radioactive residue per kg tomatoes
n.a.: Not analysed

Conclusions

Tomatoes were treated with [^{14}C]-zoxamide and the distribution and identification of degradates of zoxamide in products made from tomatoes were investigated. From this study, it appears that for certain processed commodities (e.g. tomato puree) the major residue was not zoxamide. In the puree where the whole tomatoes were cooked for 20-30 minutes the parent substance was degraded mainly to RH-129151 and RH-150721. The parent compound amounted to 3.1% (0.092 ppm) of the radioactivity applied. The main degradation products reached levels of 10.9% (0.324 ppm) of the radioactivity applied and 6.7% (0.199 ppm), respectively.

(Völkl S., 2000)

A 2.1.2.2 Nature of residues in livestock

EFSA (2019) requested in his Peer Review Conclusion (2017) a study on the *Log Po/w for RH-141288 and RH-127450 and an assessment regarding their potential fatsolubility according to FAO (2009) should be provided*. The study on RH-127450 is already available in the RAR (Tognucci, 1988), the study on RH141288 has been performed and is provided in the following.

A 2.1.2.2.1 Study 1

Comments of zRMS:	Confirmatory study for zoxamide currently being evaluated by RMS (Latvia).

Reference:	**KCA 6.7.1/01**
Report	Cashmore, A., 2019: RH-141288: Partition coefficient (n-octanol/water) Shake flask method Gowan Crop Protection Ltd., Uk Smithers ERS Ltd, UK, Report No. 3202371, GLP, Not published
Guideline(s):	OECD 107 (1995)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Test item (Lot/Batch No.)	RH-141288 (HHGCP003-00-1)
Purity	99.87 %
Expiry date	July 2019

The partition coefficient (log Pow) for RH-141288 was determined for n-octanol and aqueous buffers at pH 5, 7 and 9 using the shake flask method. Vessels were prepared containing n-octanol and aqueous buffers at three different ratios (1:1, 2:1 and 1:2) and treated with RH-141288. After shaking, the phases were separated and analysed by a method validated according to SANCO/3029/99 rev. 4 (2000).

Results and discussion / Conclusions

The mean n-octanol/water partition coefficients (log Pow) at 25±1°C were determined to be as follows:

pH 5: log Pow = 2.810 (range 2.612 to 2.959)

pH 7: log Pow = 2.892 (range 2.763 to 2.999)

pH 9: log Pow = 2.644 (range 2.512 to 2.784)

The individual log Pow values for each pH level tested were within the range of ± 0.3 units from the mean. The overall recoveries for each vessel were ≥ 90% (range 90.64 to 107.35%).

The log Pow was similar at each pH, demonstrating that the test substance does not dissociate between the range of pH 5 to pH 9.

A 2.1.3 Magnitude of residues in plants

A 2.1.3.1 Fruiting crops – wine and table grapes

Table A 11: Comparison of intended and critical EU GAPs

Type of GAP	Number of applications	Application rate per treatment (g/ha)	Interval between application	Growth stage at last application	PHI (days)
cGAP EU (RAR, Latvia, 2017)	5	180	8	BBCH 79	28
cGAP EU (Art. 12, EFSA, year)	--	--	--	--	--
Intended cGAP (13-16 for N-EU [wine and table grapes] and 17-30 [wine and table grapes] for S-EU) *	3	148.5	7-10	BBCH 89 ** or not relevant (considering the PHI of 28 days)	28

* Use number(s) in accordance with the list of all intended GAPs in Part B, Section 0
** Should be realistically BBCH 85 if considering the phi of 28 days or "not relevant", and taking into account only the phi of 28 days

The following two studies of Romanini (2011a,b; reports no. CREG 2117 and CREG 2120) were considered for the authorisation of Cymoxanil 33% + Zoxamide 33% WG and regarded valid. They are summarised in the following.

A 2.1.3.1.1 Study 1

Comments of zRMS:	Comment of the zRMS Spain during product authorisation: *Four trials in Northern and four trials in Southern Europe are available in grapes treated with 'Cymoxanil 33% + Zoxamide 33% WG' (Harpon WG, batch XE28160A11, Cymoxanil 32.7% , Zoxamide 33.5%). The 8 trials can be considered as valid according to the intended cGAP.* *For cymoxanil residues were below the method limit of quantification (LOQ = 0.05 mg/kg for bunches and 0.01 mg/kg for processed samples) in all the specimens analysed (treated, untreated and processed), except for one young wine residue trial where it was slightly above the LOQ.* *N-EU: 4x<0.05 mg/kg* *S-EU: 4x<0.05 mg/kg* *For zoxamide, four trials in Northern and four trials in Southern Europe are available in grapes according to the cGAP. Residue values for bunches were as follows:* *N-EU: 0.8598; 0.2980; 0.4586; 0.2656 mg/kg* *S-EU: 0.2594; 0.2748; 0.0733; 0.0833 mg/kg* *All the results were below the respective MRL values set forth according to Reg. (EC) No. 396/2005 for grapes (0.2 mg/kg and 5 mg/kg for cymoxanil and zoxamide, respectively).* *Applicant has demonstrated that method used Burdge E. et al. (1999), method TR 34-98-179, DP 81841 ref. KIIIA 5.3.1/08 to determine zoxamide in wine is equivalent to methods Burdge E. et al. (1999), method TR 34-99-111, DERBI 91462 (accepted for pre-registration proposes in this drr) and Burdge E. et al. (1998), method TR 34-98-150; DP81828, ref. KIIIA 5.3.1/07 to determine zoxamide in grapes (watery matrix), grape*

	juice and raisins accepted in DAR for monitoring. Thus the method could be accepted and is totally valid. The analytical method used for cymoxanil determination is a validated method into the residue study SIP1278 for tomato matrix. This method is equivalent to A0087, which was evaluated ***in Cymoxanil DAR as acceptable for grape potato and tomato matrixes with LOQ = 0.05 mg/Kg.****Both are accepted for pre-registration proposes.* (ZRMS PL: previously evaluated)

Reference:	**KCA 6.3/01**
Report:	Romanini, M., 2011: Determination of cymoxanil and zoxamide residues at harvest in raw and processed agricultural commodity grape (bunch, must young and bottled wine) following five applications of Harpon W (Cymoxanil 33% + Zoxamide 33% WG) - Four trials, Italy 2010 (Southern Europe) Gowan Comercio Internacional e Servicos Limitada, Portugal Research Centre "E. Gagliardini, Italy, Report No. CREG 2117, GLP, Not published
Guideline(s):	1607/VI/97 rev. 2, Appendix B 7029/VI/95 rev. 5 SANCO/3029/99 rev. 4 (2000) SANCO/825/00 rev. 7 ENV /JM/MONO (2007)17 ENV/MC/CHEM (98)17
Deviations:	No
GLP:	Yes
Acceptability:	Yes

and

Reference:	**KCA 6.3/02**
Report:	Romanini, M., 2011: Determination of cymoxanil and zoxamide residues at harvest in raw and processed agricultural commodity grape (bunch, must young and bottled wine) following five applications of Harpon W (Cymoxanil 33% + Zoxamide 33% WG) - Four trials, ~~Italy~~ Northern France, Germany 2010 (Northern Europe) Gowan Comercio Internacional e Servicos Limitada, Portugal Research Centre "E. Gagliardini, Italy, Report No. CREG 2120, GLP, Not published
Guideline(s):	1607/VI/97 rev. 2, Appendix B 7029/VI/95 rev. 5 SANCO/3029/99 rev. 4 (2000) SANCO/825/00 rev. 7 ENV /JM/MONO (2007)17 ENV/MC/CHEM (98)17
Deviations:	Deviation for Trial 80100 AN1 (FJCZ1 O/GR02) - shipment of specimens for processing: The transport duration of grapes processing specimens (between the expedition made just after the sampling and the receipt at the processing facility) was 10 days, whereas the study

	plan required that they should be delivered as quickly as possible. The specimens were firstly delivered at wrong address (error of the transport company), then transferred to the processing facility. It has no Impact on the study, since the specimens were shipped under refrigerated conditions. Deviation for trial 60100 AN2 (F/CZ1 O/GR03): The trial was performed on white vinegrapes instead of red vinegrapes requested in the study plan. Deviation for trial 80100 AN1 (F/CZ101GR02): No retain specimens were sampled for the specimens for processing. Deviation for trial 80100 BM1 (F/CZ10/GR04): The spray deviation from target spray volume (fixed at 500 L/ha) was superior to 5% in 1a1 and 4th application. The deviation from the target rate remained included into the range of ±10%. This deviation represents the worst case.
GLP:	Yes
Acceptability:	Yes

Materials and methods

Test item (Lot/Batch No.):	Cymoxanil 33% + Zoxamide 33% WG / Harpon WG (XE28160A11)
Active substance content (nominal):	33 % Cymoxanil + 33% Zoxamide (nominal) 32.7 % Cymoxanil + 33.5% Zoxamide (analysed)
Expiry date:	July 2011

Four trials in Northern and 4 trials in Southern Europe are available in grapes, treated with 5 applications of 'Cymoxanil 33% + Zoxamide 33% WG' (Harpon WG, batch XE28160A11, Cymoxanil 32.7%, Zoxamide 33.5%) at 0.15 kg as/ha each, approximately 7 days interval. Plant samples were collected 28 days after the last application. These trials have been conducted in accordance with the proposed GAP. Samples arising from trials I/CZ10/GR02 and F/CZ10/GR02 underwent processing steps in order to obtain must, young wine and bottled wine to be analysed.

The field and processing phase has been performed by Anadiag in France and by RESEARCH CENTRE "E. GAGLIARDINI - SIPCAM S.p.A in Italy under phase reports no. CREG2117 and CREG2120, the analytical phase has been performed at RESEARCH CENTRE "E. GAGLIARDINI - SIPCAM S.p.A in Italy under reports no. CREG2117 and CREG2120 with a method validated according to SANCO/825/00 rev. 8.1 and SANCO/3029/99 rev. 4 under reports no. CREG2117 and CREG2120 for Zoxamide and report no. RAU/038 for cymoxanil.

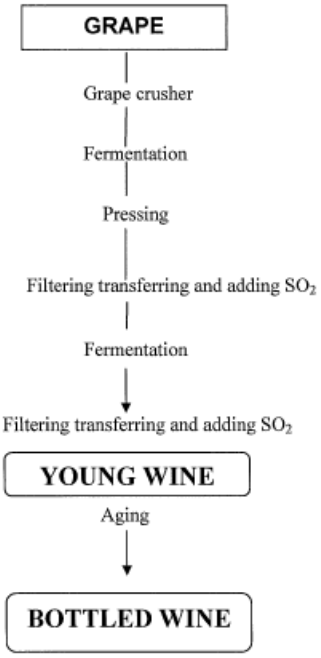


Figure A 1: Flowchart on processed grapes

A sample of bunches was taken and analysed straight after deep freezing. For Cymoxanil the sample was extracted by Ultra-Turrax (bunch samples) or by shaking (processed samples) with ethyl acetate, purified by liquid-liquid partition and analysed by gas chromatography with a Nitrogen Phosphorus Detector (GC/NPD). Zoxamide was determined using an internal analytical method that consisted of a solvent extraction by Ultraturrax (bunch samples) or by shaking (processed samples). The extract containing the active ingredient was cleaned up by liquid-liquid partition and by SPE chromatography, then analysed by gas chromatography equipped with an ECD. The Limit of Quantification (LOQ) was 0.05 mg/kg for Cymoxanil on bunches samples and 0.01 mg/kg on processed samples; for Zoxamide the LOQ was 0.01 mg/kg for all matrices.

Results and discussion

For cymoxanil residues were below the method limit of quantification (LOQ = 0.05 mg/kg for bunches and 0.01 mg/kg for processed samples) in all the specimens analysed (treated, untreated and processed), except for young wine residues were slightly above the LOQ.

For zoxamide the highest residues observed were 0.8598 mg/kg in bunches, 0.0346 mg/kg in must, 0.0228 mg/kg in young wine and 0.0186 mg/kg in bottled wine.

For cymoxanil several other trials are available that were conducted with different formulations. For more information, Tier I summaries of all available trials can be found in Appendix 4 of this document. None of these additional trials were assessed as part of this evaluation as no additional trials are necessary (all residues of cymoxanil in the trials presented above are < LOQ and therefore authorisation can be recommended on the basis of the four trials submitted with this application).

Consideration based on trials submitted with this application:

The applicant states that for zoxamide numerous additional supervised residue trials on grapes treated with a WG formulation are available on EU level and have been evaluated during Annex I inclusion of this active substance. They made reference to a range of trials however the only ones that are relevant to the proposed NEU GAP are the trials done to support a maximum of 6 applications each of 0.06 to 0.24 kg as/ha (based

on an application rate of 15 kg as/hL) at a minimum interval of 7-10 days up to BBCH 89 of grapes and a PHI of 56 days (although the majority have results for shorter PHIs as well – close to or at 28 days) in Northern EU. These trials are presented in Addendum 1 to the DAR for Zoxamide, dated June 2002, Annex B, B.7.6 (see page 24 and 25 of 37). It is possible that these trials are supportive of the proposed GAP however the details given in the DAR are inadequate (e.g. range of application rate: 0.06 to 0.24 kg as/ha) and appear to include a mistake with regard to the expression of the application rate – the units are assumed to be g a.s./hL and not kg as/hL. The original studies are no longer available to the UK (HSE) and so it is not possible for HSE to know if they are acceptable. It would therefore not be possible to recommend authorisation on the basis of these trials.

Consideration based on data evaluated in the DAR:

Authorisation of GF 1045‡ (containing zoxamide and mancozeb) was considered and recommended under HSE job number 2008/00751 (see residues covering file note at HSE ref. W 001348947 (enc 73) and registration report at HSE ref. W 001213020 (enc 6). The proposed GAP and the GAP considered for active substance approval were similar and it was concluded that the proposed use of zoxamide on grapes could be recommended on the basis of the DAR.

‡: 'Electis 75 WG' was also authorised under COP 2008/00797, based on this evaluation.

In this case the proposed GAP in NEU is 5 × 0.1485 kg as/ha (7-day minimum interval) with a PHI of 28 days (up to GS 89). The GAP considered for active substance approval was 10 × 0.15 kg as/ha with a PHI of 7 days. The proposed GAP is within the active substance approval GAP and is therefore supported by the residues trials evaluated for active substance approval of zoxamide (i.e. the same conclusion as under HSE job number 2008/00751 can be drawn).

A summary of the results is given in the following table.

(Romanini M. 2011a,b)

Table A 12: Summary of the study 1 trials on grapes / Southern EU

Reference:	Romanini, M., 2011b, CREG2117		
GLP:	Yes	Sample storage conditions:	≤ -18 °C for residues analysis
Crop/crop group:	Grapes	Analytical method:	CREG2120 for Zoxamide, RAU/038 for Cymoxanil
Indoor/Outdoor:	Outdoor	Limit of Quantification (mg/kg):	Zoxamide: 0.01 mg/kg Cymoxanil: 0.05 mg/kg
Formulation:	WG	Limit of Detection (mg/kg):	Zoxamide: 0.0051 mg/kg Cymoxanil: 0.0290 mg/kg (bunches), 0.0058 mg/kg (processed samples)
Content of active substance (g/kg or g/l):	33% Zoxamide + 33% Cymoxanil	Residues calculated as:	zoxamide, cymoxanil

Trial No./ Location/ EU zone/ Year	Commodity/ Variety	Date of 1.Sowing or planting 2.Flowering 3. Harvest	Application rate per treatment			Dates of treatment or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)		PHI (days)	Details on trial
			g a.s./ ha	Water (l/ha)	g a.s./hl				Zoxamide	Cymoxanil		
	(a)	(b)				(c)					(d)	(e)
I/CZ10/GR01 15058 Viguzzolo (AL) Italy S-EU, 2010	Grape/ Barbera	1) 1997 2) N.A. 3) End of September	149.49 147.51 151.47 146.52 152.46	1006.67 993.33 1020.00 986.67 1026.67	14.85 14.85 14.85 14.85 14.85	06/08/2010 12/08/2010 19/08/2010 26/08/2010 02/09/2010	84	Grape bunch	0.2594	< LOQ (N.D.)	28	LOQ=0.01 mg/kg (Zoxamide) /0.05 mg/kg (Cymoxanil) LOD Zoxamide =0.0051 mg/kg LOD Cymoxanil= 0.0290 mg/kg (bunches), 0.0058 mg/kg (processed samples) Analytical method validated in study CREG2117 for Zoxamide) and RAU/038 for Cymoxanil Max. Storage interval between sampling and analysis:8 days

Trial No./ Location/ EU zone/ Year	Commodity/ Variety	Date of 1.Sowing or planting 2.Flowering 3. Harvest	Application rate per treatment			Dates of treatment or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)		PHI (days)	Details on trial
			g a.s./ ha	Water (l/ha)	g a.s./hl				Zoxamide	Cymoxanil		
	(a)	(b)				(c)					(d)	(e)
I/CZ10/GR02 26857 Salerano sul Lambro (LO) Italy S-EU, 2010	Grape/ Barbera	1) 10/02/1993 2) 10/06/2010 3) 1st decade of October	145.47 141.68 149.26 148.95 150.93	685.71 667.86 703.57 702.14 711.43	21.21 21.21 21.21 21.21 21.22	18/08/2010 24/08/2010 30/08/2010 06/09/2010 13/09/2010	81	Grape bunch Must Young wine Bottled wine	0.2748 0.0166 0.0191 0.0186	< LOQ (N.D.) < LOQ (N.D.) < LOQ (N.D.) < LOQ (N.D.)	28	LOQ=0.01 mg/kg (Zoxamide) /0.05 mg/kg (Cymoxanil) LOD Zoxamide =0.0051 mg/kg LOD Cymoxanil= 0.0290 mg/kg (bunches), 0.0058 mg/kg (processed samples) Analytical method validated in study CREG2117 for Zoxamide) and RAU/038 for Cymoxanil) Max. Storage interval between sampling and analysis: 8 days
I/CZ10/GR03 64013 Corropoli (TE) Italy S-EU, 2010	Grape/ Montepulciano d'Abruzzo	1) 1999 2) N.A. 3) 30/09/2010	145.53 155.10 141.24 148.17 155.43	980.00 1045.33 951.33 997.33 1046.67	14.85 14.84 14.85 14.86 14.85	05/08/2010 12/08/2010 19/08/2010 26/08/2010 02/09/2010	85	Grape bunch	0.0733	< LOQ (N.D.)	28	LOQ=0.01 mg/kg (Zoxamide) /0.05 mg/kg (Cymoxanil) LOD Zoxamide =0.0051 mg/kg LOD Cymoxanil= 0.0290 mg/kg (bunches), 0.0058 mg/kg (processed samples) Analytical method validated in study CREG2117 for Zoxamide) and RAU/038 for Cymoxanil Max. Storage interval between sampling and analysis: 8 days

Trial No./ Location/ EU zone/ Year	Commodity/ Variety	Date of 1.Sowing or planting 2.Flowering 3. Harvest	Application rate per treatment			Dates of treatment or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)		PHI (days)	Details on trial
			g a.s./ ha	Water (l/ha)	g a.s./hl				Zoxamide	Cymoxanil		
	(a)	(b)				(c)					(d)	(e)
I/CZ10/GR04 64010 Morro D'Oro (TE) Italy S-EU, 2010	Grape/ Montepulciano d'Abruzzo	1) 1989 2) N.A. 3) 30/09/2010	151.80 151.14 154.77 149.49 154.44	1021.33 1016.67 1041.33 1006.67 1040.00	14.86 14.87 14.86 14.85 14.85	05/08/2010 12/08/2010 19/08/2010 26/08/2010 02/09/2010	85	Grape bunch	0.0883	< LOQ (N.D.)	28	LOQ=0.01 mg/kg (Zoxamide) /0.05 mg/kg (Cymoxanil) LOD Zoxamide =0.0051 mg/kg LOD Cymoxanil= 0.0290 mg/kg (bunches), 0.0058 mg/kg (processed samples) Analytical method validated in study CREG2117 for Zoxamide) and RAU/038 for Cymoxanil Max. Storage interval between sampling and analysis: 8 days

N.D. not detectable

Table A 13: Summary of the study 1 trials on grapes / Northern EU

Reference:	Romanini, M., 2011b, CREG2120		
GLP:	Yes	Sample storage conditions:	≤ -18 °C for residues analysis
Crop/crop group:	Grapes	Analytical method:	CREG2120 for Zoxamide, RAU/038 for Cymoxanil
Indoor/Outdoor:	Outdoor	Limit of Quantification (mg/kg):	Zoxamide: 0.01 mg/kg Cymoxanil: 0.05 mg/kg
Formulation:	WG	Limit of Detection (mg/kg):	Zoxamide: 0.0051 mg/kg Cymoxanil: 0.0252 mg/kg (bunches), 0.0050 mg/kg (must and young wine), 0.0058 mg/kg (bottled wine)
Content of active substance (g/kg or g/l):	33% Zoxamide + 33% Cymoxanil	Residues calculated as:	Zoxamide, cymoxanil

Trial No./ Location/ EU zone/ Year	Commodity/ Variety	Date of 1.Sowing or planting 2.Flowering 3. Harvest	Application rate per treatment			Dates of treatment or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)		PHI (days)	Details on trial
			g a.s./ ha	Water (l/ha)	g a.s./hl				Zoxamide	Cymoxanil		
G/CZ10/GR01 79356 Eichstetten, Baden-Würtenberg Germany N-EU, 2010	Grapevine/ Chardonnay	1) 1992 2) N.A. 3) End of September	152.4 152.4 146.7 145.8 149.7	513 513 494 491 504	29.7 29.7 29.7 29.7 29.7	06/08/2010 13/08/2010 19/08/2010 25/08/2010 01/09/2010	83-85	Bunch	0.8598	< LOQ (N.D.)	28	LOQ =0.01 mg/kg (Zoxamide) /0.05 mg/kg (Cymoxanil) LOD Zoxamide =0.0051 mg/kg LOD Cymoxanil = 0.0252 mg/kg (bunches), 0.0050 mg/kg (must and young wine), 0.0058 mg/kg (bottled wine) Analytical method validated in study CREG2120 for Zoxamide and RAU/038 for Cymoxanil Max. Storage interval between sampling and analysis: 15-23 days

Trial No./ Location/ EU zone/ Year	Commodity/ Variety	Date of 1.Sowing or planting 2.Flowering 3. Harvest	Application rate per treatment			Dates of treatment or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)		PHI (days)	Details on trial
			g a.s./ ha	Water (l/ha)	g a.s./hl				Zoxamide	Cymoxanil		
F/CZ10/GR02 67117 Furdenheim, Alsace Northern France N-EU, 2010	Grapevine/ Auxerrois	1) 1992 2) N.A. 3) last decade of September	142.3 155.9 145.8 155.3 155.3	479 525 491 523 523	29.7 29.7 29.7 29.7 29.7	27/07/2010 03/08/2010 09/08/2010 17/08/2010 24/08/2010	81	Bunch Must Young wine Bottled wine	0.2980 0.0346 0.0228 < 0.01 (0.0089)	< LOQ (N.D.) < 0.01 (N.D.) 0.0151 < 0.01 (N.D.)	28	LOQ =0.01 mg/kg (Zoxamide) /0.05 mg/kg (Cymoxanil) LOD Zoxamide =0.0051 mg/kg LOD Cymoxanil = 0.0252 mg/kg (bunches), 0.0050 mg/kg (must and young wine), 0.0058 mg/kg (bottled wine) Analytical method validated in study CREG2120 for Zoxamide and RAU/038 for Cymoxanil Max. Storage interval between sampling and analysis: 15-23 days
F/CZ10/GR03 67990 Osthoffen, Alsace Northern France N-EU, 2010	Grapevine/ Pinot Blanc	1) 1998 2) N.A. 3) last decade of September	155.6 153.8 142.3 146.7 155.6	524 518 479 494 524	29.7 29.7 29.7 29.7 29.7	27/07/2010 03/08/2010 09/08/2010 17/08/2010 24/08/2010	81	Bunch	0.4586	< LOQ (N.D.)	28	LOQ =0.01 mg/kg (Zoxamide) /0.05 mg/kg (Cymoxanil) LOD Zoxamide =0.0051 mg/kg LOD Cymoxanil = 0.0252 mg/kg (bunches), 0.0050 mg/kg (must and young wine), 0.0058 mg/kg (bottled wine) Analytical method validated in study CREG2120 for Zoxamide and RAU/038 for Cymoxanil Max. Storage interval between sampling and analysis: 15-23 days

Trial No./ Location/ EU zone/ Year	Commodity/ Variety	Date of 1.Sowing or planting 2.Flowering 3. Harvest	Application rate per treatment			Dates of treatment or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)		PHI (days)	Details on trial
			g a.s./ ha	Water (l/ha)	g a.s./hl				Zoxamide	Cymoxanil		
F/CZ10/GR04 49540 Martigne-Briand, Pays de la Loire Northern France N-EU, 2010	Grapevine/ Grolleau	1) 10/03/1950 2) N.A. 3) End of September	162.5 144.9 155.6 161.6 146.7	547 488 524 544 494	29.7 29.7 29.7 29.7 29.7	02/08/2010 09/08/2010 16/08/2010 23/08/2010 30/08/2010	83	Bunch	0.2656	< LOQ (N.D.)	28	LOQ =0.01 mg/kg (Zoxamide) /0.05 mg/kg (Cymoxanil) LOD Zoxamide =0.0051 mg/kg LOD Cymoxanil = 0.0252 mg/kg (bunches), 0.0050 mg/kg (must and young wine), 0.0058 mg/kg (bottled wine) Analytical method validated in study CREG2120 for Zoxamide and RAU/038 for Cymoxanil Max. Storage interval between sampling and analysis: 15-23 days

N.D. not detectable

Additional / new studies were performed with zoxamide products applied at a similar GAP as intended, but partially with a higher application number (i.e. 5) than intended (i.e. 3) – and can therefore be considered as a worst-case.
These additional / new studies are submitted for evaluation within this dossier. They have not yet been assessed beforehand.

A 2.1.3.1.2 Study 2

Comments of zRMS:	The study is acceptable. 2 grapes residue trials were conducted in NEU. The objective of the study was to determine the magnitude of residues of zoxamide (R & S and sum) and its metabolites [RH-150721 (R & S), RH-141288 (R & S), RH-129151 (A and B), RH-24549 and RH-141452] in raw agricultural commodity specimens of grapevine (RAC bunches) and processed fractions after five applications of Zoxium 240 SC. Target application rate was 0.75 L/ha and target application timing was 60 (± 4), 52 (± 4), 44 (± 3), 36 (± 3) and 28 (± 2) days before harvest. Two deviations were observed during the Analytical Phase of the Study. They were all considered without impact on the study results. The analytical method was described and fully validated according to guidelines SANCO/825/00 rev. 8.1 and SANCO/3029/99 rev. 4 in a previous study (LabAnalysis Study BPL-STUDY-18-000085).

Reference:	**KCA 6.3/03**
Report:	Peterek, S., 2020: Magnitude of the residues of zoxamide and its metabolites in grapevine (RAC bunches) and processed fractions, following applications of Zoxium 240 SC, Northern Europe – 2018[9] Gowan Crop Protection Ltd., UK Staphyt GmbH, Germany, Report No. AB2-18-35355, GLP, Not published
Guideline(s):	OECD TG 509 (2009) SANCO 7029/VI/95 rev. 5 (1997) OECD TG 508 (2008) SANCO 7035/VI/95 rev. 5 (1997)
Deviations:	Due to response instability in analytical sequence with both solvent and matrix standards, the matrix effects were not calculated. This deviation from the study plan is regarded to be not relevant for the integrity of the study since the analysis of residues has been performed by matrix matched standards. Mean recoveries below 70 % were found for RH-129151 A and RH-129151 B in young wine at 0.05 mg/L, while the corresponding mean recoveries at LOQ were in the range 70 - 110 %. However, as the residues of these analytes in young wine samples were found below the LOQ, the results were not corrected by taking into account a recovery factor. For analytes/matrices combination with mean recoveries > 110 %, the high recovery is probably due to the interconversion among the analytes, present at the same time and in the same concentration in the recovery extracts, while in the corresponding "real" samples this condition does not occur. The recovery factor was not applied to the residues results in order to remain the worst-case.

[9] Confirmatory study for zoxamide currently being evaluated by RMS (Latvia).

GLP:	Yes
Acceptability:	Yes

Materials and methods

Test item (Lot/Batch No.)	GWN 9790 EU / Zoxium 240 SC (SIPAL7001)
Active substance content (nominal)	240 g/L zoxamide (nominal), 239.47 g/L (analysed) (R/S zoxamide ratio: 50/50)
Expiry date	May 2020

The magnitude of residues of zoxamide and its metabolite RH-141452 in raw agricultural commodity specimens of grapes (bunches) and processed fractions has been determined in one harvest and one decline trial performed in Northern Europe (Offenburg and Merdingen in Germany) in 2018. In addition, the metabolites RH-150721, RH-24549, RH-129151 and RH-141288 have been analysed in processed commodities (filtered fresh and pasteurised juice and must, young and bottled red and white wine).

Two plots were established per trial: 1 plot (control) was left untreated, another plot was treated five times with each 0.75 L/ha GWN 9790 EU (180 g zoxamide/ha) at a 7-8 (+1) day's interval with the last application 28 days and 30 days (= which is within the 25 % range of the intended phi) before harvest. The test item has been applied with airblast sprayers to reflect common agricultural practice.

The raw agricultural commodities (grape bunches) for processing were collected and stored in Styrofoam boxes with frozen gel packs and shipped for processing at A.S.T.R.A. in Italy (report phase no. PTR_18_GOW_VITVI-WG_Z-P). All other specimens for residue analysis were frozen down within 4 hours after sampling and kept frozen at ≤ -18°C until analysis at Renolab S.r.l. in Italy.

During processing, limpid juice samples (specimens collected before and after the pasteurization of filtered juice) and samples from red wine and white wine making (specimens collected after grapes crushing (must), wine filtration (young wine) and an ageing period of 2-3 months (bottled wine)) were collected. The processed specimens were frozen down and kept frozen until analysis.

Limpid juice (before and after pasteurization): Both red and white grapes were processed into limpid juice. At receipt, the grapevine bunches were crushed, then the resulting product (flesh and peels) was softly pressed with a specific equipment to obtain the raw must. Stalks and wet pomace were discarded. Sulphur dioxide (preservative) and pectolytic enzyme (clarifying agent) were added to the raw must in order to obtain the settling of the coarse lees during storage in a cold room for a period of at least 24 hours. The resulting product was clarified a second time at low temperature using bentonite, the supernatant was racked. The semi-finished juice was filtered using specific filter capsules (up to 1.2 µm) in order to obtain a limpid/shiny product. The limpid juice before the pasteurization phase was packaged using suitable glass bottles. The remaining bottles of limpid juice were pasteurized at temperature >80°C for 30 minutes.

Red wine making (must, young wine and bottled wine): The stalks were removed from the grapevine bunches and the berries were softly crushed to obtain raw must. The specimens of raw must were collected. Some oenological products (sulphur dioxide as preservative, trade yeast and yeast activators) were added to the raw must. The must fermentation occurred in presence of the peels in order to extract the red colour from the latter. The drawing-off phase (separation of the macerated peels from the fermenting must) was carried out at 8-9° of alcohol, then the sugar remaining in the must was transformed into alcohol in the following days. To the raw wine three oenological products (sulphur dioxide as preservative; gelatine and bentonite as clarifying agents) were added at the end of the fermentation. The product was stored in a cold room (-5 °C) for at least 3 weeks for total precipitation of potassium bitartrate (wine tartaric stabilization) and decanted. The wine was stabilized by the addition of sulphur dioxide as preservative (at least 15 mg/L free SO_2). The wine was filtered using specific filter capsules (up to 0.65 µm). The filtered wine (young

wine) was packaged in glass bottles. Bottles of finished wine were stored in a cold room for ageing during a 2 to 3 months period. At the end of this period, the aged wine of all bottles was poured in a stainless-steel container to obtain a single homogeneous mass.

White wine (must, young wine and bottled wine): At receipt, the grapevine bunches were crushed and the resulting product (flesh and peels) softly pressed to obtain the raw must. Stalks and wet pomace were discarded. Some oenological products (sulphur dioxide as preservative; potassium caseinate and bentonite as clarifying agents; trade yeast; yeast activators) were added to the raw must to have a fast start of the fermentation process and a complete transformation of the sugars into alcohol and to obtain the dynamic clarification of the must during the fermentation process. The must fermentation occurred in absence of the fruit peels. Two oenological products (sulphur dioxide as preservative and bentonite as clarifying agent) were added. The product was stored in a cold room (-5 °C) for at least 3 weeks to obtain a total precipitation of potassium bitartrate (wine tartaric stabilization). The colloidal substances were decanted. The stabilized wine obtained at the end of this phase was enriched with sulphur dioxide (preservative; at min. 15 mg/L of free SO_2). The turbid wine was filtered using specific capsules (up to 0.65 µm) to obtain a shiny finished wine. The filtered wine (young wine) was packaged I glass bottles. The remaining bottles of finished wine were stored in a cold room for ageing (at least 2-3 months). At the end of this period, the aged wine of all bottles was poured in a stainless-steel container to obtain a single homogeneous mass.

The analytical phase has been performed at Renolab S.r.l. in Italy under report no. 18097-01R with a method developed and validated according to SANCO/825/00 rev. 8.1 and SANCO/3029/99 rev. 4 under report no. BPL-STUDY-18-000085 and applied at Renolab S.r.l.. The method has been (re-)validated according to guidelines SANCO/825/00 rev. 8.1 and SANCO/3029/99 rev. 4 during the course of this study.

Results and discussion

The concentration of zoxamide (R, S and sum) and its metabolites RH-150721 (R, S and sum), RH-141288 (R, S and sum), RH-129151 (A and B), RH-24549 and RH-141452 were determined in grapes and/or processed specimens.

For metabolite RH-129151 the correlation between the absolute configuration of the enantiomer (R) or (S) and the corresponding chromatographic peaks was not available; therefore, the first eluted peak was assigned as RH-129151 (A) and the second eluted peak as RH-129151 (B).

The metabolite RH-141452, which was known to form conjugates with matrix molecules (e.g. sugars), was released in an additional hydrolysis step to establish total fractions in addition to the free fractions in the matrices.

The limit of quantification (LOQ) was set at 0.01 mg/kg for zoxamide, RH-150721, RH-129151 and RH-141288 (sum of enantiomers), RH-24549, free RH-141452 and total RH-141452, and 0.005 mg/kg for single isomers. The limit of determination (LOD) was given as 0.003 mg/kg for RH-150721, RH-129151 and RH-141288 (sum of enantiomers) and RH-24549, free RH-141452 and total RH-141452 and 0.0015 mg/kg for single isomers.

No residues of zoxamide or any metabolites were found above LOQ in all untreated field specimens. A summary of the results from the treated plots is given in the following table.

Transfer factors were calculated for zoxamide with reliable residue concentrations (≥10x LOQ) in the raw agricultural commodity (RAC) and (≥ LOQ) in the processed commodity as the ratio between mean residue levels in treated grapevine bunches and the processed fractions. Only samples harvested around the intended phi of 28 days were taken into account. In line with OECD TG 508, no transfer factors are reported here for metabolites of zoxamide appearing at or below the LOQ in the RAC.

Table A 14: Processing factors – wine grapes

Substance	Processed commodity	Processing factor	
		Red wine	White wine
Zoxamide	Fresh juice	0.03	0.04
	Must	1.1	0.4
	Young wine	0.07	0.2
	Bottled wine	0.04	0.1

In case of residues > LOQ for isomeric compounds, they are demonstrating a stable chiral centre of zoxamide and its residues.

(Peterek S. 2020)

Table A 15: Summary of the study 2 trials on grapes / Northern EU

Reference:	Peterek S. 2020, AB2-18-35355		
GLP:	Yes	Sample storage conditions:	≤ -18°C for residues analysis
Crop/crop group:	Grapes / fruiting crops	Analytical method:	STUDY-18-000085
Indoor/Outdoor:	Outdoor	Limit of Quantification (mg/kg):	0.01 mg/kg (sum), 0.005 mg/kg (for each isomer)
Formulation:	SC	Limit of Detection (mg/kg):	0.003 mg/kg (sum), 0.0015 mg/kg (for each isomer)
Content of active substance (g/kg or g/l):	240 g/L Zoxamide	Residues calculated as:	Zoxamide (sum). (R)-Zoxamide. (S)-Zoxamide, RH-150721 (sum), (R)-RH-150721, (S)-RH-150721, RH-129151 (sum), (R)-RH-129151, (S)-RH-129151, RH-141288, (R)-RH-141288, (S)-RH141288 and RH24549, RH-141452

Trial No. Location EU zone Year	Com-modity/ Variety	Date of 1. Sowing or Planting 2. Flowering 3. Harvest	Application rate per treatment			Dates of treat-ment(s) or no of treat-ment(s) and last date	Gro wth stage at last treat-ment or date	Portion ana-lyzed	Residues (mg/kg)															PHI (days)	Remarks
			kg as/ha	Water (L/ha)	kg as/ hl				Zoxamide (sum)	Zoxamide (R)	Zoxamide (S)	RH-150721 (sum)	(R)-RH-150721	(S)-RH-150721	RH-129151 (sum)	RH-129151 (A)	RH-129151 (B)	RH-141288 (sum)	(R)-RH-141288	(S)-RH-141288	Total RH-141452	Free RH-141452	RH-24549		
AB2-18-35355 - D01 77654 Offenburg Baden-Württem-berg Germany N-EU-2018	Grapevi ne EPPO code: VITVI Pinot Noir	1) 01/05/1998 2) 22/05/2018-05/08/2018 3) 20/09/2018	0.181 0.179 0.179 0.179 0.180	0.754 0.749 0.749 0.748 0.751	-	20/07/2018 27/08/2018 04/08/2018 11/08/2018 20/08/2018	81 81 83 85 85	Bunches	0.94	0.48	0.47	NR	NR	NR	NR	NR	NR	NR	NR	NR	0.016	<LOQ (0.004)	<LOQ (0.003)	28	#
								Limpid juice *	0.030	0.015	0.015	ND	ND	ND	ND	ND	ND	ND	ND	ND	<LOQ (0.0055)	ND	ND	-	
								Limpid juice **	<LOQ (0.005)	<LOQ (0.002)	<LOQ (0.002)	0.020	0.010	0.011	ND	ND	ND	ND	ND	ND	<LOQ (0.0060)	ND	ND	-	
								Must	1.00	0.50	0.50	<LOQ (0.004)	<LOQ (0.002)	<LOQ ((0.002)	<LOQ (0.009)	<LOQ (0.004)	<LOQ (0.004)	<LOQ (0.005)	<LOQ (0.003)	<LOQ (0.003)	0.010	ND	ND	-	
								Young wine	0.070	0.036	0.034	0.013	0.0062	0.0070	<LOQ (0.002)	<LOQ (0.002)	N.D.	<LOQ (0.005)	<LOQ (0.002)	<LOQ (0.002)	0.013	<LOQ (0.0060)	ND	-	
								Bottled wine	0.045	0.020	0.025	0.026	0.012	0.014	ND	ND	ND	<LOQ (0.004)	<LOQ (0.002)	<LOQ (0.002)	0.013	<LOQ (0.004)	ND	-	

Trial No. Location EU zone Year	Commodity/ Variety	Date of 1. Sowing or Planting 2. Flowering 3. Harvest	Application rate per treatment: kg as/ha	Application rate per treatment: Water (L/ha)	Application rate per treatment: kg as/ hl	Dates of treatment(s) or no of treatment(s) and last date	Growth stage at last treatment or date	Portion analyzed	Residues (mg/kg): Zoxamide (sum)	Zoxamide (R)	Zoxamide (S)	RH-150721 (sum)	(R)-RH-150721	(S)-RH-150721	RH-129151 (sum)	RH-129151 (A)	RH-129151 (B)	RH-141288 (sum)	(R)-RH-141288	(S)-RH-141288	Total RH-141452	Free RH-141452	RH-24549	PHI (days)	Remarks
AB2-18-35355 DE02 79291 Merdingen Baden-Württemberg Germany N-EU, 2018	Grapevine EPPO code: VITVI Müller-Thurgau	1) 2001 2) 22/05/2018 to 05/06/2018 3) 12/09/2018	0.179 0.179 0.179 0.179 0.177	0.747 0.748 0.747 0.748 0.741	-	11/07/2018 19/07/2018 26/07/2018 03/08/2018 11/08/2018	79 81 83 83 85	Bunches	1.34	0.67	0.67	NR	NR	NR	NR	NR	NR	NR	NR	NR	<LOQ (0.008)	<LOQ (0.005)	<LOQ (0.004)	0	#
								Bunches	1.43	0.70	0.73	NR	NR	NR	NR	NR	NR	NR	NR	NR	0.016	<LOQ (0.008)	<LOQ (0.004)	6	
								Bunches	1.09	0.53	0.56	NR	NR	NR	NR	NR	NR	NR	NR	NR	<LOQ (0.0098)	<LOQ (0.004)	ND	14	
								Bunches	1.03	0.52	0.52	NR	NR	NR	NR	NR	NR	NR	NR	NR	0.015	<LOQ (0.005)	ND	22	
								Bunches	1.22	0.62	0.60	NR	NR	NR	NR	NR	NR	NR	NR	NR	0.021	<LOQ (0.007)	<LOQ (0.005)	30	
								Limpid juice *	0.051	0.025	0.026	ND	ND	ND	ND	ND	ND	ND	ND	ND	<LOQ (0.006)	ND	N.D.	-	
								Limpid juice **	<LOQ (0.003)	<LOQ (0.002)	<LOQ (0.003)	0.027	0.013	0.014	ND	ND	ND	ND	ND	ND	<LOQ (0.008)	<LOQ (0.004)	<LOQ (0.003)	-	
								Must	0.47	0.24	0.23	<LOQ (0.003)	<LOQ (0.002)	<LOQ (0.002)	<LOQ (0.004)	<LOQ (0.002)	<LOQ (0.002)	ND	ND	ND	<LOQ (0.007)	ND	ND	-	
								Young wine	0.18	0.10	0.082	0.031	0.014	0.017	<LOQ (0.005)	<LOQ (0.003)	<LOQ (0.002)	<LOQ (0.004)	<LOQ (0.002)	<LOQ (0.002)	<LOQ (0.007)	<LOQ (0.003)	ND	-	
								Bottled wine	0.15	0.082	0.064	0.059	0.026	0.033	N.D.	N.D.	N.D.	<LOQ (0.004)	<LOQ (0.002)	<LOQ (0.002)	<LOQ (0.007)	<LOQ (0.004)	ND	-	

\# LOQ = 0.010 mg/kg for zoxamide (sum), RH-150721 (sum), RH-1292151 (sum), RH-141288 (sum), RH-141452 and RH-24549, and 0.005 mg/kg for single isomers.
LOD = 0.003 mg/kg for zoxamide (sum), RH-150721 (sum), RH-1292151 (sum), RH-141288 (sum), RH-141452 and RH-24549, and 0.0015 mg/kg for single isomers.
Analytical method validated in study BPL-STUDY-18-000085 and during the analytical phase 18097-01R of this study
Max. Storage interval between sampling and analysis: 458 – 495 days

ND = not detectable, NR = not relevant

* pre-pasteurisation, ** post-pasteurisation

A 2.1.3.1.3 Study 3

Comments of zRMS:	The studies are acceptable. The analytical determination was carried out using a HPLC-MS/MS method validated according to SANCO/825/00 rev.8.1 and SANCO/3029/99 rev. 4 guidelines.

Reference:	**KCA 6.3/04**
Report	Sala, A., 2020: Determination of zoxamide and its metabolites in raw agricultural commodity wine grape (berries) and processed fractions (juice, wine) following five applications of Zoxium 240 SC (GWN-9790 EU) in open field condition, 2 harvest trials, Northern Europe, year 2017 - final report amendment no. 1[10] Gowan Crop Protection Ltd, UK Lab Analysis, Italy, Report No. BPL-STUDY-19-000041, GLP, Not published
and	
Reference:	**KCA 6.3/05**
Report	Thomas-Delille, E., 2020: Determination of zoxamide and its metabolite RH-150721 residues in wine grape and processed fractions following five foliar applications with Zoxium 240 SC under field conditions in Northern Europe in 2017 – amended final report Gowan Crop Protection Ltd, UK Anadiag, France, Report No. B7284, GLP, Not published
Guideline(s):	OECD TG 509 (2009) SANCO 7029/VI/95 rev. 5 (1997) OECD TG 508 (2008) 7035/VI/95 rev. 5 (1997) OECD TG 507 (2007) SANCO7525/VI/95 rev. 10.2 (2016) OECD 96 (2008)
Deviations:	Deviation on trial B7284 BW1: Due to the mechanically harvest of the vineyard by the farmer, the field principal investigator anticipated the sampling at harvest and performed is 20 days after last application instead of 28 (±2) days after last application, as required by the study plan. Deviation on trial B7284 BW1: At sampling the minimum weight of 60 kg required for the specimens B7284 BW1/UH/P and B7284 BW1/TH/P was not reached; it was only 58.438 kg and 58.091 kg, respectively. This deviation has no impact on the integrity of the data since the weights were sufficient to perform the processing phase. Deviation on trial B7284 CZ1: The samples for processing were shipped under ambient conditions instead of under refrigerated conditions as required by the study plan due to organisational reason. However, the sanitary status of

[10] Confirmatory study for zoxamide currently being evaluated by RMS (Latvia).

	the specimens was good at receipt. The processing phase of trial B7284 BW1 was started 14 days after receipt of the samples instead of the next day after receipt at the samples, as required by the study plan. However, according to the analytical results, this deviation was regarded acceptable.
GLP:	Yes
Acceptability:	Partially

Materials and methods

Test item (Lot/Batch No.)	GWN 9790 EU/Zoxium 240 SC (SB 2401)
Active substance content (nominal)	240 g/L zoxamide (nominal), 232 g/L (analysed) (R/S zoxamide ratio: 50/50)
Expiry date	April 2018

The magnitude of residues of zoxamide and its metabolite RH-141452 in raw agricultural commodity specimens of grapes (bunches) and processed fractions has been determined in two harvest trials performed in Northern Europe (Germany and Czech Republic) in 2017. In addition, the metabolites RH-150721, RH-24549, RH-129151 and RH-141288 have been analysed in processed commodities (juice, young and bottled red and white wine).

In each trial one plot was treated five times with Zoxium 240 SC at an application rate of 0.75 L/ha, one plot remained untreated. The applications were made at an interval of 7-8 days with the last application 26 days before harvest in trial B7284 CZ1 (which is within a 25% deviation from the intended phi of 28 days) and 20 days before harvest in trial B7284 BW1 (which is outside a 25% deviation from the intended phi of 28 days). Samples were taken both for residues analysis and for processing.

Residues samples were stored in the freezer within 4 hours and kept frozen at ≤ -18°C during shipment and storage until extraction.

The field phase has been performed by Anadiag France under phase report no. B7284. The samples for processing were brought by car from the field test sites in Germany and in Czech Republic to the processing facility of Anadiag in Haguenau, France. Here, the samples were processed as detailed in the following figures. The analytical phase has been performed at LabAnalysis in Italy under report no. BPL-STUDY-19-000041 with a method validated according to SANCO/825/00 rev. 8.1 and SANCO/3029/99 rev. 4 under report no. BPL-STUDY-18-000085.

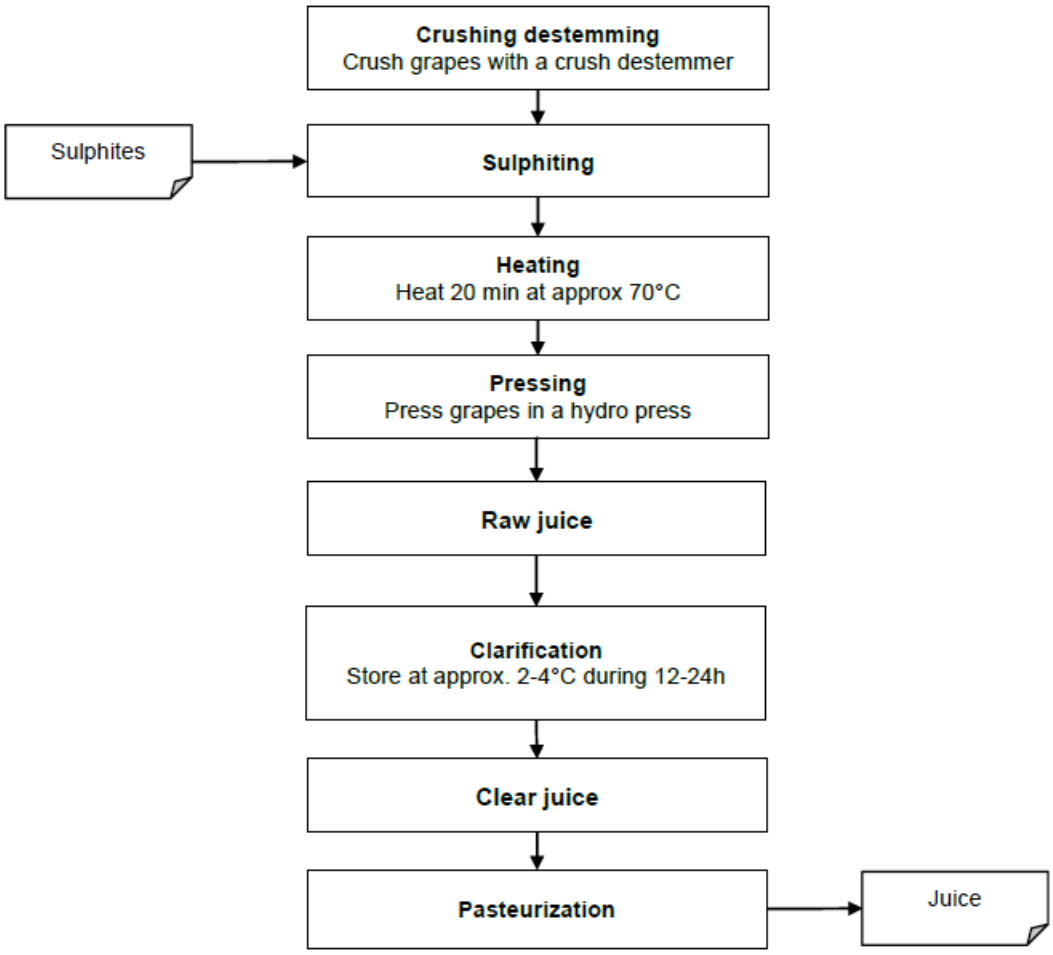


Figure A 2: Processing of white grape juice

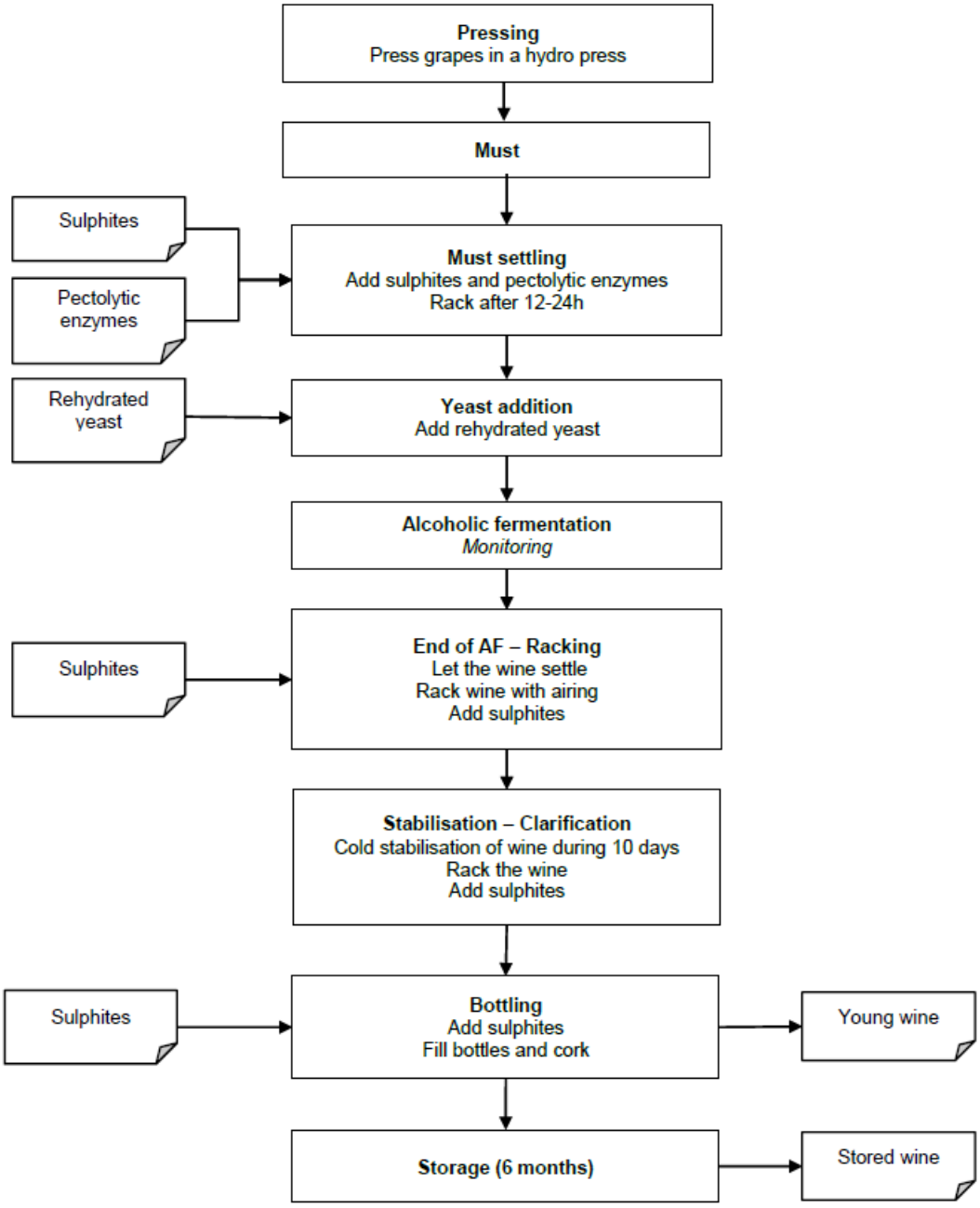


Figure A 3: Processing of white wine

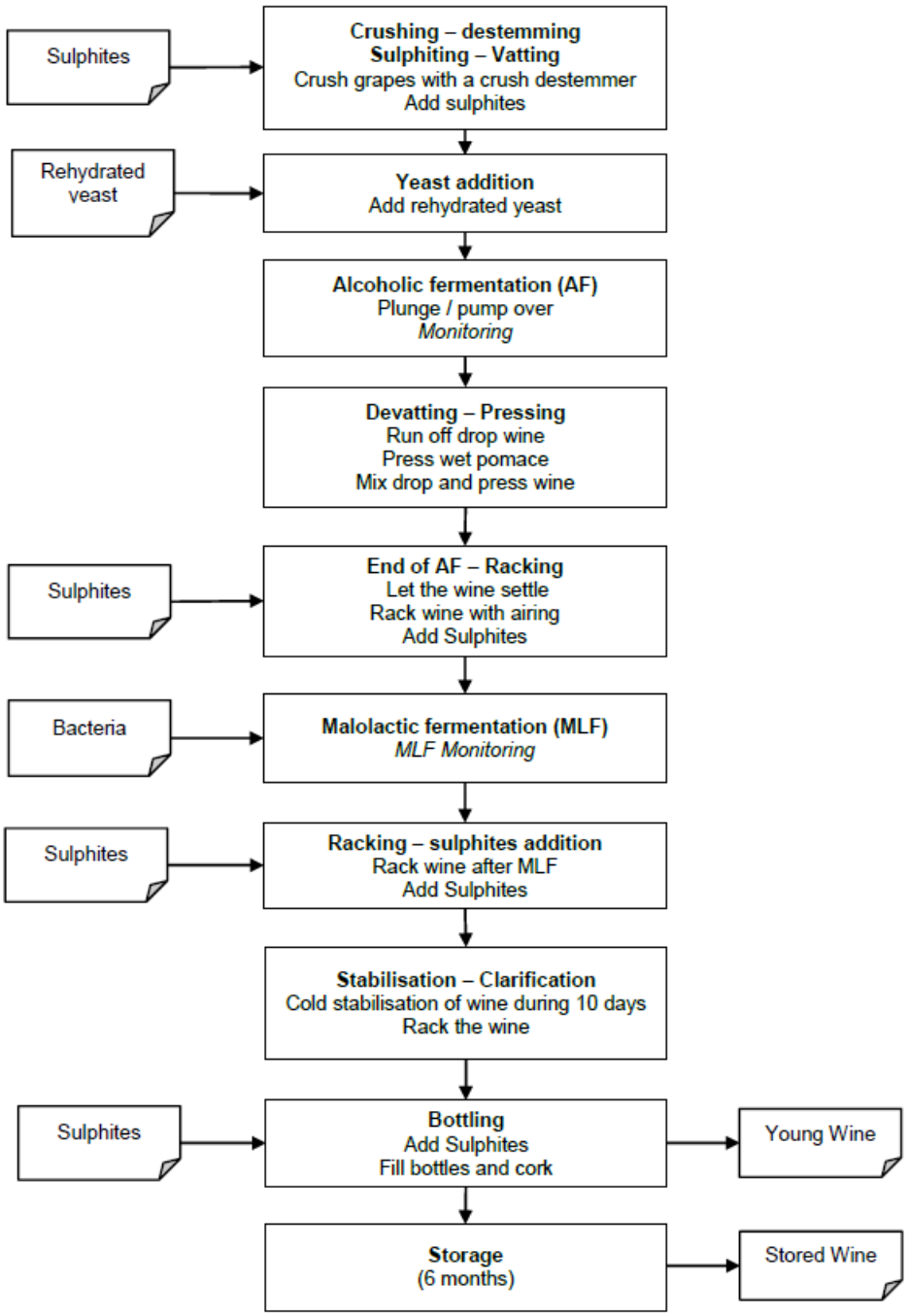


Figure A 4: Processing of red wine

Results and discussion

The concentration of zoxamide (R, S and sum) and its metabolites RH-150721 (R, S and sum), RH-141288 (R, S and sum), RH-129151 (A and B), RH-24549 and RH-141452 were determined in grapes and/or processed specimens.

For metabolite RH-129151 the correlation between the absolute configuration of the enantiomer (R) or (S) and the corresponding chromatographic peaks was not available; therefore, the first eluted peak was assigned as RH-129151 (A) and the second eluted peak as RH-129151 (B).

The metabolite RH-141452, which was known to form conjugates with matrix molecules (e.g. sugars), was released in an additional hydrolysis step to establish total fractions in addition to the free fractions in the matrices.

The limit of quantification (LOQ) was set at 0.01 mg/kg for zoxamide, RH-150721, RH-129151 and RH-141288 (sum of enantiomers), RH-24549, free RH-141452 and total RH-141452, and 0.005 mg/kg for single isomers. The limit of determination (LOD) was given as 0.003 mg/kg for RH-150721, RH-129151 and RH-141288 (sum of enantiomers) and RH-24549, free RH-141452 and total RH-141452 and 0.0015 mg/kg for single isomers.

No residues of zoxamide or any metabolites were found above LOQ in all untreated field specimens. A summary of the results from the treated plots is given in the following table.

In case of residues > LOQ for isomeric compounds, they are demonstrating a stable chiral centre of zoxamide and its residues.

(Sala A. 2020)

(Thomas-Delille E. 2020)

Table A 16: Summary of the study 3 trials on grapes / Northern EU

Reference:	Sala A. 2020, BPL-STUDY-19-000041; field part study no. B7284		
GLP:	Yes	Sample storage conditions:	≤ -18 °C for residues analysis
Crop/crop group:	Grapes / fruiting crops	Analytical method:	BPL-STUDY-18-000085
Indoor/Outdoor:	Outdoor	Limit of Quantification (mg/kg):	0.01 mg/kg (sum), to 0.005 mg/kg (single isomers)
Formulation:	SC	Limit of Detection (mg/kg):	0.003 mg/kg (sum), 0.0015 mg/kg (single isomer)
Content of active substance (g/kg or g/l):	240 g/L Zoxamide	Residues calculated as:	Zoxamide (as sum of R and S isomers), (R)-Zoxamide, (S)-Zoxamide, metabolites RH-141452, RH-150721 (as sum of R and S isomers), (R)-RH-150721, (S)-RH-150721, RH-129151 (as sum of R and S isomers), (R)-RH-129151, (S)- RH-129151, RH-24549, RH-141288 (as sum of R and S isomers), (R)-RH-141288, (S)-RH-141288

Trial No. Location EU zone Year	Commodity/ Variety	Date of 1. Sowing or Planting 2. Flowering 3. Harvest	Application rate per treatment			Dates of treatment(s) or no of treatment(s) and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)													PHI (days)	Remarks
			kg as/ha	Water (L/ha)	kg as/hl				Zoxamide (sum)	Zoxamide (R)	Zoxamide (S)	RH-141452	RH-24549	RH-150721 (sum)	(R)-RH-150721	(S)-RH-150721	RH-129151 (sum)	RH-129151 (A)	RH-129151 (B)	RH-141288 (sum)	(R)-RH-141288	(S)-RH-141288		
B7284 BW1	Grape / Chasselas	1) Year 2009 2) from 27/06/2017 to 10/07/2017 3)05/09/2017	0.180	997	0.018	19/07/2017	75-77	Grape (berries)	1.400	0.757	0.643	ND	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	20	h
			0.193	1072		26/07/2017	79		0.805	0.435	0.370	ND	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR		i
79238 Norsingen, Baden-Württemberg, Germany			0.180	1000		02/08/2017	79-81		0.933	0.503	0.430	< LOQ (0.0059)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR		l
			0.177	981		09/08/2107	81-83	Juice	0.0108	0.0057	0.0051	ND	ND	0.0106	0.0059	< LOQ (0.0047)	ND	ND	ND	ND	ND	ND		*
			0.195	1083		16/08/2017	83-85	Young wine	0.0173	0.0101	0.0072	ND	ND	0.0135	0.0061	0.0074	ND	ND	ND	ND	ND	ND		
N-EU, 2017								Stored wine	< LOQ (0.0066)	< LOQ (0.0033)	< LOQ (0.0032)	< LOQ (0.0035)	ND	0.0130	0.0051	0.0080	ND	ND	ND	ND	ND	ND		

Trial No. Location EU zone Year	Commodity/ Variety	Date of 1. Sowing or Planting 2. Flower-ing 3. Harvest	Application rate per treatment			Dates of treat-ment(s) or no of treat-ment(s) and last date	Gro wth stage at last treat-ment or date	Portion ana-lysed	Residues (mg/kg)														PHI (days)	Remarks
			kg as/ha	Water (L/ha)	kg as/hl				Zoxamide (sum)	Zoxamide (R)	Zoxamide (S)	RH-141452	RH-24549	RH-150721 (sum)	(R)-RH-150721	(S)-RH-150721	RH-129151 (sum)	RH-129151 (A)	RH-129151 (B)	RH-141288 (sum)	(R)-RH-141288	(S)-RH-141288		
B7284 CZ1 69301 Horni Bojanov-ice, South Moravia, Czech Re-public N-EU, 2017	Grape / Vavrinecke	1) Year 2004 2) from 10/06/2017 to 15/06/2017 3)10/10/2017	0.183 0.193 0.191 0.185 0.187	662 696 688 667 676	0.028	15/08/2017 22/08/2017 30/08/2017 07/09/2017 14/09/2017	81 81-83 83 83-85 85-87	Grape (berries)	1.353**	0.731	0.622	0.014	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	26	h
									1.550**	0.835	0.715	< LOQ (0.0067)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR		i
									1.620**	0.878	0.742	< LOQ (0.0077)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR		l
								Juice	0.0284	0.0146	0.0139	< LOQ (0.0061)	ND	0.0471	0.0258	0.0213	ND	ND	ND	ND	ND	ND		*
								Young wine	0.0508	0.0288	0.0221	< LOQ (0.0088)	ND	0.0235	0.0096	0.0140	ND	ND	ND	ND	ND	ND		
								Stored wine	0.0503	0.0288	0.0215	< LOQ (0.0087)	< LOQ (0.005)	0.0448	0.0211	0.0237	ND	ND	ND	< LOQ (0.0052)	< LOQ (0.0026)	< LOQ (0.0025)		

* LOQ = 0.010 mg/kg for zoxamide (sum), RH-150721 (sum), RH-1292151 (sum), RH-141288 (sum), RH-141452 and RH-24549, and 0.005 mg/kg for single isomers.
LOD = 0.003 mg/kg for zoxamide (sum), RH-150721 (sum), RH-1292151 (sum), RH-141288 (sum), RH-141452 and RH-24549, and 0.0015 mg/kg for single isomers.
Analytical method validated in study BPL-STUDY-18-000085
Max. Storage interval between sampling and analysis: for grapes (berries): 605-643 days
for juice: 604-632 days
for young wine: 550-591 days
for stored wine: 368-406 days

** geometric mean value = 1.503 mg/kg (n=3)

ND = not detectable, NR = not relevant

h = grape sampled directly on field

i = Grape sample just before processing phase start

l = Grape sample just before processing phase start

A 2.1.3.1.4 Study 4

Comments of zRMS:	The studies are not relevant.

Reference:	**KCA 6.3/06**
Report	Sala, A., 2020: Determination of zoxamide and its metabolites in raw agricultural commodity wine grape (berries) and processed fractions (juice, wine) following five applications of Zoxium 240 SC (GWN-9790 EU) in open field condition 2 harvest trials, Southern Europe, year 2017 - final report amendment no. 1[11] Gowan Crop Protection Limited, UK LabAnalysis, Italy, Report No. BPL-STUDY-19-000051, GLP, Not published
and	
Reference:	**KCA 6.3/07**
Report	Casalinouvo, L., 2020: Determination of zoxamide and his metabolite RH-150721 residues raw agricultural commodity red grapes and processed fraction following five applications of Zoxium 240 SC (zoxamide 240 g/L) (South Europe – 2 trials year 2017) - plus amendment no. 1 to final report Gowan Crop Protection Limited, UK BioSphereS by Biotecnologie B.T., Italy, Report No. BIU-005-17, GLP, Not published
Guideline(s):	OECD TG 509 (2009) OECD TG 508 (2008)
Deviations:	Due to the dry season the bunches did not reach the quantity assumed by the study plan. As a result, from trial I/ZO17/GR01 only 26.5 and 24 kg were collected from the control and the treated plot, respectively, and from trial I/ZO17/GR02 only 20 and 22 kg from the control and the treated plot, respectively. However, these amounts were regarded sufficient for the intended processing phase and thus the study plan deviation regarded to not impact the integrity of the study. Due to the limited bunch weights the juice samples received from trial I/ZO17/GR02 were also reduced, but still sufficient for the following residue analysis. This deviation was therefore regarded to not impact the integrity of the study. The following recovery checks were slightly higher than the intended accuracy validation range (70-110%): 19/51/GJ/RC3/NH/2: 118.9% for analyte (S)-Zoxamide 19/51/GJ/RC1/NH: 118.8% for analyte (S)-RH-141288 19/51/GJ/RC2/NH: 113.6% for analyte (S)-RH-141288 19/51/WI/RC1/NH: 121.7% for analyte (R)-RH-150721 19/51/WI/RC2/NH: 118.5% for analyte (R)-RH-150721 19/51/WI/RC1/NH: 114.8% for analyte (S)-RH-150721

[11] Confirmatory study for zoxamide currently being evaluated by RMS (Latvia).

	19/51/WI/RC2/NH: 124.4% for analyte (S)-RH-150721 However, these deviations were regarded to have no impact on the integrity of the study results since the recoveries reported above are slightly higher than maximum allowed (70-110%) – and therefore represent worst-case values.
GLP:	Yes

Materials and methods

Test item (Lot/Batch No.)	GWN 9790 EU/Zoxium 240 SC (SB2401)
Active substance content	240 g/L zoxamide (nominal), 232 g/L (analysed) (R/S zoxamide ratio: 50/50)
Expiry date	April 2018

The magnitude of residues of zoxamide and its metabolite RH-141452 in raw agricultural commodity specimens of grapes (bunches) and processed fractions has been determined in two harvest trials performed in Southern Europe (Italy) in 2017. In addition, the metabolites RH-150721, RH-24549, RH-129151 and RH-141288 have been analysed in processed commodities (juice, young and bottled red wine).

In each trial one plot was treated five times with Zoxium 240 SC at an application rate of 0.75 L/ha, one plot remained untreated. The applications were made at an interval of 7-8 (+1) days with the last application 27 and 28 days before harvest. Samples were taken both for residues analysis and for processing.

The residues samples were frozen within 5 hours and 10 minutes in the field. They were stored at ≤ -18°C until analysis.

The field and processing phase has been performed by BioSphereS under phase report no. BIU-005-17. The samples for processing were put in boxes and sent at ambient temperatures for processing. The processing started at the day of arrival. The samples were processed as detailed in the following figure. The analytical phase has been performed at LabAnalysis in Italy under report no. BPL-STUDY-19-000051 with a method validated according to SANCO/825/00 rev. 8.1 and SANCO/3029/99 rev. 4 under report no. BPL-STUDY-18-000085.

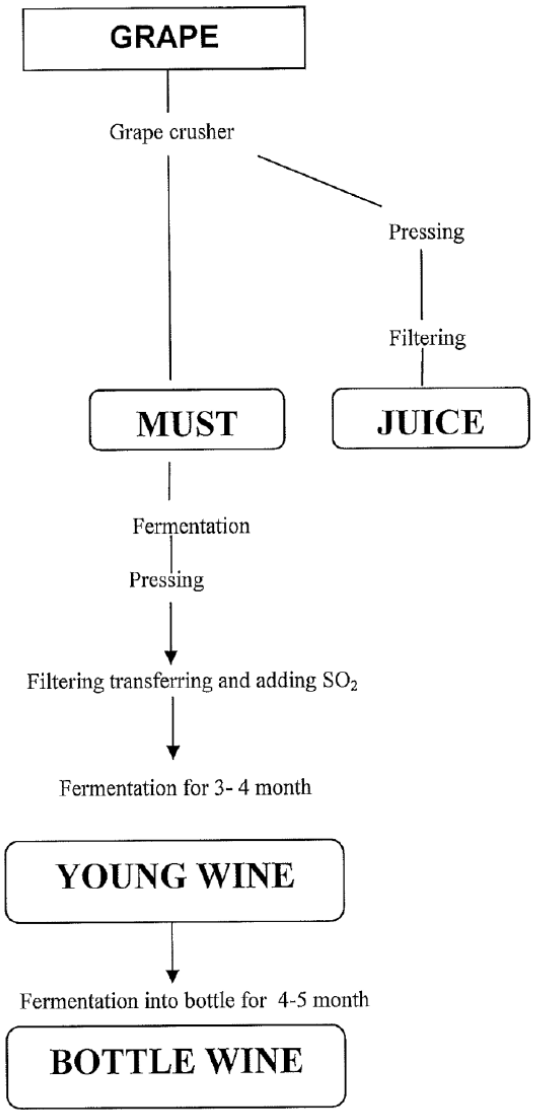


Figure A 5: Processing of juice and red wine

Results and discussion

The concentration of zoxamide (R, S and sum) and its metabolites RH-150721 (R, S and sum), RH-141288 (R, S and sum), RH-129151 (A and B), RH-24549 and RH-141452 were determined in grapes and/or processed specimens.

For metabolite RH-129151 the correlation between the absolute configuration of the enantiomer (R) or (S) and the corresponding chromatographic peaks was not available; therefore, the first eluted peak was assigned as RH-129151 (A) and the second eluted peak as RH-129151 (B).

The metabolite RH-141452, which was known to form conjugates with matrix molecules (e.g. sugars), was released in an additional hydrolysis step to establish total fractions in addition to the free fractions in the matrices.

The limit of quantification (LOQ) was set at 0.01 mg/kg for zoxamide, RH-150721, RH-129151 and RH-141288 (sum of enantiomers), RH-24549, free RH-141452 and total RH-141452, and 0.005 mg/kg for single isomers. The limit of determination (LOD) was given as 0.003 mg/kg for RH-150721, RH-129151 and RH-141288 (sum of enantiomers) and RH-24549, free RH-141452 and total RH-141452 and 0.0015 mg/kg

for single isomers.

No residues of zoxamide or any metabolites were found above LOQ in all untreated field specimens. A summary of the results from the treated plots is given in the following table.

In case of residues > LOQ for isomeric compounds, they are demonstrating a stable chiral centre of zoxamide and its residues.

(Sala A. 2020)

(Casalinouvo L. 2020)

Table A 17: Summary of the study 4 trials on grapes / Southern EU

Reference:	Sala A. 2020, BPL-STUDY-19-000051; field report no. BIU-005-17		
GLP:	Yes	Sample storage conditions:	≤ -18 °C for residues analysis
Crop/crop group:	Grapes / fruiting crops	Analytical method:	BPL-STUDY-18-000085
Indoor/Outdoor:	Outdoor	Limit of Quantification (mg/kg):	0.01 mg/kg (sum), to 0.005 mg/kg (single isomers)
Formulation:	SC	Limit of Detection (mg/kg):	0.003 mg/kg (sum), 0.0015 mg/kg (single isomer)
Content of active substance (g/kg or g/l):	240 g/L Zoxamide	Residues calculated as:	Zoxamide (as sum of R and S isomers), (R)-Zoxamide, (S)-Zoxamide, metabolites RH-141452, RH-150721 (as sum of R and S isomers), (R)-RH-150721, (S)-RH-150721, RH-129151 (as sum of R and S isomers), (R)-RH-129151, (S)- RH-129151, RH-24549, RH-141288 (as sum of R and S isomers), (R)-RH-141288, (S)-RH-141288

Trial No. Location EU zone Year	Commodity/ Variety (a)	Date of 1. Sowing or Planting 2. Flowering 3. Harvest (b)	Application rate per treatment: kg as/ha	Water (L/ha)	kg as/hl	Dates of treatment(s) or no of treatment(s) and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg): Zoxamide (R)	Zoxamide (S)	Zoxamide (sum)	RH-150721 (R)	RH-150721 (S)	RH-150721 (sum)	RH-141288 (R)	RH-141288 (S)	RH-141288 (sum)	RH-129151 (A)	RH-129151 (B)	RH-129151 (sum)	RH-24549	RH-141452	PHI (days)	Remarks:
I/ZO17/GR01 48025 Riolo Terme (RA) Italy S-EU, 2017	Grape / Sangiovese	1) 10/12/2010 2) N.A. 3) 27/09/2017	0.181 0.179 0.179 0.185 0.183	1005.6 991.7 991.7 1025.0 1013.9	0.018	31/07/2017 08/08/2017 16/08/2017 24/08/2017 31/08/2017	81 82 83 85 86-88	Grape (berries)	0.776	0.630	1.406	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	ND	27	* **
								Juice	0.0598	0.0571	0.116	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		
								Young wine	0.00508	< LOQ (0.00395)	< LOQ (0.00903)	0.0265	0.0312	0.0577	ND	ND	ND	ND	ND	ND	< LOQ (0.00509)	< LOQ (0.00538)		
								Bottled wine	ND	ND	ND	0.0247	0.0287	0.0535	ND	ND	ND	ND	ND	ND	0.0204	< LOQ (0.00535)		
I/ZO17/GR02 27050 Retorbido (PV) Italy S-EU, 2017	Grape / Croatina	1) 04/2010 2) N.A. 3) 14/09/2017	0.172 0.178 0.178 0.188 0.186	957.7 990.6 990.6 1047.1 1035.3	0.018	18/07/2017 25/07/2017 01/08/2017 08/08/2017 17/08/2017	79 81 81-83 83 85-87	Grape (berries)	0.250	0.202	0.452	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	0.0188	28	* ***
								Juice	0.0931	0.0907	0.183	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	< LOQ (0.00622)		
								Young wine	< LOQ (0.00460)	< LOQ (0.00402)	< LOQ (0.00862)	0.0187	0.0198	0.0384	ND	ND	ND	ND	ND	ND	< LOQ (0.00460)	0.0121		
								Bottled wine	ND	ND	ND	0.0151	0.0158	0.0309	ND	ND	ND	ND	ND	ND	0.0140	0.0127		

* LOQ = 0.010 mg/kg for zoxamide (sum), RH-150721 (sum), RH-1292151 (sum), RH-141288 (sum), RH-141452 and RH-24549, and 0.005 mg/kg for single isomers.
LOD = 0.003 mg/kg for zoxamide (sum), RH-150721 (sum), RH-1292151 (sum), RH-141288 (sum), RH-141452 and RH-24549, and 0.0015 mg/kg for single isomers.
Analytical method validated in study BPL-STUDY-18-000085
Max. Storage interval between sampling and analysis: for grapes (barriers): 617-634 days

for juice: 614-629 days
for young wine: 480-482 days
for bottled wine: 343-345 days

** NOTE: LWA (Leaf Wall Area) = (soil surface of the orchard x treated height/row distance) x 2
LWA= (10000*1.4 m / 3) *2= 9333 m^2

*** NOTE: LWA (Leaf Wall Area) = (soil surface of the orchard x treated height/row distance) x 2
LWA= (10000 * 1.4 * / 2.5) *2 = 11200 m^2

ND = not detectable, NR = not relevant

A 2.1.3.1.5 Study 5

Comments of zRMS:	The study is acceptable. The objective of this study was the determination of the residues of its enantiomers (R)-Zoxamide and (S)-Zoxamide (an the sum), and of the following Zoxamide metabolites: RH-150721 (as sum of R and S enantiomers) and its enantiomers (R)-RH-150721, (S)-RH-150721; RH-129151 (as sum of R and S enantiomers) and its enantiomers (R)-RH-129151 and (S)-RH-129151; RH-141288 (as sum of R and S enantiomers) and its enantiomers (R)-RH-141288 and (S)-RH-141288; RH-24549 and RH-141452 in grape wine, coming from 1 harvest trial and 3 decline trials performed in open field in NEU. Each trial was carried out performing 5 and 3 applications of the product ZOXIUM 240 SC (GWN 9790 EU). The analytical method was validated according to the SANCO/825/00 rev.8.1 and SANCO/3029/99 rev.4 guidelines in the BPL-STUDY-18-000085.

Reference:	**KCA 6.3/08**
Report	Longhi, D., 2020: Determination of zoxamide and its metabolites in raw agricultural commodity of grape wine in open field following five and three applications of the formulated product GWN-9790 EU (North Europe - 4 trials year 2019)[12] Gowan Crop Protection Ltd, UK Lab Analysis, Italy, Report No. BPL-STUDY-19-000057, GLP, Not published
Guideline(s):	OECD TG 509 (2009)
Deviations:	In the field trial CMN-19-38746-FR01 on the T1 plot at applications A4 and A5 a deviation from the target dose of GWN 9790 EU was noted at +10.5%, being slightly above the intended +/- 10% because of a fluctuation of the sprayer flow rate. For correction, the correct amount of the applied test item dose has been recalculated. This deviation has been regarded to not influence the integrity of the study results. In the field trials CMN-19-38746-HU03 and CMN-19-38746-FR02 the T1 plots were sampled before the T2 plots though it should have been inversely, as T1 plots were treated less times. This deviation has been regarded to not influence the integrity of the study results. In the field trial CMN-19-38746-HU03 the application no. 4 was done 10 days after the application no. 3. However, this is still within the ±25 % range of an intended application interval of 8 days. Besides, other applications were spaced by 7 days, balancing the interval to a target range of nominally 7-8 (+1) days.
GLP:	Yes
Acceptability:	Yes

Materials and methods

Test item (Lot/Batch No.)	GWN 9790 EU / Zoxium 240 SC (SIPAL7001)

[12] Confirmatory study for zoxamide currently being evaluated by RMS (Latvia).

Active substance content	240 g/L zoxamide (nominal), 242.9 g/L (analysed) (R/S) zoxamide ratio: 50/50)
Expiry date	May 2020

The magnitude of residues of zoxamide (as sum of R and S isomers, (R)-zoxamide and (S)-zoxamide) and its metabolite RH-141452 in raw agricultural commodity specimens of grapes (bunches) has been determined in four trials performed in Northern Europe (France and Hungary) in 2019. In each trial one plot was treated either three or five times with Zoxium 240 SC at an application rate of 0.75 L/ha, one plot remained untreated. The applications were made at an interval of 7-8 days with the last application 27 and 28 days before harvest. Samples were taken only for residues analysis.

The residues samples were frozen within 5 hours. They were stored and shipped at ≤ -18°C until analysis.

The field phase has been conducted by Staphyt France and Hungary under field phase number CMN-19-38746. The analytical work has been performed at LabAnalysis in Italy under report no. BPL-STUDY-19-000057 with a method validated according to SANCO/825/00 rev. 8.1 and SANCO/3029/99 rev. 4 under report no. BPL-STUDY-18-000085.

Results and discussion

The concentration of zoxamide (R, S and sum) and its metabolite RH-141452 were determined in grape fruits/bunches.

The metabolite RH-141452, which was known to form conjugates with matrix molecules (e.g. sugars), was released in an additional hydrolysis step to establish total fractions in addition to the free fractions in the matrices.

The limit of quantification (LOQ) was set at 0.01 mg/kg for zoxamide (sum) and free RH-141452 and total RH-141452, and 0.005 mg/kg for single isomers. The limit of determination (LOD) was given as 0.003 mg/kg for zoxamide (sum of enantiomers) and free RH-141452 and total RH-141452 and 0.0015 mg/kg for single isomers.

No residues of zoxamide or any metabolites were found above LOQ in all untreated field specimens.

The analyses samples of the trials CMN-19-38746 HU03 and CMN-19-38746 HU04 showed abnormal results, and because of this, “retain” samples of the above-mentioned trials were analysed. However, the unexpected results stayed without a possible explanation. Therefore, these results were not considered for the risk assessment.

A summary of the results from the treated plots is given in the following table.

In case of residues > LOQ for isomeric compounds, they are demonstrating a stable chiral centre of zoxamide and its residues.

(Longhi D. 2020)

Table A 18: Summary of the study 5 trials on grapes / Northern EU

Reference:	Longhi D. 2020, BPL-STUDY-19-000057		
GLP:	Yes	Sample storage conditions:	≤ -18 °C for residues analysis
Crop/crop group:	Grape / wine grape	Analytical method:	BPL-STUDY-18-000085
Indoor/Outdoor:	Outdoor	Limit of Quantification (mg/kg):	0.01 mg/kg (sum), to 0.005 mg/kg (single isomers)
Formulation:	SC	Limit of Detection (mg/kg):	0.003 mg/kg (sum), 0.0015 mg/kg (single isomer)
Content of active substance (g/kg or g/l):	240 g/L Zoxamide	Residues calculated as:	Zoxamide (as sum of R and S isomers), (R)-Zoxamide, (S)-Zoxamide, metabolite RH-141452

Trial No. Location EU zone Year	Commodity/ Variety	Date of 1. Sowing or Planting 2. Flowering 3. Harvest	Application rate per treatment			Dates of treatment(s) or no of treatment(s) and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)					PHI (days)	Remarks:
			kg as/ha	Water (L/ha)	kg as/hl				Zoxamide (sum)	Zoxamide (R)	Zoxamide (S)	RH-141452 total	RH-141452 free		
CMN-19-38746 FR01 41150 Veuzain (Centre Val de Loire)-France N-EU, 2019	Grape vine Gamay	1) 1985 2) 17/06/2019 to 30/06/2019 3)05/09/2019	Plot T1												
			0.193 0.189 0.196 0.201 0.201	370 363 377 387 387	0.0521 0.0521 0.0521 0.0521 0.0521	22/07/2019 30/07/2019 08/08/2019 16/08/2019 23/08/2019	77 77 77-79 81 83	Grape wine (bunches)	0.541	0.267	0.274	<LOQ (0.00605)	<LOD	28	*
			Plot T2												
			0.195 0.198 0.182	375 380 348	0.0521 0.0521 0.0521	08/08/2019 16/08/2019 23/08/2019	77-79 81 83	Grape wine (bunches)	0.460	0.224	0.235	<LOQ (0.00387)	<LOD	28	*
CMN-19-38746 FR02 49700 Brossay (Centre Val de Loire)-France	Grape vine Cabernet franc	1) 1960 2) 03/06/2019 to 20/06/2019 3)23/08/2019 30/08/2019 05/09/2019	Plot T1												
			0.0506 0.0506 0.0506 0.0506	444 444 457 419	0.180 0.180 0.185 0.170	23/07/2019 31/07/2019 08/08/2019 15/08/2019	79 79 79 81	Grape wine (bunches)	0.304 0.309	0.155 0.156	0.150 0.153	ND ND	ND ND	0 7	*

Trial No. Location EU zone Year	Commodity/ Variety	Date of 1. Sowing or Planting 2. Flowering 3. Harvest	Application rate per treatment			Dates of treatment(s) or no of treatment(s) and last date	Growth stage at last treat-ment or date	Portion analysed	Residues (mg/kg)					PHI (days)	Remarks:
			kg as/ha	Water (L/ha)	kg as/hl				Zoxamide (sum)	Zoxamide (R)	Zoxamide (S)	RH-141452 to-tal	RH-141452 free		
N-EU, 2019		13/09/2019 09/09/2019	0.0506	429	0.174	23/08/2019	83		0.410	0.203	0.208	ND	ND	13	
									0.268	0.132	0.136	ND	ND	21	
									<u>0.231</u>	0.115	0.116	ND	ND	27	
			Plot T2												
			0.178 0.174 0.177	439 429 437	0.0506 0.0506 0.0506	08/08/2019 15/08/2019 23/08/2019	79 81 83	Grape wine (bunches)	0.168	0.0869	0.0813	ND	ND	0	*
									0.129	0.0641	0.0650	ND	ND	7	
									0.119	0.0595	0.0597	ND	ND	13	
									0.125	0.0600	0.0649	ND	ND	21	
									<u>0.163</u>	0.0833	0.0800	ND	ND	27	
CMN-19-38746 HU03 8308 Zalahulap (Zala county) Hungary N-EU, 2019	Grape vine Ezerjo	1) 1987 2) 08/06/2019 to 20/06/2019 3)12/08/2019 19/08/2019 26/08/2019 02/09/2019 09/09/2019	Plot T1												
			0.182 0.185 0.199 0.185 0.177	400 406 437 406 389	0.0546 0.0456 0.0456 0.0456 0.0456	11/07/2019 19/07/2019 26/07/2019 05/08/2019 12/08/2019	75 75 79 82 83	Grape wine (bunches)	0.585	0.285	0.300	<LOQ (0.00426)	ND	0	*
									0.532	0.268	0.265	<LOQ (0.00600)	ND	7	
									0.474	0.237	0.237	<LOQ (0.00450)	ND	14	
									0.342	0.170	0.171	<LOQ (0.00481)	ND	21	
									<u>0.768</u>	0.385	0.384	<LOQ (0.00959)	ND	28	
			Plot T2												

Trial No. Location EU zone Year	Commodity/ Variety	Date of 1. Sowing or Planting 2. Flowering 3. Harvest	Application rate per treatment			Dates of treatment(s) or no of treatment(s) and last date	Growth stage at last treat-ment or date	Portion analysed	Residues (mg/kg)					PHI (days)	Remarks:
			kg as/ha	Water (L/ha)	kg as/hl				Zoxamide (sum)	Zoxamide (R)	Zoxamide (S)	RH-141452 to-tal	RH-141452 free		
			0.187 0.200 0.187	411 438 411	0.0455 0.0455 0.0455	26/07/2019 05/08/2019 12/08/2019	79 82 83	Grape wine (bunches)	0.490	0.241	0.250	ND	ND	0	*
									0.318	0.158	0.161	ND	ND	7	
									0.396	0.200	0.197	<LOQ (0.00367)	ND	14	
									0.299	0.150	0.149	ND	ND	21	
									0.302	0.149	0.153	<LOQ (0.00553)	ND	28	
CMN-19-38746 HU04 8790 Zala-szentgrot (Zala County) Hungary N-EU, 2019	Grape vine Cerszegi füszeres	1) 1912 2NAV 3)09/08/2019 15/08/2019 23/08/2019 30/08/2019 06/09/2019	Plot T1												
			0.190 0.181 0.187 0.168 0.181	625 596 615 553 598	0.0304 0.0304 0.0304 0.0304 0.0304	08/07/2019 16/07/2019 24/07/2019 01/08/2019 09/08/2019	75 77 81 81 83	Grape wine (bunches)	1.30	0.613	0.688	<LOQ (0.00904)	ND	0	* **
									1.61	0.815	0.790	<LOQ (0.00806)	ND	6	
									<LOQ (0.00390)	<LOQ (0.00206)	<LOQ (0.00184)	ND	ND	14	
									0.711	0.355	0.357	<LOQ (0.00703)	ND	21	
									1.19	0.593	0.594	0.0183	<LOQ (0.00439)	28	
			Plot T2												
			0.194 0.186 0.180	638 611 593	0.0304 0.0304 0.0304	24/07/2019 01/08/2019 09/08/2019	81 81 83	Grape wine (bunches)	0.890	0.436	0.454	ND	ND	0	*
									1.07	0.544	0.527	ND	ND	6	
									1.00	0.501	0.498	ND	ND	14	
									0.377	0.189	0.188	ND	ND	21	

Trial No. Location EU zone Year	Commodity/ Variety	Date of 1. Sowing or Planting 2. Flowering 3. Harvest	Application rate per treatment			Dates of treatment(s) or no of treatment(s) and last date	Growth stage at last treat-ment or date	Portion analysed	Residues (mg/kg)					PHI (days)	Remarks:
			kg as/ha	Water (L/ha)	kg as/hl				Zoxamide (sum)	Zoxamide (R)	Zoxamide (S)	RH-141452 to-tal	RH-141452 free		
									0.910	0.456	0.454	<LOQ (0.00651)	ND	28	

* LOQ = 0.010 mg/kg for zoxamide (sum) and RH-141452 and 0.005 mg/kg for single isomers.
LOD = 0.003 mg/kg for zoxamide (sum) and RH-141452 and 0.0015 mg/kg for single isomers.
Analytical method validated in study BPL-STUDY-18-000085
Max. Storage interval between sampling and analysis: 42-45 days

** Abnormal results for specimens sampled at 14 PHI: no explanation was found. It is presumed an error during sampling (e.g. C plot sampled instead T plot). Analyses on retain sample confirmed this anomaly

ND = not detectable, NR = not relevant

A 2.1.3.1.6 Study 6

Comments of zRMS:	Not relevant. For SEU.

Reference:	**KCA 6.3/09**
Report	Longhi, D., 2020: Determination of zoxamide and its metabolites in raw agricultural commodity of table grape and processed (raisin) in open field following five and three applications of the formulated product GWN 9790 EU (South Europe – 1 trial year 2019)[13] Gowan Crop Protection Ltd., UK LabAnalysis s.r.l., Italy, Report No. BPL-STUDY-19-000058, GLP, Not published
Guideline(s):	OECD TG 509 (2009) OECD TG 508 (2008)
Deviations:	The recovery values for the analytes (R)-RH-150721 and (S)-RH-150721 on recovery check samples BPL-SMPL-19-002114/NH RC1 and BPL-SMPL-19-002114/NH RC2 were found to be above the range allowed by the SANCO/3030/99 rev. 4 and SANCO/825/00 rev. 8.1 (70 - 110%). Since the recovery values were above the permitted range, the concentrations of the analytes under examination have been overestimated, what is regarded a worst-case. Since the analytes (R)-RH-150721 and (S)-RH-150721 were still detected at concentrations <LOQ, the overestimation was regarded to have no impact on the integrity of the results.
GLP:	Yes
Acceptability:	Yes

Materials and methods

Test item (Lot/Batch No.)	GWN 9790 EU/Zoxium 240 SC (SIPAL7001)
Active substance content (nominal)	240 g/L Zoxamide (nominal), 242.9 g/L (analysed) (R/S Zoxamide ratio : 50/50)
Expiry date	May 2020

The magnitude of residues of zoxamide (sum and R and S isomers) and RH-141452 in raw commodities (grape fruits) and of zoxamide (sum and R and S isomers), RH-150721 (as sum of R and S isomers), (R)-RH-150721, (S)-RH-150721, RH-129151 (as sum of R and S isomers), (R)-RH-129151, (S)- RH-129151, RH-24549, RH-141288 (as sum of R and S isomers), (R)-RH-141288 and (S)-RH-141288 in raisins coming from 1 decline trial (DEC) in the open field in Southern Europe (Italy) were determined. Besides an untreated control plot, two further plots were performed with either 5 or 3 applications of Zoxium 240 SC (containing nominally 240 g/L zoxamide). The formulated product was applied with a high-pressure mist sprayer at a rate of nominally 750 mL/ha (corresponding to 180 g/ha of zoxamide) in 1000 L water/ha at an interval of 8 days and with a last application 27 days before harvest.

[13] Confirmatory study for zoxamide currently being evaluated by RMS (Latvia).

Specimens were sampled, weighed, packed and coded separately. The residues samples were stored within 24 hours after sampling and kept at ≤-18°C until analysis. Processing of the samples (i.e. drying of grape fruits) started on the day of harvest.

For processing, single layers of grape berries were dried at 45-65°C in a flow of dry and warm air until they reached a moisture content of < 18%.

The field and processing parts were performed by ASTRA, Italy under the phase report number PFR_19_GOW_VITVI_TG_Z-D. The analytical phase has been performed at LabAnalysis in Italy under report no. BPL-STUDY-19-000058 with a method validated according to SANCO/825/00 rev. 8.1 and SANCO/3029/99 rev. 4 under report no. BPL-STUDY-18-000085.

Results and discussion

The residues of the zoxamide metabolites containing a chiral centre (RH-150721, RH-129151 and RH-141288) were expressed as sum of 2 enantiomers and per single enantiomer (R and S). However, for metabolite RH-129151 the correlation between the absolute configuration of the enantiomer (R) or (S) and the corresponding chromatographic peaks was not available; therefore, the first eluted peak was identified as RH-129151 (A) and the second eluted peak as RH-129151 (B).

The metabolite RH-141452, which is known to form conjugates with matrix molecules (e.g. sugars), was released in an additional hydrolysis step to establish total fractions in addition to the free fractions in the matrices. For RH-141452 the total fractions are considered as worst-case.

The limit of quantification (LOQ) was set at 0.01 mg/kg for zoxamide, RH-150721, RH-129151 and RH-141288 (sum of enantiomers), RH-141452 and RH-24549, and 0.005 mg/kg for single isomers. The limit of determination (LOD) was given as 0.003 mg/kg for RH-150721, RH-129151, RH-141288 (sum of enantiomers), RH-141452 and RH-24549, and 0.0015 mg/kg for single isomers.

No residues were found in the control plot.

A summary of the results is given in the following table.

(Longhi D. 2020)

Table A 19: Summary of the study 6 trial on grapes / Southern EU

Reference:	Longhi D. 2020, BPL-STUDY-19-000058		
GLP:	Yes	Sample storage conditions:	≤ -18 °C for residues analysis
Crop/crop group:	Grapes / fruiting crops	Analytical method:	BPL-STUDY-18-000085
Indoor/Outdoor:	Outdoor	Limit of Quantification (mg/kg):	0.01 mg/kg (sum), to 0.005 mg/kg (single isomers)
Formulation:	SC	Limit of Detection (mg/kg):	0.003 mg/kg (sum), 0.0015 mg/kg (single isomer)
Content of active substance (g/kg or g/l):	240 g/L Zoxamide	Residues calculated as:	Zoxamide (as sum of R and S isomers), (R)-Zoxamide, (S)-Zoxamide, metabolites RH-141452, RH-150721 (as sum of R and S isomers), (R)-RH-150721, (S)-RH-150721, RH-129151 (as sum of R and S isomers), (R)-RH-129151, (S)- RH-129151, RH-24549, RH-141288 (as sum of R and S isomers), (R)-RH-141288, (S)-RH-141288

Trial No. Location EU-zone Year	Commodity/ Variety	Date of 1. Sowing or Planting 2. Flowering 3. Harvest	Application rate per treatment			Dates of treatment(s) or no of treatment(s) and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)														Phi (days)	Remarks
			kg as/ha	Water (L/ha)	kg as/hl				Zoxamide (R)	Zoxamide (S)	Zoxamide (sum)	RH-150721 (R)	RH-150721 (S)	RH-150721 (sum)	RH-141288 (R)	RH-141288 (S)	RH-141288 (sum)	RH-129151 (A)	RH-129151 (B)	RH-129151 (sum)	RH-24549	RH-141452		
PFR_19_ GOW_VI-TVI-TG_Z-D01 48013 Brisighella (RA) Italy S-EU, 2019	Table Grape / ARRA 15/K5BB	1) Year 2012 2) N.A. 3) 09/08/2019 16/08/2019 23/08/2019 30/08/2019 05/09/2019 Raisin 17/09/2019 (processed from the grape sampled on 05/09/2019)	Plot T1																					
			0.1817 0.1834 0.1785 0.1820 0.1903	997.5 1006.6 980.0 999.0 1044.3	0.018218 0.018218 0.018218 0.018218 0.018218	08/07/2019 16/07/2019 24/07/2019 01/08/2019 09/08/2019	75 75 75 75 81-83	Table grape (bunches)	0.556	0.541	1.097	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	<LOQ (0.00929)	0	*
									0.345	0.352	0.697	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	0.0104	7	
									0.365	0.362	0.728	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	0.0113	14	
									0.349	0.316	0.665	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	0.0208	21	
									0.281	0.255	0.537	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	0.0133	27	
								Raisin	0.300	0.296	0.596	0.0183	0.0241	0.0424	<LOQ (0.00430)	<LOQ (0.00183)	<LOQ (0.00613)	0.0101	0.0113	0.0214	<LOQ (0.00374)	0.0572	27	

Trial No. Location EU-zone Year	Commodity/ Variety	Date of 1. Sowing or Planting 2. Flowering 3. Harvest	Application rate per treatment			Dates of treatment(s) or no of treatment(s) and last date	Growth stage at last treat-ment or date	Portion ana-lysed	Residues (mg/kg)														Phi (days)	Remarks
			kg as/ha	Water (L/ha)	kg as/hl				Zoxamide (R)	Zoxamide (S)	Zoxamide (sum)	RH-150721 (R)	RH-150721 (S)	RH-150721 (sum)	RH-141288 (R)	RH-141288 (S)	RH-141288 (sum)	RH-129151 (A)	RH-129151 (B)	RH-129151 (sum)	RH-24549	RH-141452		
			Plot T2																					
			0.1815 0.1844 0.1874	996.3 1012.1 1028.5	0.018218 0.018218 0.018218	24/07/2019 01/08/2019 09/08/2019	75 75 81-83	Table grape (bunches)	0.381	0.371	0.751	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	N.D.	0	*
									0.296	0.294	0.590	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	<LOQ (0.00458)	7	
									0.336	0.315	0.651	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	<LOQ (0.00653)	14	
									0.194	0.176	0.370	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	<LOQ (0.00727)	21	
									0.226	0.204	0.430	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	<LOQ (0.00636)	27	

* LOQ = 0.010 mg/kg for zoxamide (sum), RH-150721 (sum), RH-1292151 (sum), RH-141288 (sum), RH-141452 and RH-24549, and 0.005 mg/kg for single isomers.
LOD = 0.003 mg/kg for zoxamide (sum), RH-150721 (sum), RH-1292151 (sum), RH-141288 (sum), RH-141452 and RH-24549, and 0.0015 mg/kg for single isomers.
Analytical method validated in study BPL-STUDY-18-000085
Max. Storage interval between sampling and analysis: table grapes: 26 days
raisins: 6-7 days

ND = not detectable, NR = not relevant

A 2.1.3.1.7 Study 7

Comments of zRMS:	Not relevant. For SEU.

Reference:	**KCA 6.3/10**
Report:	Maccaferri, L., 2020: Magnitude of the residues of zoxamide in table grape bunches and in raisins processed fraction, following applications of Zoxium 240 SC. One harvest trial, Southern Europe – 2018[14] Gowan Crop Protection Ltd., UK Renolab S.r.l. Italy, Report No. 18097-03R, GLP, Not published
Guideline(s):	SANCO/7029/VI/95 rev. 5 (1997) OECD TG 509 (2009) SANCO/7035/VI/95 rev. 5 (1997) OECD TG 508 (2008)
Deviation(s):	Due to response instability in analytical sequence with both solvent and matrix standards, the matrix effects were not calculated. This deviation was regarded as not relevant for the integrity of the study since the analysis is performed with matrix matched standards. For some analyte / matrix combinations the mean recovery was >110 % but <120 %. Since recovery values of 70-120% for the here implied concentration ranges of 0.01-0.1 mg/kg are acceptable according to SANCO/825/00 rev. 8.1 (2010), this deviation from the study plan is regarded as not relevant.
GLP:	Yes
Acceptability:	Yes

Materials and methods

Test item (Lot/Batch No.):	GWN 9790 EU/Zoxium 240 SC (SIPAL7001)
Active substance content (nominal):	240 g/L Zoxamide (nominal), 242.9 g/L (analysed) (R/S Zoxamide ratio: 50/50)
Expiry date:	May 2020

The magnitude of residues of zoxamide (sum and R and S isomers) and RH-141452 in raw commodities (grape fruits) and of zoxamide (sum and R and S isomers), RH-150721 (as sum of R and S isomers), (R)-RH-150721, (S)-RH-150721, RH-129151 (as sum of R and S isomers), (R)-RH-129151, (S)- RH-129151, RH-24549, RH-141288 (as sum of R and S isomers), (R)-RH-141288 and (S)-RH-141288 in raisins coming from 1 harvest trial in the open field in Southern Europe (Italy) were determined.

Besides an untreated control plot, one further plot was performed with 5 applications of Zoxium 240 SC (containing nominally 240 g/L Zoxamide). The formulated product was applied with a high-pressure mist sprayer at a rate of nominally 750 mL/ha (corresponding to 180 g/ha of zoxamide) in 1000 L water/ha at an interval of 7-8 days.

[14] Confirmatory study for zoxamide currently being evaluated by RMS (Latvia).

Commodity (RAC) samples were collected at commercial harvest time (28 DALA). They were kept a temperature until processing. Samples for residues analysis were frozen at ≤ -18°C within 8 hours after sampling in the field and remained deep frozen until extraction and analysis.

For processing, single layers of grape berries were dried at about 51-54°C in a flow of dry and warm air until they reached a moisture content of 16.5-17.5%.

The field and processing parts were performed by ASTRA, Italy under the phase report number PFR_18_GOW_VITVI_TG_Z-H. The analytical phase was performed by Renolab S.r.l. in Italy under report no. 18097-03R according to method BPL-STUDY-18-000085, which has been validated according to SANCO/825/00 rev. 8.1 and SANCO/3029/99 rev. 4. The method has been re-validated for the determination of zoxamide (as sum of R and S isomers), (R)-Zoxamide, (S)-Zoxamide, metabolites RH-141452, RH-150721 (as sum of R and S isomers), (R)-RH-150721, (S)-RH-150721, RH-129151 (as sum of R and S isomers), (R)-RH-129151, (S)- RH-129151, RH-24549, RH-141288 (as sum of R and S isomers), (R)-RH-141288 and (S)-RH-141288 in Raw Agricultural Commodity grapes (bunches) and processed samples (raisins) during the course of this study.

The metabolite RH-141452, which is known to form conjugates with matrix molecules (e.g. sugars), were released in an additional hydrolysis step to establish total fractions in addition to the free fractions in the matrices.

Results and discussion

The residues of the zoxamide metabolites containing a chiral centre (RH-150721, RH-129151 and RH-141288) were expressed as sum of 2 enantiomers and per single enantiomer (R and S). However, for metabolite RH-129151 the correlation between the absolute configuration of the enantiomer (R) or (S) and the corresponding chromatographic peaks was not available; therefore, the first eluted peak was identified as RH-129151 (A) and the second eluted peak as RH-129151 (B).

The metabolite RH-141452, which is known to form conjugates with matrix molecules (e.g. sugars), was released in an additional hydrolysis step to establish total fractions in addition to the free fractions in the matrices. For RH-141452 the total fractions are considered as worst-case.

The limit of quantification (LOQ) was set at 0.01 mg/kg for zoxamide, RH-150721, RH-129151 and RH-141288 (sum of enantiomers), RH-141452 and RH-24549, and 0.005 mg/kg for single isomers. The limit of determination (LOD) was given as 0.003 mg/kg for RH-150721, RH-129151, RH-141288 (sum of enantiomers), RH-141452 and RH-24549, and 0.0015 mg/kg for single isomers.

No residues were found in the control plot.

A summary of the results is given in the following tables.

Processing factors were calculated for analytes with reliable residue concentrations (≥10x LOQ) in the raw agricultural commodity (RAC) and (≥ LOQ) in the processed commodity as the ratio between mean residue levels in treated grapevine fruits and the processed fraction (raisins). Only samples harvested at the intended phi of 28 days were taken into account.

Table A 20: Summary of processing factors

Analyte	Residue (mg/kg)		Processing factor
	Grape fruits	Raisins	
Zoxamide sum R+S	0.52	0.90	1.7
(R) - Zoxamide	0.27	0.45	1.7
(S) - Zoxamide	0.25	0.45	1.8

In case of residues > LOQ for isomeric compounds, they are demonstrating a stable chiral centre of zoxamide and its residues.

(Maccaferri L. 2020)

Table A 21: Summary of the study 7 trial on grapes / Southern EU

Reference:	Maccaferri L. 2020, 18097-03R		
GLP:	Yes	Sample storage conditions:	≤ -18°C for residues samples
Crop/crop group:	Grapes / Fruiting crops	Analytical method:	BPL-STUDY-18-000085, re-validated in study 18097-03R
Indoor/Outdoor:	Outdoor	Limit of Quantification (mg/kg):	0.01 mg/kg (sum), to 0.005 mg/kg (single isomers)
Formulation:	SC	Limit of Detection (mg/kg):	0.003 mg/kg (sum), 0.0015 mg/kg (single isomer)
Content of active substance (g/kg or g/l):	240 g/L Zoxamide	Residues calculated as:	Zoxamide (as sum of R and S isomers), (R)-Zoxamide, (S)-Zoxamide, metabolites RH-141452, RH-150721 (as sum of R and S isomers), (R)-RH-150721, (S)-RH-150721, RH-129151 (as sum of R and S isomers), (R)-RH-129151, (S)- RH-129151, RH-24549, RH-141288 (as sum of R and S isomers), (R)-RH-141288, (S)-RH-141288

Trial No. Location EU zone Year	Commodity/ Variety	Date of 1. Sowing or Planting 2. Flowering 3. Harvest	Application rate per treatment			Dates of treatment(s) or no of treatment(s) and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)																		
			kg as/ha	Water (L/ha)	kg as/hl				Zoxamide (sum)	Zoxamide (R)	Zoxamide (S)	RH-141452 (total)	RH-141452 (free)	RH-24549	RH-150721 (sum)	(R)-RH-150721	(S)-RH-150721	RH-129151 (sum)	(A)-RH-129151	(B)-RH-129151	RH-141288 (sum)	(R)-RH-141288	(S)-RH-141288	PHI (days)	Remarks
PFR_18_GOW_VITVI-TG_Z-H01 40026 – Bologna, Imola Italy S-EU-2018	Table grape (Sultanina)	1) - 2) – 3) 17/08/2018	0.180	1000	0.018	19/06/2018 26/06/2018 04/07/2018 12/07/2018 20/07/2018	75 75 75 75 81-83	Bunches	0.52	0.27	0.25	0.011	<LOQ (0.005)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	28	*
								Raisin	0.90	0.45	0.45	0.043	0.034	<LOQ (0.005)	0.056	0.025	0.031	<LOQ (0.0094)	<LOQ (0.0047)	<LOQ (0.0047)	0.013	0.0067	0.0066	28	

* LOQ = 0.010 mg/kg for zoxamide (sum), RH-150721 (sum), RH-1292151 (sum), RH-141288 (sum), RH-141452 and RH-24549, and 0.005 mg/kg for single isomers.
LOD = 0.003 mg/kg for zoxamide (sum), RH-150721 (sum), RH-1292151 (sum), RH-141288 (sum), RH-141452 and RH-24549, and 0.0015 mg/kg for single isomers.
Analytical method validated in study BPL-STUDY-18-000085

Max. Storage interval between sampling and analysis:	table grapes: 481 days raisins: 484 days

ND = not detectable, NR = not relevant

A 2.1.3.1.8 Study 8

Comments of zRMS:	Not relevant. For SEU.

Reference:	**KCA 6.3/11**
Report:	Maccaferri, L. 2019: Determination of the residues of zoxamide and/or phosphorous acid in table grape raw agricultural commodity following five applications of GOW F 716, Zoxium 240 SC, GOW F 316 in open field conditions (one harvest trial, Italy 2017)[15] Gowan Crop Protection Ltd., UK Renolab S.r.l., Italy, Report No. 17120-01R, GLP, Not published
and	
Reference:	**KCA 6.3/12**
Report:	Maccaferri, L. 2019: Determination of the residues of zoxamide and/or phosphorous acid in raw agricultural commodity of grapevine and processed commodities (juice, must, young wine and bottled wine) following five applications of GOW F 716, Zoxium 240 SC, GOW F 316 in open field conditions (one harvest trial, Italy 2017) Gowan Crop Protection Ltd. UK, Renolab S.r.l., Italy, Report No. 17120-02R, GLP, Not published
and	
Reference:	**KCA 6.3/13**
Report:	Maccaferri, L., 2020: Magnitude of the residues of zoxamide enantiomers and metabolites in grapes and processed commodities (juice, must, young wine and bottled wine) following five applications of GOW 716 and Zoxium 240 SC in open field condition (Italy 2017) Gowan Crop Protection Ltd., UK Renolab S.r.l. Italy, Report No. 19200-01R, GLP, Not published
Guideline(s):	OECD TG 509 (2009) OECD TG 508 (2008)
Deviation(s):	Due to response instability in analytical sequence with both solvent and matrix standards, the matrix effects were not calculated. This deviation was regarded as not relevant for the integrity of the study since the analysis is performed with matrix matched standards. For some analyte / matrix combinations the mean recovery was >110 % but ≤120 %. Since recovery values of 70-120% for the here implied concentration ranges of 0.01-0.1 mg/kg and below (0.005-0.05 mg/kg) are acceptable according to SANCO/825/00 rev. 8.1 (2010), this deviation from the study plan is regarded as not relevant. For the following analyte / matrix combination the mean recovery was < 70 %: (A) RH-129151 and (B) RH-129151 at 0.05 mg/L in young wine. How-

[15] Confirmatory study for zoxamide currently being evaluated by RMS (Latvia).

	ever, the overall mean recovery for young wine (at different fortification levels) was 72.7 ±15.9 %.
GLP:	Yes
Acceptability:	Yes

Materials and methods

Test item (Lot/Batch No.):	GWN 9790 EU/Zoxium 240 SC (SB 2401)
Active substance content (nominal):	240 g/L Zoxamide (nominal), 232 g/L (analysed) (R/S Zoxamide ratio: 50/50)
Expiry date:	April 2018
Test item (Lot/Batch No.):	GOW F 716 (P1612669001)
Active substance content (nominal):	60 g/L Zoxamide (nominal), 65.5 g/L (analysed) (R/S Zoxamide ratio: 50/50) Dipotassium phosphonate (K_2HPO_3) and Monopotassium phosphonate (KH_2PO_3) at a concentration of 504 g/L phosphorous acid (nominal), 498 g/L (analysed)
Expiry date:	December 2018

The magnitude of residues of zoxamide (sum and R and S isomers) and RH-141452 in raw commodities (grape fruits) and of zoxamide (sum and R and S isomers), RH-150721 (as sum of R and S isomers), (R)-RH-150721, (S)-RH-150721, RH-129151 (as sum of R and S isomers), (R)-RH-129151, (S)- RH-129151, RH-24549, RH-141288 (as sum of R and S isomers), (R)-RH-141288 and (S)-RH-141288 in juice, must, young and bottled wine coming from 4 harvest trials in the open field in Southern Europe (Italy) were determined. For this analysis, the retain samples of studies no. 17120-01R and 17120-02R were taken forward.

Besides untreated control plots, further plots were performed with 5 applications of either Zoxium 240 SC (containing nominally 240 g/L Zoxamide) or GOW F 716 (containing nominally 60 g/L Zoxamide) at a rate corresponding to 180 g/ha zoxamide in 1000 L water/ha at an interval of 8 ± 1 days.

Commodity (RAC) samples were collected at commercial harvest time (27 or 28 DALA). They were kept at 5°C until processing. Samples for residues analysis were frozen at ≤ -18°C within 3 hours (report 17120-02R) and 24 hours (report 17120-01R) after sampling in the field and remained deep frozen until extraction and analysis.

The processing of the grapes began within 24 hours after sampling.

For the juice production the following steps were taken:

1. Crushing-pressing of the grapes to obtain the raw must
2. Addition of the following oenological products: preservative - sulphur dioxide, 100-120 mg/L; pectolytic enzyme, 2-5 g/100 kg
3. Settling of the raw must under conditions of low temperature (2-5°C) for minimum 24 hours (temperature monitoring)
4. Racking of the must at the end of settling process and visual control of its turbidity
5. Possible further addition to the limpid must of the following oenological products: vegetable gelatine 10-30 g/100 kg; bentonite, 20-50 g/100 kg
6. Racking of the settled must at the end of the second settling process

7. Filtration of must by means of cartridges with different porosity (up to 1.2 μm) in order to obtain a limpid product
8. Packaging of limpid juice into glass bottles
9. Pasteurization of the packaged juice at 80-90°C for at least 30 min., monitoring the temperature of the product

For the white wine production, the following steps were taken:

1. Crushing-pressing of the grapes to obtain the must
2. Addition to the raw must of following oenological products: preservative - sulphur dioxide, 50-60 mg/L; clarifying agents – potassium caseinate 5-10 g/100 Kg and/or bentonite 20-30 g/100 Kg; yeasts nutrient – ammonium sulphate, up to 180 mg/L of nitrogen (if necessary); selected yeasts for must fermentation – suitable trade strain, 20-30 g/100 kg
3. Placement of the must sample in a thermo-conditioned room (15-20°C) for the fermentation phase (temperature monitoring)
4. Daily control of the fermentation process by means of a specific instrument (non-GLP assessment): recording of sugar content and temperature (°C)
5. Racking of the raw wine at the end of fermentation process
6. Addition to the raw wine of following oenological products: preservative - sulphur dioxide, up to 90-100 mg/L; clarifying agents – potassium caseinate 5-20 g/100 kg and/or bentonite 20-30 g/100 kg
7. Storage of the raw wine at -5° C for the tartaric stabilization – duration of the phase minimum 3 weeks (temperature monitoring)
8. Racking of the stabilised wine at the end of the phase described above
9. Addition of sulphur dioxide to the stabilised wine (maximum limit of 150 mg/L)
10. Filtration of the wine by means of cartridges with different porosity (up to 0.65 μm) to obtain a limpid/shiny product

The field and processing parts were performed by ASTRA, Italy under the phase report number PCR_17_GOW_VITVI-UV_ZP-H. The analytical phase was performed by Renolab S.r.l. in Italy under report no. 19200-01R according to method BPL-STUDY-18-000085, which has been validated according to SANCO/825/00 rev. 8.1 and SANCO/3029/99 rev. 4. The method has been re-validated for the determination of zoxamide (as sum of R and S isomers), (R)-Zoxamide, (S)-Zoxamide, metabolites RH-141452, RH-150721 (as sum of R and S isomers), (R)-RH-150721, (S)-RH-150721, RH-129151 (as sum of R and S isomers), (R)-RH-129151, (S)- RH-129151, RH-24549, RH-141288 (as sum of R and S isomers), (R)-RH-141288 and (S)-RH-141288 in Raw Agricultural Commodity grapes (bunches) and processed samples (raisins) during the course of this study.

Results and discussion

The residues of the zoxamide metabolites containing a chiral centre (RH-150721, RH-129151 and RH-141288) were expressed as sum of 2 enantiomers and per single enantiomer (R and S). However, for metabolite RH-129151 the correlation between the absolute configuration of the enantiomer (R) or (S) and the corresponding chromatographic peaks was not available; therefore, the first eluted peak was identified as RH-129151 (A) and the second eluted peak as RH-129151 (B).

The metabolite RH-141452, which is known to form conjugates with matrix molecules (e.g. sugars), was released in an additional hydrolysis step to establish total fractions in addition to the free fractions in the matrices. For RH-141452 the total fractions are considered as worst-case.

The limit of quantification (LOQ) was set at 0.01 mg/kg for zoxamide, RH-150721, RH-129151 and RH-141288 (sum of enantiomers), RH-141452 and RH-24549, and 0.005 mg/kg for single isomers. The limit

of determination (LOD) was given as 0.003 mg/kg for RH-150721, RH-129151, RH-141288 (sum of enantiomers), RH-141452 and RH-24549, and 0.0015 mg/kg for single isomers.

No residues were found in the control plot.

A summary of the results is given in the following table.

(Maccaferri L. 2019a,b)

(Maccaferri L. 2020)

Table A 22: Summary of the study 8 trial on grapes / Southern EU

Reference:	Maccaferri L. 2020, 19200-01R; field report no. 17120-01R		
GLP:	Yes	Sample storage conditions:	≤ -18°C for residues analysis
Crop/crop group:	Grapes / Fruiting crops	Analytical method:	STUDY-18-000085
Indoor/Outdoor:	Outdoor	Limit of Quantification (mg/kg):	0.01 mg/kg (sum), 0.005 mg/kg (for each isomer)
Formulation:	SC	Limit of Detection (mg/kg):	0.003 mg/kg (sum), 0.0015 mg/kg (for each isomer)
Content of active substance (g/kg or g/l):	60 g/L Zoxamide	Residues calculated as:	Zoxamide (sum). (R)-Zoxamide. (S)-Zoxamide, RH-150721 (sum), (R)-RH-150721, (S)-RH-150721, RH-129151 (sum), (R)-RH-129151, (S)-RH-129151, RH-141288, (R)-RH-141288, (S)-RH141288 and RH24549, RH-141452

Trial No. Location EU zone Year	Com-modity/ Variety	Date of 1. Sowing or Planting 2. Flower-ing 3. Harvest	Application rate per treatment			Dates of treat-ment(s) or no of treat-ment(s) and last date	Gro wth stage at last treat-ment or date	Portion ana-lysed	Residues (mg/kg)																
			kg as/ha	Water (L/ha)	kg as/hl				Zoxamide (sum)	Zoxamide (R)	Zoxamide (S)	RH-150721 (sum)	(R)-RH-150721	(S)-RH-150721	RH-129151 (sum)	RH-129151 (A)	RH-129151 (B)	RH-141288 (sum)	(R)-RH-141288	(S)-RH-141288	Total RH-141452	Free RH-141452	RH-24549	PHI (days)	Remarks
PCR_17_GOW_VITVI UT_ ZP-H01 40026 Linaro di Imola (Bologna) Italy S-EU-2017	Plot 2																								
	Table grape (Italia)	1) - 2) - 3) 11/09/2017	0.180	1000	0.018	13/07/2017 20/07/2017 29/07/2017 07/08/2017 14/08/2017	77 79 81 83 85	Bunches	0.31	0.16	0.15	NR	NR	NR	NR	NR	NR	NR	NR	NR	< LOQ (0.008)	ND	NR	28	*

* LOQ = 0.010 mg/kg for zoxamide (sum), RH-150721 (sum), RH-1292151 (sum), RH-141288 (sum), RH-141452 and RH-24549, and 0.005 mg/kg for single isomers.
LOD = 0.003 mg/kg for zoxamide (sum), RH-150721 (sum), RH-1292151 (sum), RH-141288 (sum), RH-141452 and RH-24549, and 0.0015 mg/kg for single isomers.
Analytical method validated in study BPL-STUDY-18-000085
Max. Storage interval between sampling and analysis: 821 days
ND = not detectable, NR = not relevant

Table A 23: Summary of the study 8 trial on grapes / Southern EU

Reference:	Maccaferri L. 2020, 19200-01R; field report no. 17120-01R		
GLP:	Yes	Sample storage conditions:	≤ -18°C for residues analysis
Crop/crop group:	Grapes / Fruiting crops	Analytical method:	STUDY-18-000085
Indoor/Outdoor:	Outdoor	Limit of Quantification (mg/kg):	0.01 mg/kg (sum), 0.005 mg/kg (for each isomer)
Formulation:	SC	Limit of Detection (mg/kg):	0.003 mg/kg (sum), 0.0015 mg/kg (for each isomer)
Content of active substance (g/kg or g/l):	240 g/L Zoxamide	Residues calculated as:	Zoxamide (sum). (R)-Zoxamide. (S)-Zoxamide, RH-150721 (sum), (R)-RH-150721, (S)-RH-150721, RH-129151 (sum), (R)-RH-129151, (S)-RH-129151, RH-141288, (R)-RH-141288, (S)-RH141288 and RH24549, RH-141452

Trial No. Location EU zone Year	Com-modity/ Variety	Date of 1. Sowing or Planting 2. Flowering 3. Harvest	Application rate per treatment			Dates of treat-ment(s) or no of treat-ment(s) and last date	Gro wth stage at last treat-ment or date	Portion ana-lysed	Residues (mg/kg)																
			kg as/ha	Water (L/ha)	kg as/hl				Zoxamide (sum)	Zoxamide (R)	Zoxamide (S)	RH-150721 (sum)	(R)-RH-150721	(S)-RH-150721	RH-129151 (sum)	RH-129151 (A)	RH-129151 (B)	RH-141288 (sum)	(R)-RH-141288	(S)-RH-141288	Total RH-141452	Free RH-141452	RH-24549	PHI (days)	Remarks
PCR_17_GOW_VITVIUT_ZP-H01 40026 Imola (Bologna) Italy S-EU-2017	Plot 3																								
	Table grape (Italia)	1) - 2) - 3) 11/09/2017	0.180	1000	0.018	13/07/2017 20/07/2017 29/07/2017 07/08/2017 14/08/2017	77 79 81 83 85	Bunches	0.39	0.20	0.19	NR	NR	NR	NR	NR	NR	NR	NR	NR	< LOQ (0.006)	< LOQ (0.003)	ND	28	*

* LOQ = 0.010 mg/kg for zoxamide (sum), RH-150721 (sum), RH-1292151 (sum), RH-141288 (sum), RH-141452 and RH-24549, and 0.005 mg/kg for single isomers.
LOD = 0.003 mg/kg for zoxamide (sum), RH-150721 (sum), RH-1292151 (sum), RH-141288 (sum), RH-141452 and RH-24549, and 0.0015 mg/kg for single isomers.
Analytical method validated in study BPL-STUDY-18-000085
Max. Storage interval between sampling and analysis: 821 days
ND = not detectable, NR = not relevant

Table A 24: Summary of the study 8 trial on grapes / Southern EU

Reference:	Maccaferri L. 2020, 19200-01R; field report no. 17120-02R		
GLP:	Yes	Sample storage conditions:	≤ -18°C for residues analysis
Crop/crop group:	Grapes / Fruiting crops	Analytical method:	STUDY-18-000085
Indoor/Outdoor:	Outdoor	Limit of Quantification (mg/kg):	0.01 mg/kg (sum), 0.005 mg/kg (for each isomer)
Formulation:	SC	Limit of Detection (mg/kg):	0.003 mg/kg (sum), 0.0015 mg/kg (for each isomer)
Content of active substance (g/kg or g/l):	60 g/L Zoxamide	Residues calculated as:	Zoxamide (sum). (R)-Zoxamide. (S)-Zoxamide, RH-150721 (sum), (R)-RH-150721, (S)-RH-150721, RH-129151 (sum), (R)-RH-129151, (S)-RH-129151, RH-141288, (R)-RH-141288, (S)-RH141288 and RH24549, RH-141452

Trial No. Location EU zone Year	Com-modity/ Variety	Date of 1. Sow-ing or Planting 2. Flow-ering 3. Har-vest	Application rate per treatment			Dates of treat-ment(s) or no of treat-ment(s) and last date	Growth stage at last treat-ment or date	Portion ana-lysed	Residues (mg/kg)																
			kg as/ha	Water (L/ha)	kg as/hl				Zoxamide (sum)	Zoxamide (R)	Zoxamide (S)	RH-150721 (sum)	(R)-RH-150721	(S)-RH-150721	RH-129151 (sum)	RH-129151 (A)	RH-129151 (B)	RH-141288 (sum)	(R)-RH-141288	(S)-RH-141288	Total RH-141452	Free RH-141452	RH-24549	PHI (days)	Remarks
PCR_17_GOW_VITVIUT_ZP-H01 40026 Linaro di Imola (Bologna) Italy S-EU-2017	Plot 2																								
	Grape-vine (Char-donnay)	1) - 2) - 3) 17/08/2017	0.180	1000	0.018	19/06/2017 27/06/2017 05/07/2017 13/07/2017 21/07/2017	77 77-79 79 79 83	Bunches	0.22	0.11	0.11	NR	NR	NR	NR	NR	NR	NR	NR	NR	0.014	ND	ND	27	*
								Limpid juice	0.014	0.0071	0.067	< LOQ (0.008)	< LOQ (0.004)	< LOQ (0.004)	ND	ND	ND	ND	ND	ND	0.013	< LOQ (0.006)	ND		
								Must	0.24	0.12	0.12	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.012	ND	ND		
								Young wine	0.0058	0.032	0.025	0.018	0.0076	0.020	ND	ND	ND	ND	ND	ND	0.014	< LOQ (0.009)	ND		
								Bottled wine	0.0047	0.026	0.021	0.024	0.011	0.015	ND	ND	ND	ND	ND	ND	0.013	< LOQ (0.008)	ND		

* LOQ = 0.010 mg/kg for zoxamide (sum), RH-150721 (sum), RH-1292151 (sum), RH-141288 (sum), RH-141452 and RH-24549, and 0.005 mg/kg for single isomers.
LOD = 0.003 mg/kg for zoxamide (sum), RH-150721 (sum), RH-1292151 (sum), RH-141288 (sum), RH-141452 and RH-24549, and 0.0015 mg/kg for single isomers.
Analytical method validated in study BPL-STUDY-18-000085
Max. Storage interval between sampling and analysis: barriers: 846 days
limpid juice: 846 days
must: 853 days
young wine: 743 days
bottled wine: 660 days

ND = not detectable, NR = not relevant

Table A 25: Summary of the study 8 trial on grapes / Southern EU

Reference:	Maccaferri L. 2020, 19200-01R; field report no. 17120-02R		
GLP:	Yes	Sample storage conditions:	≤ -18°C for residues analysis
Crop/crop group:	Grapes / Fruiting crops	Analytical method:	STUDY-18-000085
Indoor/Outdoor:	Outdoor	Limit of Quantification (mg/kg):	0.01 mg/kg (sum), 0.005 mg/kg (for each isomer)
Formulation:	SC	Limit of Detection (mg/kg):	0.003 mg/kg (sum), 0.0015 mg/kg (for each isomer)
Content of active substance (g/kg or g/l):	240 g/L Zoxamide	Residues calculated as:	Zoxamide (sum). (R)-Zoxamide. (S)-Zoxamide, RH-150721 (sum), (R)-RH-150721, (S)-RH-150721, RH-129151 (sum), (R)-RH-129151, (S)-RH-129151, RH-141288, (R)-RH-141288, (S)-RH141288 and RH24549, RH-141452

Trial No. Location EU zone Year	Commodity/ Variety	Date of 1. Sowing or Planting 2. Flowering 3. Harvest	Application rate per treatment			Dates of treatment(s) or no of treatment(s) and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)																
			kg as/ha	Water (L/ha)	kg as/hl				Zoxamide (sum)	Zoxamide (R)	Zoxamide (S)	RH-150721 (sum)	(R)-RH-150721	(S)-RH-150721	RH-129151 (sum)	RH-129151 (A)	RH-129151 (B)	RH-141288 (sum)	(R)-RH-141288	(S)-RH-141288	Total RH-141452	Free RH-141452	RH-24549	PHI (days)	Remarks
PCR_17_GOW_VITVIUT_ZP-H01 40026 Linaro di Imola (Bologna) Italy S-EU-2017	Plot 3																								
	Grapevine (Chardonnay)	1) - 2) - 3) 17/08/2017	0.180	1000	0.018	19/06/2017 27/06/2017 05/07/2017 13/07/2017 21/07/2017	77 77-79 79 79 83	Bunches	0.26	0.13	0.13	NR	NR	NR	NR	NR	NR	NR	NR	NR	0.012	ND	ND	27	*
								Limpid juice	ND	ND	ND	0.010	< LOQ (0.0048)	0.0053	ND	ND	ND	ND	ND	ND	0.011	< LOQ (0.008)	ND		
								Must	0.22	0.11	0.11	ND	N.D:	N.D.	ND	ND	ND	ND	ND	ND	0.012	ND	ND		
								Young wine	0.051	0.029	0.022	0.015	0.0064	0.0087	ND	ND	ND	ND	ND	ND	0.013	< LOQ (0.007)	ND		
								Bottled wine	0.043	0.024	0.019	0.021	0.0090	0.012	ND	ND	ND	ND	ND	ND	0.011	< LOQ (0.006)	ND		

* LOQ = 0.010 mg/kg for zoxamide (sum), RH-150721 (sum), RH-1292151 (sum), RH-141288 (sum), RH-141452 and RH-24549, and 0.005 mg/kg for single isomers.
LOD = 0.003 mg/kg for zoxamide (sum), RH-150721 (sum), RH-1292151 (sum), RH-141288 (sum), RH-141452 and RH-24549, and 0.0015 mg/kg for single isomers.
Analytical method validated in study BPL-STUDY-18-000085
Max. Storage interval between sampling and analysis: barriers: 846 days

limpid juice: 846 days
must: 853 days
young wine: 743 days
bottled wine: 660 days

ND = not detectable, NR = not relevant

A 2.1.3.1.9 Study 9

Comments of zRMS:	Not relevant. For SEU.

Reference:	**KCA 6.3/14**
Report	Luciani, P.G., 2016: Determination of zoxamide and benalaxyl-m residues after three applications of GWN-10392 on wine grapes under field conditions – Italian trial, year 2015[16] Gowan Comércio Internacional e Servicos, Limitada, Portugal Tentamus AgriParadigma srl, Italy, Report No. AGRI 009/15 GLP HAR, GLP, Not published
Guideline(s):	OECD TG 509 (2009) SANCO 7029/Vl/95 - rev. 5 (1997) OECD TG 508 (2008) SANCO 7035/VI/95 - rev. 5 (1997)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Test item (Lot/Batch No.):	GWN-10392 (G005/15)
Active substance content (nominal):	Zoxamide: 225 g/L (nominal), 228.68 g/L (analysed) Benalaxyl-m: 150 g/L (nominal), 143.10 g/L (analysed)
Expiry date:	31 July 2017

The magnitude of residues of zoxamide and benalaxyl-m in black wine grapes and its processed commodities (limpid juice, must, young and old wine) has been determined in one harvest trial performed in Southern Europe (Italy) in 2015. The test item GWN-10392 has been applied three times to the foliar with an 8 days interval and a last application 28 days before harvest.

Two plots were established per trial, one plot (control) was left untreated, another plot was treated three times with each 0.7 L product/ha (157.5 g/ha zoxamide and 105 g/ha benalaxyl-m). The test item was applied with a mist sprayer to reflect common agricultural practice.

For processing, the raw agricultural commodity (grape bunches) was collected and stored at 5°C until the beginning of the processing at Astra in Italy. All other specimens for residue analysis were immediately frozen down in the field and transported in dry ice to the laboratory for storage at ≤ -18°C until analysis.

The limpid juice production was characterised by the following steps:

- Pressing of the grapes to obtain the raw must.
- Addition of 100-150 mg/L sulphur dioxide (depending on pH of must) and 2-5 g/100 kg pectolytic enzymes (depending by the turbidity of must).
- Settling of raw must at 2-5°C for a minimum 24 hours.
- Racking of must.

[16] Confirmatory study for zoxamide currently being evaluated by RMS (Latvia).

- Addition of 10-30 g/100 vegetable gelatine and/or 20-50 g/100 kg bentonite – as needed.
- Racking of must.
- Filtration of must.
- Packaging of limpid juice into glass bottles.
- Pasteurization at 90-98°C for at least 30 min.

The wine production was characterised by the following steps:

- Removal of grape stalks and crushing of the berries.
- Addition of 50-70 mg/L sulphur dioxide, yeast, etc.
- Fermentation/maceration at 15-20°C, incl. maceration of the peels.
- Separation of the solid part (macerated peels) from the liquid part (fermenting must).
- Racking of raw wine.
- Addition of up to 90-100 mg/L sulphur dioxide, 5-10 g/100 kg gelatine and/or 30-40 g/100 kg bentonite – as needed.
- Stabilisation of the wine for at min. 3 weeks at -5°C.
- Racking of stabilised wine.
- Addition of sulphur dioxide (max. of 150 mg/L).
- Filtration of the wine.

The analytical phase has been performed by Tentamus Agriparadigma Srl in Italy under analytical phase report number AGRI 009/15 GLP HAR with an LC-MS/MS method developed and validated according to SANCO/3029/99 rev. 4 under report no. Agri BPL 064 rev. 1.

Results and discussion

The concentrations of zoxamide and benalaxyl-m were determined in grapes (RAC) or processed specimens. The limit of quantification (LOQ) for zoxamide and benalaxyl-m in wine grapes, limpid juice, must and wine was set at 0.01 mg/kg.

As a result, no residue of zoxamide and benalaxyl-m were found above LOQ in untreated RAC and processed samples. A summary of the results from the treated plots is given in the following table.

(Luciani G.P. 2016)

Table A 26: Summary of the study 9 trial on grapes / Southern EU

Reference:	Luciani G.P. 2016, AGRI 009/15 GLP HAR		
GLP:	Yes	Sample storage conditions:	at ≤ -18 °C for residues analysis
Crop/crop group:	Wine grape	Analytical method:	Agri BPL 064 rev. 1
Indoor/Outdoor:	Outdoor	Limit of Quantification (mg/kg):	0.01 mg/kg
Formulation:	SC	Limit of Detection (mg/kg):	--
Content of active substance (g/kg or g/l):	225 g/L zoxamide 150 g/L benalaxyl-m	Residues calculated as:	Zoxamide

Trial No./ Location/ EU zone/ Year	Commodity/ Variety	Date of 1.Sowing or planting 2.Flowering 3. Harvest	Application rate per treatment			Dates of treat-ment or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			kg a.s./ ha	Water (L/ha)	kg a.s./hL				Zoxamide		
AGRI 009/15 GLP HAR 40060 Dozza (BO) Italy EU-South 2015	Wine grape	1) - 2) - 3) 22/09/15	0.164 0.164 0.170	921.8 923.9 953.8	- - -	10/08/15 18/08/15 26/08/15	83-85 85 85	Wine grape Limpid juice Must Young wine Aged wine	0.27 < 0.01 0.05 0.02 0.01	28	LOQ: 0.01 mg/kg Analytical method Agri BPL 064 rev. 1 Max. freezer storage period: 167 days

A 2.1.3.1.10 Study 10

Comments of zRMS:	The study is acceptable. 2 trials were conducted in Northern France. The analytical method (LC-MS/MS) was validated in terms of accuracy, precision, linearity, selectivity, LOQ and LOD in compliance with guidelines SANCO/825/00 rev. 8.1 (16/11/2010) and SANCO/3029/99, rev. 4 (11/07/2000) by means of recovery tests and analysis of blank samples on bunches, must and wine. A mean recovery of 70-110% with a Relative Standard Deviation lower than 20% was adopted as acceptability criteria. The validation parameters were in required range

Reference:	**KCA 6.3/15**
Report	Perboni, A., 2017: Determination of ~~cymoxanil~~ benalaxyl-m and zoxamide residues in raw agricultural commodity grapes (wine and table) and processed commodity (must, fermenting must, wine and aged wine) following three applications of GWN-10392 (benalaxyl-m 150 g/L + zoxamide 225 g/L) in open field condition (3 harvest trials, northern and southern Europe, year 2015)[17] Gowan Crop Protection Limited, UK BioTecnologie BT Srl., Italy, Report No. RAU-049-15, GLP, Not published
Guideline(s):	OECD TG 509 (2009)
Deviations:	Deviation during processing phase of trial F/BZ15/GR03: The must commodity was sampled 7 days later than planned since the fermentation did not start in time. This deviation was regarded to have no impact on the results and the integrity of the study.
GLP:	Yes
Acceptability:	Yes

Materials and methods

Test item (Lot/Batch No.)	GWN-10392 (G005/15)
Active substance content (nominal)	Zoxamide: 225 g/L (nominal), 228.68 g/L (analysed) Benalaxyl-m: 150 g/L (nominal), 143.10 g/L (analysed)
Expiry date	31.07.2017

The magnitude of cymoxanil and zoxamide residues in Raw Agricultural Commodity grapes (wine and table) and processed commodities (must, fermenting must, wine and aged wine) has been determined in 3 harvest trials in Southern and Northern Europe (Greece and Northern France) in 2015.

Two plots were established per trial, one plot (control) was left untreated, another plot was treated three times with each 0.7 L/ha GWN-10392 (containing 150 g/L benalaxyl-m + 225 g/L zoxamide) at nominal rates of 105 g/ha benalaxyl-m and 157.5 g/ha zoxamide in 200-1000 L/ha water at an interval of 8 days and with the last application 28 days before harvest.

The raw agricultural commodities (grape bunches) were harvested and stored at room temperature until processing at Research Centre Biospheres in Italy. All residues samples were frozen down within 3 hours and kept frozen at ≤ -18°C until analysis at Research Centre Biospheres in Italy.

[17] Confirmatory study for zoxamide currently being evaluated by RMS (Latvia).

During processing, must, fermenting must, young and aged wine samples were collected.

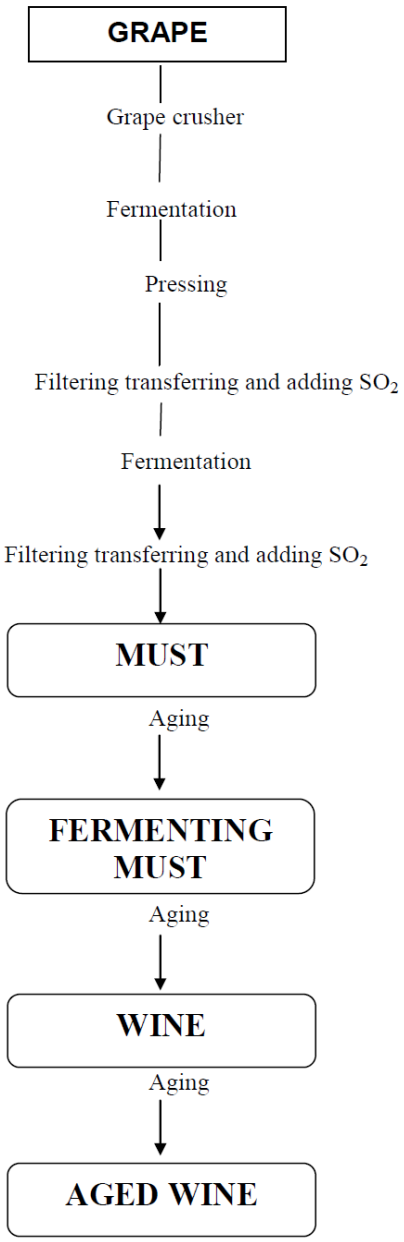


Figure A 6: Processing of must and red wine

The analytical method has been validated according to SANCO/825/00 rev. 8.1 (2010) and SANCO/3029/99 rev. 4 (2000) by means of recovery experiments on blank samples of bunches, must and wine.

Results and discussion

The concentrations of zoxamide and benalaxyl-m were determined in grapes (wine and table) and processed commodities (must, fermenting must, wine and aged wine) at a Limit of Quantification (LOQ) of 0.01 mg/kg for both analytes. The LOD was defined as the lowest measurable concentration at which the signal is higher than at least three times the background noise – at 0.003 mg/kg for both analytes. As a result, no residue of zoxamide and benalaxyl-m were found below the LOQ in the (untreated) control samples. A summary of the results of the treated specimens is given in the following table. (Perboni A. 2017)

Table A 27: Summary of study 10 trial on grapes / Southern EU

Reference:	Perboni A. 2017, RAU-049-15		
GLP:	Yes	Sample storage conditions:	at ≤ -18 °C for residues analysis
Crop/crop group:	Table grapes	Analytical method:	RAU-049-15
Indoor/Outdoor:	Outdoor	Limit of Quantification (mg/kg):	0.01 mg/kg
Formulation:	SC	Limit of Detection (mg/kg):	0.003 mg/kg
Content of active substance (g/kg or g/l):	225 g/L zoxamide 150 g/L benalaxyl-m	Residues calculated as:	zoxamide

Trial No./ Location/ EU zone/ Year	Commodity/ Variety	Date of 1.Sowing or planting 2.Flowering 3. Harvest	Application rate per treatment			Dates of treatment or no. of treatments and last date	Growth stage at last treatment or date BBCH	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			g a.s./ ha	Water (L/ha)	g a.s./hL				Zoxamide		
G/BZ15/GR01 64007 Elaiochori, Kavala Greece S-EU, 2015	Table grapes Soultanina	1) Year 2008 2) 10/05/2015 – 20/05/2015 3) 15/09/2015 – 30/09/2015	161.68 163.17 162.30	807.45 814.91 810.56	19.7 19.7 19.7	3 20/08/2015	81	Grapes	1.026	28	LOQ: 0.01 mg/kg LOD: 0.003 mg/kg Analytical method validated under report no. RAU-049-15 Max. freezer storage period: 233 days

Table A 28: Summary of the study 10 trials on grapes / Northern EU

Reference:	Perboni A. 2017, RAU-049-15		
GLP:	Yes	Sample storage conditions:	at ≤ -18 °C for residues analysis
Crop/crop group:	Table grapes	Analytical method:	RAU-049-15
Indoor/Outdoor:	Outdoor	Limit of Quantification (mg/kg):	0.01 mg/kg
Formulation:	SC	Limit of Detection (mg/kg):	0.003 mg/kg
Content of active substance (g/kg or g/l):	225 g/L zoxamide 150 g/L benalaxyl-m	Residues calculated as:	zoxamide

Trial No./ Location/ EU zone/ Year	Commodity/ Variety	Date of 1.Sowing or planting 2.Flowering 3. Harvest	Application rate per treatment			Dates of treatment or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			g a.s./ ha	Water (L/ha)	g a.s./hL				Zoxamide		
F/BZ15/GR02 72340 Ruillè sur Loir France N- EU, 2015	Wine grapes Chardonnay	1Year 1995 2) 28/05/2015-03/06/2015 3) 24/09/2015 – 25/09/2015	156.4 163.0 167.4	684 713 732	22.9 22.9 22.9	28/08/2015	85	Grapes	0.4796	28	LOQ: 0.01 mg/kg LOD: 0.003 mg/kg Analytical method validated under report no. RAU-049-15 Max. freezer storage period: 233 days
F/BZ15/GR03 51220 Brimont France N-EU, 2015	Wine grapes Pinot noir	1Year 1995 2) 10/06/2015 – 30/06/2015 3) 02/09/2015	159.4 157.8 156.2	697 690 683	22.9 22.9 22.9	12/08/2015	81	Grapes	0.2892	28	LOQ: 0.01 mg/kg LOD: 0.003 mg/kg Analytical method validated under report no. RAU-049-15 Max. freezer storage period: 233 days
								Must	0.0157		
								Fermenting must	< 0.01		
								Wine	< 0.01		
								Aged wine	< 0.01		

A 2.1.3.2 Tomato

Table A 29: Comparison of intended and critical EU GAPs

Type of GAP	Number of applications	Application rate per treatment (precise unit)	Interval between application	Growth stage at last application	PHI (days)
cGAP EU (--)	--	--	--	--	--
cGAP EU (Art. 12, EFSA, year)	--	--	--	--	--
Intended cGAP (30-36; 31-37*)	3	148.5 g/ha	7-10	Not relevant	3

* Use number(s) in accordance with the list of all intended GAPs in Part B, Section 0

The following study of Romanini (2011; report no. CREG2118) was considered for the authorisation of Cymoxanil 33% + Zoxamide 33% WG and has been regarded as valid. It is summarised in the following.

A 2.1.3.2.1 Study 1

Comments of zRMS:	No tomatoes in the GAP for CEU. The study is for SEU. Not relevant. Previously evaluated.

Reference:	**KCA 6.3/16**
Report:	Romanini, M., 2011: Determination of cymoxanil and zoxamide residues at harvest in raw and processed agricultural commodity tomato (fruit, juice, puree and canned) following five applications of Harpon WG (Cymoxanil 33% + Zoxamide 33% WG) - Four trials, Italy 2010 Gowan Comercio Internacional e Servicos Limitada, Portugal Research Centre "E. Gagliardini, Italy, Report No. CREG2118, GLP, Not published
Guideline(s):	1607/VI/97 rev. 2, Appendix B 7029/VI/95 rev. 5 ENV /JM/MONO (2007)17 ENV/MC/CHEM (98)17
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Test item (Lot/Batch No.):	Cymoxanil 33% + Zoxamide 33% WG / Harpon WG (XE28160A11)
Active substance content (nominal):	33 % Cymoxanil + 33% Zoxamide (nominal) 32.7 % Cymoxanil + 33.5% Zoxamide (analysed)
Expiry date:	July 2011

Four trials in southern Europe are available on tomatoes treated with Cymoxanil 33% + Zoxamide 33% WG (Harpon WG, batch XE28160A11, Cymoxanil 32.7%, Zoxamide 33.5%) with 5 applications at 0.15 kg as/ha, 7 days interval. Samples were collected 3 days after the last application. These trials have been conducted in SEU whereas the proposed GAP is in NEU; therefore, these trials do not support (according to a strict reading of the guidance) the intended GAP. However, the trials are supportive of the GAP (number of applications, timing and PHI are the same as the intended GAP) in all other respects and may therefore be considered supportive of the intended GAP if the issue relating to the definition of Romania as being in the northern EU, climatically, is left aside.

Samples arising from trial I/CZ10/TO02 underwent processing steps in order to obtain juice, puree and canned tomato to be analysed. See section on processing, below.

The field phase has been performed by Research Centre "E. Gagliardini – SIPCAM S.p.A. in Italy and SOCIETA COOPERATIV ARES AGRARIA A R.L in Italy under phase report no. CREG2118, the processing and analytical phase have been performed by Research Centre "E. Gagliardini – SIPCAM S.p.A. in Italy under phase report no. CREG2118 with a method validated according to SANCO/825/00 rev. 7 and SANCO/3029/99 rev. 4 under report no. RAU/038 for Cymoxanil and TR 34-99-111 (Burdge, E., et al, (1999)) for Zoxamide.

Figure 1 – Flowchart of the processed tomato

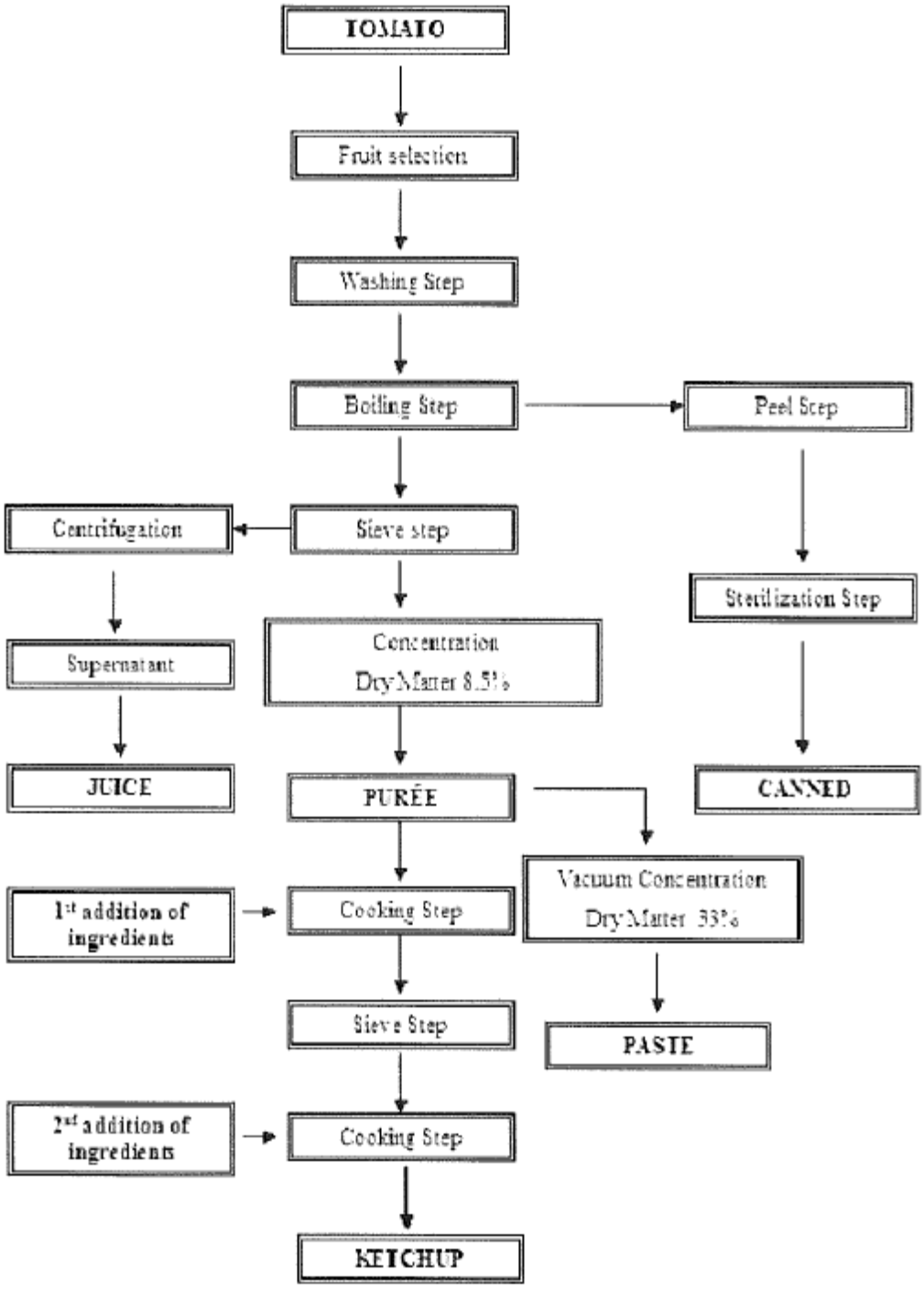


Figure A 7: Flowchart on processed tomato

For cymoxanil the sample was extracted by Ultra-Turrax with ethyl acetate, purified by liquid-liquid partition and analysed by gas chromatography with a Nitrogen Phosphorus Detector (GC/NPD). Zoxamide was determined using an internal analytical method that consisted in a solvent extraction by Ultraturrax (bunch samples) or by shaking (processed samples). The extract containing the active ingredient was cleaned up by liquid-liquid partition and by SPE chromatography, then analysed by gas chromatography equipped with

an ECD. The Limit of Quantification (LOQ) was 0.05 mg/kg for Cymoxanil on tomato samples and 0.01 mg/kg on processed samples; for Zoxamide the LOQ was 0.01 mg/kg for all matrices.

Results and discussion

For cymoxanil residues were below the limit of quantification (LOQ) and below the limit of detection (LOD) in all the specimens analysed (treated, untreated and processed).

For Zoxamide the highest residue observed in tomato fruits was 0.3073 mg/kg. Residues in processed tomato matrices were below the LOQ.

For cymoxanil 3 other trials in southern EU are available conducted with different formulations (WG and WP), results of these studies confirm the above (i.e. LOQ residues are expected). For more information, Tier I summaries of available trials can be found in Appendix 4 of this document (trials were done outdoors at or within 25% of the intended GAP and are considered supportive of the intended GAP). The trials are:

1) SIP 1551 I/CZ/07/TO01; ref. in Appendix 4 is [5 CY]
2) SIP 1551 CY-ZO1/TO/S-03; ref. in Appendix 4 is [6 CY]
3) CY2-PO (location: I-26857, Salerano sul Lambro, (Lodi) Italy; ref. in Appendix 4 is [7 CY]

1) Trial SIP 1551 I/CZ/07/TO01 and

2) Trial SIP 1551 CY-ZO1/TO/S-03

The method of analysis used 'RAU/038' was validated above. Procedural recoveries were acceptable. Samples were stored for a maximum of 68 days prior to analysis, within the period they have been shown to be stable.

3) Trial CY2-PO [7 CY]

See 'IIIA 8.3-25 CY2 I 01PO.pdf' for the field phase summary report and 'IIIA 8.3-26 SIP1299.pdf' for the final report.

The method of analysis used 'SIP 1276' (GC/NPD method, POS H0CYMV01) was validated above. Procedural recoveries were acceptable. Samples were stored for a maximum of 5 days therefore no storage stability data are required.

For Zoxamide 15 additional GLP supervised residue trials on tomatoes were conducted in Southern Europe (outdoor) in different years. In each trial Zoxamide, formulated as either a water dispersible granule (DG or WG) or soluble concentrate (SC), was applied five times at a nominal application rate of 0.15 kg ai/ha each. The last application was made 3 days before typical harvest (i.e. the trials were done at the same rate and timings as the proposed GAP). These trials were not included in the Draft Assessment Report (DAR) for Zoxamide or its addenda and are therefore presented here. The highest residue for Zoxamide in tomatoes was determined at 0.3 mg/kg. The residue analytical method for tomatoes (via GC/ECD) plus an ILV and confirmatory method is given in chapter IIIA 5.3.1 (please refer to ref. K IIIA 5.3.1/01 and KIIIA 5.3.1/02).

For the tomato use in the central zone it should be noted that Cymoxanil 33% + Zoxamide 33% WG is only intended for application in Romania. Romania was listed as belonging to southern Europe as far as the climatic conditions and weather influences are concerned until beginning 2011 (7525_VI_95 - rev.8 appd). This allocation was modified with the new revision of the document (rev 9, March 2011). However the applicant has argued that since ideal growing conditions for tomatoes in terms of climate still reflect those of southern EU countries, it is expected that tomato growing areas in Romania are reflecting this requirement. Taking this into account, the applicant put forward the view that supervised residue trials on tomato performed in southern EU will cover the requirement for registration in Romania.

This argument was not considered acceptable and as a consequence it is concluded that there are no trials to support the proposed GAP. The applicant was informed of this and their response is included below (see HSE ref. W 001568197, enc 83).

"*For Zoxamide, beside the 4 trials performed with Cymoxanil 33% + Zoxamide 33% WG in southern EU, 17 of additional GLP supervised residue trials on tomatoes are available from Southern Europe in different years. Furthermore, 4 trials were conducted under Northern EU growing conditions in the field – a summary of these trials can be found in the revised Part B Section 4 document [HSE ref. 'dRR Part B Section 4_CYM+ZOXrev 1.docx']. In each trial Zoxamide, formulated as either a water dispersible granule (DG or WG) or soluble concentrate (SC), was applied five times at a nominal application rate of 0.15 kg ai/ha each. The highest residue for Zoxamide in tomatoes was determined at 0.3 mg/kg (Southern EU) and 0.34 (Northern EU). Thus, the residue levels under Northern and Southern EU growing conditions are similar and support each other. Besides, all residues in tomato fruits at PHI of 3 days are below the MRL of 0.5 mg/kg set forth in Reg. (EC) No. 396/2005 for Zoxamide in tomato fruits.*

The method used for the determination of Zoxamide in the study of Romanini (2011c) is equivalent to the fully validated tolerance enforcement methods of

- *Burdge E. et al. (1999a), method TR 34-99-111, DERBI 91462, ref. KIIIA 5.3.1/01 to determine Zoxamide in tomato RAC (watery matrix) and processed fractions,*
- *Burdge E. et al. (1998), method TR 34 98 150 (evaluated during Annex I inclusion of Zoxamide; please refer to Part B Section 2) to determine Zoxamide in grapes (watery matrix), grape juice and raisins and*
- *Burdge E. et al. (1999), method TR 34-98-179, DP 81841 (evaluated during Annex I inclusion of Zoxamide; please refer to Part B Section 2) to determine Zoxamide in wine.*

Further validation data on the Zoxamide method are summarised in amendments to the final study report of Romanini (2011c). In addition, an expert statement is provided (Freschi, 2012; ref. KIIIA 8.3.2/03 [see 'KIIIA 8.3.2-03_Freschi 2012_Expert statement.pdf' resource location at HSE]), demonstrating the equivalence of the methods used in the Romanini (2011a,b,c) studies with the fully validated tolerance enforcement methods.

Thus, further residue trials are regarded to be not required.

However, please note the following: The tomato use of Cymoxanil 33% + Zoxamide 33% WG in the central zone is only intended in Romania. Romania was listed as belonging to southern Europe as far as the climatic conditions and weather influences are concerned until beginning 2011 (7525_VI_95 - rev.8 app-d), when the authorisation project was started. This allocation was modified with the new revision of the document (rev 9, March 2011). However, growing of tomatoes in the field is only possible under southern EU growing conditions inclusive Northern France and Romania. ***Therefore, since approval for use of Cymoxanil 33% + Zoxamide 33% WG in tomatoes in the Central zone is only intended in Romania, the applicant requests to restrict the use of the formulated product on tomatoes to only Romania or to handle the decision on the tomato use on national Romanian level."***

Consideration of applicant's response

Regarding the additional trials

The additional 4 trials were conducted under Northern EU growing conditions in the field (as mentioned above) are supportive of the intended GAP.

Regarding the intention to authorise use on tomatoes in Romania only

The applicant's argument is justified; although the UK (as zonal RMS) cannot propose that authorisation be recommended on the basis of the southern EU outdoor trials other MSs (in this case only Romania) may

wish to consider granting a national authorisation on the basis of the data considered herein. In the interests of facilitating a decision by the Romanian competent authority the SEU trials have been evaluated. In all respects the trials and supporting data are acceptable and therefore the only reason not to give authorisation on the basis of the SEU trials is that Romania is, according to SANCO 7525/VI/95 - rev.9, a northern EU MS.

The applicant agrees with the RMS that final decision on acceptability of tomato SEU trials should be done on country level, i.e. by Romanian authorities. Romania changed the residue zone according to SANCO in 2011.

(Romanini M. 2011)

Table A 30: Summary of the study 1 trials on tomato / Southern EU

Reference:	Romanini, M., 2011c, CREG2118		
GLP:	Yes	Sample storage conditions:	at ≤ -18 °C for residues analysis
Crop/crop group:	Tomato	Analytical method:	TR 34-99-111 (for Zoxamide), RAU/038 (for Cymoxanil)
Indoor/Outdoor:	Outdoor	Limit of Quantification (mg/kg):	Zoxamide: 0.01 mg/kg Cymoxanil: 0.05 mg/kg
Formulation:	WG	Limit of Detection (mg/kg):	Zoxamide: 0.0 051 mg/kg Cymoxanil: 0.0199 (tomato samples), 0.0249 mg/kg (processed samples)
Content of active substance (g/kg or g/l):	33% Zoxamide + 33% Zoxamide	Residues calculated as:	Zoxamide, cymoxanil

Trial No./ Location/ EU zone/ Year	Commodity/ Variety	Date of 1.Sowing or planting 2.Flowering 3. Harvest	Application rate per treatment			Dates of treatment or no. of treatments and last date	Growth stage at last treatment or date	Portion analyzed	Residues (mg/kg)		PHI (days	Details on trial
			g a.s./ ha	Water (l/ha)	g a.s./hl				Zoxamide	Cymoxanil		
I/CZ10/TO01 26857 Salerano sul Lambro (LO) Italy S-EU, 2010	Tomato/Heinz 3402	1) 03/06/2010 2) Beginning of July 3) End of August	152.21 146.64 155.31 142.31 149.74	820.0 790.0 836.7 766.7 806.7	18.56 18.56 18.56 18.56 18.56	27/08/2010	805	Fruit	0.3073	< LOQ	3	LOQ=0.01 mg/kg (Zoxamide) /0.05 mg/kg (Cymoxanil) LOD Zoxamide =0.0051 mg/kg LOD Cymoxanil= 0.0199 (tomato samples), 0.0249 mg/kg (processed samples) Analytical method validated in study TR 34-99-111 (for Zoxamide) and RAU/038 (for Cymoxanil) Max. Storage interval between sampling and analysis:43 days

Trial No./ Location/ EU zone/ Year	Commodity/ Variety	Date of 1.Sowing or planting 2.Flowering 3. Harvest	Application rate per treatment			Dates of treatment or no. of treatments and last date	Growth stage at last treatment or date	Portion analyzed	Residues (mg/kg)		PHI (days	Details on trial
			g a.s./ ha	Water (l/ha)	g a.s./hl				Zoxamide	Cymoxanil		
I/CZ10/TO02 29029 Rivergaro (PC) Italy S-EU, 2010	Tomato/Leader	1) 17/05/2010 2) 25/06/2010 3) 1st decade of October	141.49 147.68 149.32 150.97 142.31	762.2 795.6 804.4 813.3 766.7	18.56 18.56 18.56 18.56 18.56	03/09/2010	89	Fruit Juice Puree Canned	0.3031 <0.01(ND) <0.01 (ND) <0.01 (ND)	< LOQ < LOQ < LOQ < LOQ	3	LOQ=0.01 mg/kg (Zoxamide) /0.05 mg/kg (Cymoxanil) LOD Zoxamide =0.0051 mg/kg LOD Cymoxanil= 0.0199 (tomato samples), 0.0249 mg/kg (processed samples) Analytical method validated in study CREG2117 for Zoxamide) and RAU/038 for Cymoxanil Max. Storage interval between sampling and analysis:43 days
I/CZ10/TO03 64013 Corropoli (TE) Italy S-EU, 2010	Tomato/Doget	1) 02/05/2010 2) N.A. 3) End of August	143.22 150.81 141.24 155.76 155.43	772.7 813.0 761.3 839.3 838.0	18.54 18.55 18.55 18.56 18.55	23/08/2010	89	Fruit	0.1613	< LOQ	3	LOQ=0.01 mg/kg (Zoxamide) /0.05 mg/kg (Cymoxanil) LOD Zoxamide =0.0051 mg/kg LOD Cymoxanil=0.0199 (tomato samples), 0.0249 mg/kg (processed samples) Analytical method validated in study CREG2117 for Zoxamide) and RAU/038 for Cymoxanil Max. Storage interval between sampling and analysis:43 days
I/CZ10/TO04 64010 Morro D'Oro (TE) Italy S-EU, 2010	Tomato/Perfect peel	1) 03/05/2010 2) N.A. 3) End of August	155.10 155.43 155.76 155.43 141.90	837.0 837.3 839.3 838.7 765.0	18.53 18.56 18.56 18.53 18.55	23/08/2010	88	Fruit	0.2746	< LOQ	3	LOQ=0.01 mg/kg (Zoxamide) /0.05 mg/kg (Cymoxanil) LOD Zoxamide =0.0051 mg/kg LOD Cymoxanil= 0.0199 (tomato samples), 0.0249 mg/kg (processed samples) Analytical method validated in study CREG2117 for Zoxamide) and RAU/038 for Cymoxanil Max. Storage interval between sampling and analysis:43 days

(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Days after last application (Label pre-harvest interval, PHI, underline)
(e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included
ND: Not Detectable, residue lower than instrumental limit of detection (LOD)

A 2.1.3.2.2 Study 2

Comments of zRMS:	No tomatoes in the GAP for CEU. Not relevant.

Reference:	**KCA 6.3/17**
Report:	Devine, H.C., 2008: Residues of mancozeb and zoxamide in field and protected tomatoes at intervals and at harvest following multiple applications of Electis, Northern France and The United Kingdom – 2006[18] Dow Agrosciences Ltd., UK CEM Analytical Services Ltd. (CEMAS), UK, Report No. CEMS-2967, pomidory, GLP, Not published
Guideline(s):	7029/VI/95 rev. 22 (1997) 96/98 EC 91/414/EEC (1996)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Test item (Lot/Batch No.):	Electis (TL0888R2D1)
Active substance content (nominal):	83 g a.s./kg Zoxamide and 667 g a.s./kg Mancozeb (nominal)
Expiry date:	8 June 2007

As part of the safety evaluation, five trials were conducted to determine the residue of zoxamide and mancozeb in tomatoes at harvest or at intervals following five foliar applications of Electis at a nominal application rate of 1200 g a.s./ha of mancozeb and 150 g a.s./ha of zoxamide, spray volume 600L/ha.

The formulation was applied using conventional small plot application equipment at the proposed normal use rates and timings. The trials were conducted in regions of Northern France and the United Kingdom typical of Northern European tomato growing areas. The French trials were conducted outdoor. The UK trial was protected.

The field part has been performed at AGRISEARCH in France and UK under report no. CEMS-2967, analytical phase has been performed at CEM Analytical Services Ltd (CEMAS) in UK under report no. CEMS-2967, with a method developed and validated according to Rohm and Hass Technical report No. 34-99-111 (for zoxamide) and CEM-3333 (for mancozeb).

Mancozeb: Samples were weighed into a crimp cap glass tube followed by the extraction solvent 2,2,4-trimethylpentane. The decomposition solution of tin chloride in hydrochloric acid was then added and the vial was immediately capped with a PTFE/Silicone septum. The analyte was decomposed through the reaction with the tin ions in the acidic solution. Heating the vial increased the reaction. The degradation product, CS2 was extracted into the 2,2,4-trimethylpentane by agitating the vial in an incubator shaker. The extract was then transferred to a fresh auto sampler vial. The analytes were detected using capillary gas chromatography with mass-selective detection.

[18] Confirmatory study for zoxamide currently being evaluated by RMS (Latvia).

Zoxamide: Samples were weighed into a centrifuge bottle and extracted with acetonitrile. The acetonitrile was concentrated by rotary evaporation. This extract was cleaned-up using liquid-liquid partitioning into ethyl acetate before being dried by rotary evaporation and reconstituted in hexane. The extract was then purified using a carbon solid phase extraction (SPE) cartridge. The eluate was dried by rotary evaporation and re-constituted in hexane before analysis by Gas Chromatography with Electron Capture Detection (GC-ECD).

Results and discussion

Procedural recoveries were between 74% and 117% for zoxamide and 70% and 100% for mancozeb. The detector response was shown to be linear over the range 0.005 – 1.0 µg/mL for zoxamide and 0.01 – 5.0 µg/mL for mancozeb.

Residues of zoxamide were determinded in tomato using the procedure detailed in Rohm and Hass Technical report No. 34-99-111 with a Limit of Quantification (LOQ) of 0.01 mg/kg.

Residues of mancozeb were determined in tomato using the procedure detailed in CEMAS SOP CEM-3333 with a Limit of Quantification (LOQ) of 0.05 mg/kg.

(Devine H.C. 2008)

Table A 31: Summary of the study 2 trials on tomato / Northern EU

Reference:	Devine, H.C., 2008, GHE-P-11604, CEMS-2967		
GLP:	Yes	Sample storage conditions:	at ≤ -18(+3)°C for residues analysis
Crop/crop group:	Tomato	Analytical method:	Rohm and Haas report 34-99-111 (for zoxamide) CEMS-3007 (form mancozeb)
Indoor/Outdoor:	Outdoor	Limit of Quantification (mg/kg):	0.01 mg/kg (zoxamide) 0.05 mg/kg (mancozeb)
Formulation:	WG	Limit of Detection (mg/kg):	--
Content of active substance (g/kg or g/l):	Electis (83 g a.s./kg Zoxamide and 667 g a.s./kg Mancozeb)	Residues calculated as:	Zoxamide

Trial No./ Location/ EU zone/ Year	Commodity/ Variety	Date of 1.Sowing or planting 2.Flowering 3. Harvest	Application rate per treatment			Dates of treatment or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Details on trial
			kg / hL	Water (l/ha)	kg /ha				Zoxamide		
	(a)	(b)				(c)				(d)	(e)
CEMS-2967A 95000 Cergy village France N-EU, 2006	Tomato/ Roma	1)23/05/06 2) n/a 3) 18/09/06	0.025 0.025 0.025 0.025 0.025	601 611 591 596 600	0.150 0.153 0.148 0.149 0.150	18/08/06 25/08/06 01/09/06 08/09/06 15/09/06	79 81 83 85 87	Whole plants	0.24 0.23	3 7	LOQ = LOD = Analytical method validated in study 34-99-111 (for zoxamide) and CEMS-3007 (form mancozeb) Max. Storage interval between sampling and analysis: 350 days

Trial No./ Location/ EU zone/ Year	Commodity/ Variety	Date of 1.Sowing or planting 2.Flowering 3. Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Details on trial
			kg / hL	Water (l/ha)	kg /ha				Zoxamide		
	(a)	(b)				(c)				(d)	(e)
CEMS-2967B 49650 Allonnes France N-EU, 2006	Tomato/ Topkapi	1)15/06/06 2) n/a 3) 04/09/06	0.025 0.025 0.025 0.025 0.025	605 612 593 632 603	0.151 0.153 0.148 0.158 0.151	04/08/06 11/08/06 18/08/06 25/08/06 01/09/06	71-72 72-73 73-74 77 82	Whole plants	0.20 0.08	3 7	LOQ = LOD = Analytical method validated in study 34-99-111 (for zoxamide) and CEMS-3007 (form mancozeb) Max. Storage interval between sampling and analysis: 364 days
CEMS-2967C 49650 Allonnes France N-EU, 2006	Tomato/ Cobra	1)26/05/06 2) n/a 3) 26/08/06	0.025 0.025 0.025 0.025 0.025	598 605 649 615 594	0.150 0.151 0.162 0.154 0.149	26/07/06 02/08/06 09/08/06 16/08/06 23/08/06	79 81 82 83-84 84-85	Whole plants	0.13 0.28 0.36 0.24 0.34	0- 0+ 1 3 7	LOQ = LOD = Analytical method validated in study 34-99-111 (for zoxamide) and CEMS-3007 (form mancozeb) Max. Storage interval between sampling and analysis: 416 days
CEMS-2967D 49650 Allonnes France N-EU, 2006	Tomato/ Hector	1)15/06/06 2) n/a 3) 02/09/06	0.025 0.025 0.025 0.025 0.025	615 609 608 596 600	0.154 0.152 0.152 0.149 0.150	01/08/06 08/08/06 15/08/06 22/08/06 30/08/06/	71 73-75 81 82-83 85	Whole plants	0.13 0.19 0.10 0.08 0.07	0- 0+ 1 3 7	LOQ = LOD = Analytical method validated in study 34-99-111 (for zoxamide) and CEMS-3007 (form mancozeb) Max. Storage interval between sampling and analysis: 413 days

Trial No./ Location/ EU zone/ Year	Commodity/ Variety	Date of 1.Sowing or planting 2.Flowering 3. Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Details on trial
			kg / hL	Water (l/ha)	kg /ha				Zoxamide		
	(a)	(b)				(c)				(d)	(e)
CEMS-2967E 49650 Allonnes France N-EU, 2006	Tomato/ Carosel	1)02/05/06 2) n/a 3) 29/07/06	0.025 0.025 0.025 0.025 0.025	613 625 613 613 610	0.153 0.156 0.153 0.153 0.153	28/06/06 04/07/06 12/07/06 19/07/06 26/07/06	75-76 76 77-79 79-81 81-85	Whole plants	0.19 0.80 0.68 0.71 0.76	0- 0+ 1 3 7	LOQ = LOD = Analytical method validated in study 34-99-111 (for zoxamide) and CEMS-3007 (form mancozeb) Max. Storage interval between sampling and analysis: 320 days

ND: Not Detectable, residue lower than instrumental limit of detection (LOD)

A 2.1.3.2.3 Study 3

Comments of zRMS:	No tomatoes in the intended GAP for CEU. The study is for SEU. Not relevant.

Reference:	**KCA 6.3/18**
Report	Longhi, D., 2020: Determination of (R) and (S) zoxamide residues and its metabolites RH-150721, RH-129151, RH-141452, RH-141288, RH-24549 in raw agricultural commodity of industrial tomato and its processed products (juice, puree and peeled tomatoes) following five applications of formulated product Zoxium 240 SC (Sponsor code GWN-9790 EU) in open field (South Europe – 4 trials year 2018)[19] Gowan Crop Protection Ltd., UK LabAnalysis, Italy, Report No. BPL-STUDY-18-000014, GLP, Not published
Guideline(s):	OECD TG 509 (2009) OECD TG 508 (2008)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Test item (Lot/Batch No.)	GWN 9790 EU / Zoxium 240 SC (SIPAL7001)
Active substance content (nominal)	240 g/L Zoxamide (nominal), 242.9 g/L (analysed) (R/S zoxamide ratio: 50/50)
Expiry date	May 2020

The magnitude of residues of zoxamide (sum and R and S isomers) and RH-141452 in raw commodities (industrial tomatoes) and of zoxamide (sum and R and S isomers), RH-150721 (as sum of R and S isomers), (R)-RH-150721, (S)-RH-150721, RH-129151 (as sum of R and S isomers), (R)-RH-129151, (S)- RH-129151, RH-24549, RH-141288 (as sum of R and S isomers), (R)-RH-141288 and (S)-RH-141288 in processed fractions (juice, puree and peeled tomatoes – before and after pasteurisation) has been determined in 2 harvest trials (RAC) and 2 decline curve trials (with harvest at 0 and 3 DALA) performed indoor in 2018.

The field and processing phase has been performed by ASTRA, Italy, under report no. PFR_18_GOW_LYPES-IT_Z-HD. The formulated product Zoxium 240 SC was applied five times at a single rate of 0.75 L/ha, corresponding to 180 g/ha of zoxamide, at an interval of 8 ±1 days and with the last application 3 days before harvest. One untreated control plot per trial was included. The application was performed with a backpack sprayer.

The residues samples were stored under deep frozen conditions within 8 hours after sampling and shipped and stored deep frozen at ≤ -18 °C. The processing samples were shipped within 48 hours and stored at 5 ±3°C until processing.

[19]Confirmatory study for zoxamide currently being evaluated by RMS (Latvia).

Processing of the samples started one day after collection in the field.

For peeled tomatoes, tomatoes were blanched in boiling water for at min 2 minutes, cooled down in tap water and peeled manually. Part of the packed peeled tomatoes was pasteurised at 90-95°C for at least 60 minutes.

For juice production, tomatoes were grinded, heated at 85-95°C, and sieved to receive the juice. Citric acid was added in case of a pH > 4.4. The hot juice was filled in glass bottles. Part of the samples were pasteurised at 90-95°C for at least 30 minutes.

For tomato puree production, tomatoes were grinded, heated at 85-95°C, and sieved to receive the puree. Citric acid was added in case of a pH > 4.4. The puree was concentrated by high temperatures and filled in glass bottles. Part of the samples were pasteurised at 90-95°C for at least 30 minutes.

The analytical phase has been performed at LabAnalysis in Italy under report no. BPL-STUDY-18-000014 with a method validated according to SANCO/825/00 rev. 8.1 and SANCO/3029/99 rev. 4 under report no. BPL-STUDY-18-000085.

Additional procedural recoveries were determined during the course of this study.

Results and discussion

The residues of the zoxamide metabolites containing a chiral centre (RH-150721, RH-129151 and RH-141288) were expressed as sum of 2 enantiomers and per single enantiomer (R and S). However, for metabolite RH-129151 the correlation between the absolute configuration of the enantiomer (R) or (S) and the corresponding chromatographic peaks was not available; therefore, the first eluted peak was identified as RH-129151 (A) and the second eluted peak as RH-129151 (B).

The metabolite RH-141452, which is known to form conjugates with matrix molecules (e.g. sugars), was released in an additional hydrolysis step to establish total fractions in addition to the free fractions in the matrices. For RH-141452 the total fractions are considered as worst-case.

The limit of quantification (LOQ) was set at 0.01 mg/kg for zoxamide, RH-150721, RH-129151 and RH-141288 (sum of enantiomers), RH-141452 and RH-24549, and 0.005 mg/kg for single isomers. The limit of determination (LOD) was given as 0.003 mg/kg for RH-150721, RH-129151, RH-141288 (sum of enantiomers), RH-141452 and RH-24549, and 0.0015 mg/kg for single isomers.

No residues were found in the control plot.

In case of residues >> LOQ, they are demonstrating a stable chiral centre of zoxamide and its residues.

A summary of the results is given in the following table.

(Longhi D. 2020)

Table A 32: Summary of the study 3 trials on tomato / indoor

Reference:	Longhi D. 2020, BPL-STUDY-18-000014		
GLP:	Yes	Sample storage conditions:	at ≤ -18 °C for residues analysis
Crop/crop group:	Tomato / fruiting vegetable	Analytical method:	BPL-STUDY-18-000085
Indoor/Outdoor:	Indoor	Limit of Quantification (mg/kg):	0.01 mg/kg (sum), to 0.005 mg/kg (single isomers)
Formulation:	SC	Limit of Detection (mg/kg):	0.003 mg/kg (sum), 0.0015 mg/kg (single isomer)
Content of active substance (g/kg or g/l):	240 g/L zoxamide	Residues calculated as:	Zoxamide (as sum of R and S isomers), (R)-Zoxamide, (S)-Zoxamide, metabolites RH-141452, RH-150721 (as sum of R and S isomers), (R)-RH-150721, (S)-RH-150721, RH-129151 (as sum of R and S isomers), (R)-RH-129151, (S)- RH-129151, RH-24549, RH-141288 (as sum of R and S isomers), (R)-RH-141288, (S)-RH-141288

Trial No. Location EU zone Year	Commodity/ Variety	Date of 1. Sowing or Planting 2. Flowering 3. Harvest (b)	Application rate per treatment			Dates of treatment(s) or no of treatment(s) and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)													PHI (days)	Remarks:
			kg as/ha	Water (L/ha)	kg as/hl				Zoxamide (R)	Zoxamide (S)	Zoxamide (sum)	RH-150721 (R)	RH-150721 (S)	RH-150721 (sum)	RH-141288 (R)	RH-141288 (S)	RH-141288 (sum)	RH-129151 (A)	RH-129151 (B)	RH-129151 (sum)	RH-24549	RH-141452		
PFR_18_GOW_LYPES_IT_Z-H01 40026 Sesto Imolsese Italy Indoor, 2018	Tomato H4107	1) 02/05/2018 2) N.A. 3) 14/08/2018	0.1858 0.1765 0.1873 0.1849 0.1821	1020.0 968.9 1028.3 1015.0 1000.0	0.01822 0.01822 0.01822 0.01822 0.01822	12/07/2018 20/07/2018 27/07/2018 04/08/2018 11/08/2018	71 82 74/81 74/84 88-89	Tomato fruit	0.519	0.485	0.100	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	ND	3	*
PFR_18_GOW_LYPES_IT_Z-H02 41011 Panzano di Campogalliano (MO) Italy Indoor, 20018	Tomato Delfo	1) 15/05/2018 2) N.A. 3)07/09/2018	0.1822 0.1845 0.1840 0.1845 0.1936	1000.0 1013.0 1010.2 1013.0 1062.5	0.01822 0.01822 0.01822 0.01822 0.01822	03/08/2018 10/08/2018 18/08/2018 26/08/2018 04/09/2018	72/81 72-73/82 73/83-84 73-74/84-85 88	Tomato fruit	0.101	0.101	0.203	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	ND	3	*

Trial No. Location EU zone Year	Commodity/ Variety	Date of 1. Sowing or Planting 2. Flowering 3. Harvest (b)	Application rate per treatment			Dates of treatment(s) or no of treatment(s) and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)														PHI (days)	Remarks:
			kg as/ha	Water (L/ha)	kg as/hl				Zoxamide (R)	Zoxamide (S)	Zoxamide (sum)	RH-150721 (R)	RH-150721 (S)	RH-150721 (sum)	RH-141288 (R)	RH-141288 (S)	RH-141288 (sum)	RH-129151 (A)	RH-129151 (B)	RH-129151 (sum)	RH-24549	RH-141452		
PFR_18_GOW_LYPES_IT_Z-H02 40014 Crevelcore (BO) Italy Indoor, 2018	Tomato Delfo	1) 10/05/2018 2) N.A. 3) 26/08/2018 29/08/2018	0.1811 0.1828 0.1858 0.1819 0.1935	994.1 1003.6 1019.6 998.2 1061.9	0.01822 0.01822 0.01822 0.01822 0.01822	25/07/2018 02/08/2018 09/08/2018 18/08/2018 26/08/2018	72 72/81 73/81 73-74/84-85 88-89	Tomato fruit	0.149	0.147	0.296	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	ND	0	*
									0.107	0.108	0.215	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	ND	3	
								Processed before Pasteurisation																
								Juice	0.0255	0.0228	0.0453	ND	0.00519	<LOQ (0.00519)	ND	ND	ND	<LOQ (0.00272)	<LOQ (0.00284)	<LOQ (0.00555)	ND	ND	3	
								Puree	ND	ND	ND	<LOQ (0.00259)	<LOQ (0.00261)	<LOQ (0.00520)	ND	ND	ND	ND	<LOQ (0.00153)	ND	0.0125	ND		
								Pelled to-mato	0.0142	0.0136	0.0278	N.D.	<LOQ (0.00251)	N.D.	ND	ND	ND	<LOQ (0.00185)	<LOQ (0.00200)	<LOQ (0.00384)	ND	ND		
								Processed after pasteurisation																
								Juice	<LOQ (0.00260)	<LOQ (0.00394)	<LOQ (0.00654)	<LOQ (0.00291)	0.0912	0.0120	ND	ND	ND	<LOQ (0.00357)	<LOQ (0.00366)	<LOQ (0.00722)	<LOQ (0.00346)	ND	3	
								Puree	ND	ND	ND	<LOQ (0.00216)	<LOQ (0.00202)	<LOQ (0.00417)	ND	ND	ND	ND	ND	ND	0.0198	ND		
								Pelled to-mato	ND	ND	ND	<LOQ (0.00254)	<LOQ (0.00399)	<LOQ (0.00652)	ND	ND	ND	ND	<LOQ (0.00173)	ND	ND	ND		
PFR_18_GOW_LYPES_IT_Z-D02 44015 Bando di Portomaggiore (FE) Italy Indoor, 2018	Tomato H1211	1) 19/05/2018 2) N.A. 3) 31/08/2018 03/09/2018	0.2000 0.1947 0.01802 0.1831 0.1859 0.01822 0.01822 0.01822 0.01822 0.01822	1097.7 1068.5 988.9 1005.1 1020.4	0.01822 0.01822 0.01822 0.01822 0.01822	30/07/2018 07/08/2018 16/08/2018 23/08/2018 31/08/2018	71-71/81 72/82 73/83 74/85 88	Tomato fruit	0.133	0.135	0.268	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	ND	0	*
									0.0650	0.0627	0.128	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	ND	3	
								Processed before pasteurisation																
								Juice	0.0233	0.0232	0.0465	0.0241	0.0209	0.0450	<LOQ (0.00153	ND	ND	0.00942	0.00928	0.0187	ND	ND	3	
								Puree	ND	<LOQ (0.00182)	ND	0.0188	0.0196	0.0384	ND	ND	ND	<LOQ (0.00488)	<LOQ (0.00490)	<LOQ (0.00978)	0.0282	ND		
								Pelled to-mato	00101	0.0104	0.0205	0.0081	0.0123	0.0205	ND	ND	ND	0.00536	0.00569	0.0111	N0D	ND		
								Processed after Pasteurisation																
								Juice	0.00974	0.0102	0.0199	0.0273	0.0283	0.0556	<LOQ (0.00187)	<LOQ (0.00153)	<LOQ (0.00340)	0.00762	0.00752	0.0151	<LOQ (0.00712)	ND	3	
								Puree	ND	ND	ND	0.0172	0.0175	0.0347	<LOQ (0.00182)	<LOQ (0.00204)	<LOQ (0.00387)	<LOQ (0.00293)	<LOQ (0.00307)	<LOQ (0.00600)	0.0358	ND		
								Pelled to-mato	<LOQ (0.00168	ND	ND	0.0190	0.0315	0.0504	ND	ND	ND	0.00571	0.00612	0.0118	0.0199	ND		

* LOQ = 0.010 mg/kg for zoxamide (sum), RH-150721 (sum), RH-1292151 (sum), RH-141288 (sum), RH-141452 and RH-24549, and 0.005 mg/kg for single isomers.
LOD = 0.003 mg/kg for zoxamide (sum), RH-150721 (sum), RH-1292151 (sum), RH-141288 (sum), RH-141452 and RH-24549, and 0.0015 mg/kg for single isomers.
Analytical method validated in study BPL-STUDY-18-000085
Max. storage interval between sampling and analysis: industrial tomato: 538 days

	tomato juice: 523-529 days tomato puree: 529 days peeled tomato: 532 days

ND = not detectable, NR = not relevant

A 2.1.3.2.4 Study 4

Comments of zRMS:	No tomatoes in the intended GAP. Not relevant.

Reference:	**KCA 6.3/19**
Report	Longhi, D., 2020: Determination of (R) and (S) zoxamide residues and its metabolites RH-150721, RH-129151, RH-141452, RH-141288, RH-24549 in fresh market tomato raw agricultural commodity following five applications of the formulated product Zoxium 240 SC (sponsor code GWN-9790 EU) in greenhouse (South Europe - 4 trials year 2018) - final report amendment no. 1[20] Gowan Crop Protection Ltd., UK LabAnalysis, Italy, Report No. BPL-STUDY-18-000015, GLP, Not published
Guideline(s):	OECD TG 508 (2008) OECD TG 509 (2009)
Deviations:	In the field phase of trial PCR_18_GOW_LYPES-FMT_Z-D01 only few tomatoes reached the optimal degree of maturation 3 DALA sampling (July 21, 2018). This delay in maturation (quantified in at least 3-4 days compared to usual) was caused by anomalous climatic conditions which characterised the months May and June in the area of the trial, with cloudy weather, frequent rainfalls and temperatures below the seasonal averages. As a consequence, at the harvest of tomatoes 3 DALA some fruits did not appear with the optimal degree of maturation (characterized by red /light red colour at the fruit apex) typical of the variety used in this trial. However, this deviation is regarded to not change the integrity of this residues study.
GLP:	Yes
Acceptability:	Yes

Materials and methods

Test item (Lot/Batch No.)	GWN 9790 EU / Zoxium 240 SC (SIPAL7001)
Active substance content (nominal)	240 g/L Zoxamide (nominal), 242.9 g/L (analysed) (R/S Zoxamide ratio: 50/50)
Expiry date	May 2020

The magnitude of residues of zoxamide (R, S and sum) and RH-141452 in raw commodities (tomatoes) has been determined in 2 harvest trials (RAC) and 2 decline curve trials (with harvest at 0 and 3 DALA) performed in the greenhouse in 2018.

The field phase has been performed by ASTRA, Italy, under report no. PCR_18_GOW_LYPES-FMT_Z-HD. The formulated product Zoxium 240 SC was applied five times at a single rate of 0.75 L/ha, corresponding to 180 g/ha of zoxamide, at an interval of 8 ±1 days and with the last application 3 days before harvest. One untreated control plot per trial was included. The application was performed with a T-boom micro-sprayer.

[20] Confirmatory study for zoxamide currently being evaluated by RMS (Latvia).

The residues samples were stored under deep frozen conditions within 5 hours after sampling and shipped and stored deep frozen at ≤ -18 °C.

The analytical phase has been performed at LabAnalysis in Italy under report no. BPL-STUDY-18-000015 with a method validated according to SANCO/825/00 rev. 8.1 and SANCO/3029/99 rev. 4 under report no. BPL-STUDY-18-000085.

Additional procedural recoveries were determined during the course of this study.

Results and discussion

The residues of the zoxamide containing a chiral centre were expressed as sum of 2 enantiomers and per single enantiomer (R and S).

The metabolite RH-141452, which is known to form conjugates with matrix molecules (e.g. sugars), was released in an additional hydrolysis step to establish total fractions in addition to the free fractions in the matrices. For RH-141452 the total fractions are considered as worst-case.

The limit of quantification (LOQ) was set at 0.01 mg/kg for zoxamide and RH-141452 and 0.005 mg/kg for (R)-zoxamide and (S)-zoxamide. The limit of determination (LOD) was given as 0.003 mg/kg for zoxamide (sum of enantiomers) and RH-141452, and 0.0015 mg/kg for the single isomers of zoxamide.

No residues were found in the control plot.

In case of residues >> LOQ, they are demonstrating a stable chiral centre of zoxamide and its residues.

A summary of the results is given in the following table.

(Longhi D. 2020)

Table A 33: Summary of the study 4 trials on tomato / greenhouse

Reference:	Longhi D. 2020, GLP-STUDY- 18-000015 - final report amendment		
GLP:	Yes	Sample storage conditions:	at ≤ -18°C for residues analysis
Crop/crop group:	Tomato /fruiting vegetable	Analytical method:	BPL-STUDY-18-000085
Indoor/Outdoor:	Indoor	Limit of Quantification (mg/kg):	0.01 mg/kg (sum), to 0.005 mg/kg (single isomer)
Formulation:	SC	Limit of Detection (mg/kg):	0.003 mg/kg (sum), 0.0015 mg/kg (single isomer)
Content of active substance (g/kg or g/l):	240 g/L zoxamide	Residues calculated as:	Zoxamide (sum), (R)-Zoxamide, (S)-Zoxamide, metabolite RH-141452

Trial No. Location EU zone Year	Commod-ity/ Variety	Date of 1. Sowing or Planting 2. Flowering 3. Harvest	Application rate per treatment			Dates of treatment(s) or no of treatment(s) and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)														PHI (days)	Remarks
			kg as/ha	Water (L/ha)	kg as/hL				Zoxamide (sum)	Zoxamide (R)	Zoxamide (S)	RH-141452	RH-24549	RH-150721 (sum)	(R)-RH-150721	(S)-RH-150721	RH-129151 (sum)	RH-129151 (A)	RH-129151 (B)	RH-141288 (sum)	(R)-RH-141288	(S)-RH-141288		
PCR_18_GOW_LY-PES_FMT_Z-H01 40054 Maddalena di Cazzano di Budrio (BO) Italy Indoor, 2018	Tomato (*Solanum lycopersi-cum*) / Pozzano	1) 06/04/2018 2) N.A. 3) 18/06/2018	0.1675 0.1793 0.1865 0.1770 0.1891	735.4 787.5 818.8 777.1 830.4	0.022772 0.022772 0.022772 0.022772 0.022772	14/05/2018 22/05/2018 29/05/2018 07/06/2018 15/06/2018	62-63 63-64 64-65 65/71-72 66-73	Tomato fruit	0.313	0.156	0.157	ND	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	3	*
PCR_18_GOW_LY-PES_FMT_Z-H02 47521 Martorano di Cesena (FC) Italy	Tomato (*Solanum lycopersi-cum*) / Portento	1) 07/08/2018 2) N.A. 3) 24/10/2018	0.1856 0.1772 0.1767 0.1881 0.1931	815.2 778.3 776.1 826.1 847.8	0.022772 0.022772 0.022772 0.022772 0.022772	18/09/2018 26/09/2018 04/10/2018 12/10/2018 21/10/2018	63-64 64-65 66-67 67-68/72-73 67-68/73	Tomato fruit	0.210	0.108	0.103	ND	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	3	*

Trial No. Location EU zone Year	Commod-ity/ Variety	Date of 1. Sowing or Planting 2. Flowering 3. Harvest	Application rate per treatment			Dates of treatment(s) or no of treatment(s) and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)														PHI (days)	Remarks
			kg as/ha	Water (L/ha)	kg as/hL				Zoxamide (sum)	Zoxamide (R)	Zoxamide (S)	RH-141452	RH-24549	RH-150721 (sum)	(R)-RH-150721	(S)-RH-150721	RH-129151 (sum)	RH-129151 (A)	RH-129151 (B)	RH-141288 (sum)	(R)-RH-141288	(S)-RH-141288		
L Indoor, 2018																								
PCR_18_GOW_LY-PES_FMT_Z-D01 47521 Martorano di Cesena (FC) Italy Indoor, 2018	Tomato (*Solanum lycopersi-cum*) / Ca-ramba	1) 19/04/2018 2) N.A. 3) 18/06/2018 21/06/2018	0.022772 0.022772 0.022772 0.022772 0.022772	788.9 807.4 793.0 760.3 780.7	0.1796 0.1839 0.1806 0.1731 0.1778	17/05/2018 25/05/2018 02/06/2018 11/06/2018 18/06/2018	61-62 62-63 63-64 66-67/71 66/73	Tomato fruit	0.106	0.0530	0.0530	ND	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	0	*
									0.111	0.0569	0.0541	ND	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	3	
PCR_18_GOW_LY-PES_FMT_Z-D02 40026 Imola (BO) Italy Indoor, 2018	Tomato (*Solanum lycopersi-cum*) / Secolo	1) 20/08/2018 2) N.A. 3) 28/10/2018 31/10/2018	0.022772 0.022772 0.022772 0.022772 0.022772	808.0 785.0 760.0 825.0 790.0	0.1833 0.1788 0.1731 0.1879 0.1799	25/09/2018 03/10/2018 11/10/2018 19/10/2018 28/10/2018	63-64 65 65-71 65/72-73 65/72-73	Tomato fruit	0.380	0.192	0.188	ND	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	0	*
									0.468	0.234	0.233	ND	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	3	

* LOQ = 0.010 mg/kg for zoxamide (sum), RH-141452, and 0.005 mg/kg for single isomers.
LOD = 0.003 mg/kg for zoxamide (sum), RH-141452, and 0.0015 mg/kg for single isomers.
Analytical method validated in study BPL-STUDY-18-000085
Max. Storage interval between sampling and analysis: 525-526 days

ND = not detectable, NR = not relevant

A 2.1.3.2.5 Study 5

Comments of zRMS:	No tomatoes in the GAP for CEU. The study is for SEU. Not relevant.

Reference:	**KCA 6.3/20**
Report	Longhi, D., 2020: Determination of zoxamide and its metabolites in raw agricultural commodity of industrial tomato in open field following five applications of the formulated product GWN 9790 EU (South Europe - 4 trials year 2019)[21] Gowan Crop Protection Ltd., UK LabAnalysis, Italy, Report No. BPL-STUDY-19-000059, GLP, Not published
Guideline(s):	OECD TG 509 (2009)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Test item (Lot/Batch No.)	GWN 9790 EU / Zoxium 240 SC (SIPAL7001)
Active substance content (nominal)	240 g/L Zoxamide (nominal), 242.9 g/L (analysed) (R/S Zoxamide ratio: 50/50)
Expiry date	May 2020

The magnitude of residues of zoxamide (R, S and sum) and RH-141452 in raw commodities (tomatoes) has been determined in 4 decline curve trials (with harvest at 0 and 3 DALA) performed in the open field in 2019.

The field phase has been performed by ASTRA, Italy, under report no. PCR_19_GOW_LYPES-IT_Z-D. The formulated product Zoxium 240 SC was applied five times at a single rate of 0.75 L/ha, corresponding to 180 g/ha of zoxamide, at an interval of 8 ±1 days and with the last application 3 days before harvest. One untreated control plot per trial was included. The application was performed with a T-boom micro-sprayer.

The residues samples were stored under deep frozen conditions within 4 hours after sampling and shipped and stored deep frozen at ≤ -18 °C.

The analytical phase has been performed at LabAnalysis in Italy under report no. BPL-STUDY-18-000059 with a method validated according to SANCO/825/00 rev. 8.1 and SANCO/3029/99 rev. 4 under report no. BPL-STUDY-18-000085.

Additional procedural recoveries were determined during the course of this study.

Results and discussion

The residues of the zoxamide containing a chiral centre were expressed as sum of 2 enantiomers and per single enantiomer (R and S).

[21] Confirmatory study for zoxamide currently being evaluated by RMS (Latvia).

The metabolite RH-141452, which is known to form conjugates with matrix molecules (e.g. sugars), was released in an additional hydrolysis step to establish total fractions in addition to the free fractions in the matrices. For RH-141452 the total fractions are considered as worst-case.

The limit of quantification (LOQ) was set at 0.01 mg/kg for zoxamide and RH-141452 and 0.005 mg/kg for (R)-zoxamide and (S)-zoxamide. The limit of determination (LOD) was given as 0.003 mg/kg for zoxamide (sum of enantiomers) and RH-141452, and 0.0015 mg/kg for the single isomers of zoxamide.

No residues were found in the control plot.

In case of residues >> LOQ, they are demonstrating a stable chiral centre of zoxamide and its residues.

A summary of the results is given in the following table.

(Longhi D. 2020)

Table A 34: Summary of the study 5 trials on tomato / Southern EU

Reference:	Longhi D. 2020, BPL-STUDY-19-000059		
GLP:	Yes	Sample storage conditions:	at ≤ -18 °C for residues analysis
Crop/crop group:	Tomato / fruiting vegetables	Analytical method:	BPL-STUDY-18-000085
Indoor/Outdoor:	Outdoor	Limit of Quantification (mg/kg):	0.01 mg/kg (sum), to 0.005 mg/kg (single isomers)
Formulation:	SC	Limit of Detection (mg/kg):	0.003 mg/kg (sum), 0.0015 mg/kg (single isomers)
Content of active substance (g/kg or g/l):	240 g/L zoxamide	Residues calculated as:	Zoxamide (sum), (R)-Zoxamide, (S)-Zoxamide, metabolite RH-141452

Trial No. Location EU zone Year	Commodity/ Variety	Date of 1. Sowing or Planting 2. Flowering 3. Harvest (b)	Application rate per treatment			Dates of treat-ment(s) or no of treat-ment(s) and last date	Growth stage at last treat-ment or date	Portion analysed	Residues (mg/kg)														PHI (days)	Remarks:
			kg as/ha	Water (L/ha)	kg as/hL				Zoxamide (sum)	Zoxamide (R)	Zoxamide (S)	RH-141452	RH-24549	RH-150721 (sum)	(R)-RH-150721	(S)-RH-150721	RH-129151 (sum)	RH-129151 (A)	RH-129151 (B)	RH-141288 (sum)	(R)-RH-141288	(S)-RH-141288		
PCR_19_GOW_LYPES-IT_Z-D01 71029 Troia (FG) Italy S-EU, 2019	Tomato (*Solanum lycopersicum*) / Taylor	1) 19/04/2019 2) N.A.: 3) 11/08/2019 14/08/2019	0.1903 0.1925 0.1796 0.1891 0.1927	1044.4 1056.6 985.6 1037.8 1057.8	0.018218 0.018218 0.018218 0.018218 0.018218	11/07/2019 19/07/2019 27/07/2019 04/08/2019 11/08/2019	74 75/81 84 87 88	Tomato fruit	0.206	0.110	0.0953	ND	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	0	-
									0.0825	0.0450	0.0375	ND	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	3	-
PCR_19_GOW_LYPES-IT_Z-D02 71014 San Marco in Lamis (FG) Italy	Tomato (*Solanum lycopersicum*) / Docet	1) 11/05/2019 2) N.A. 3) 19/08/2019 22/08/2019	0.1929 0.1842 0.1840 0.1796 0.1844	1058.9 1011.1 1010.0 985.6 1012.2	0.018218 0.018218 0.018218 0.018218 0.018218	19/07/2019 27/07/2019 04/08/2019 12/08/2019 19/08/2019	73 74/81 75/83 87 88	Tomato fruit	0.157	0.0850	0.0723	ND	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	0	-
									0.113	0.0630	0.0501	ND	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	3	

Trial No. Location EU zone Year	Commodity/ Variety	Date of 1. Sowing or Planting 2. Flowering 3. Harvest (b)	Application rate per treatment			Dates of treat-ment(s) or no of treat-ment(s) and last date	Growth stage at last treat-ment or date	Portion analysed	Residues (mg/kg)														PHI (days)	Remarks:
			kg as/ha	Water (L/ha)	kg as/hL				Zoxamide (sum)	Zoxamide (R)	Zoxamide (S)	RH-141452	RH-24549	RH-150721 (sum)	(R)-RH-150721	(S)-RH-150721	RH-129151 (sum)	RH-129151 (A)	RH-129151 (B)	RH-141288 (sum)	(R)-RH-141288	(S)-RH-141288		
S-EU, 2019																								
PCR_19_ GOW_LY PES-IT_Z-D03 45030 Ceneselli (RO) Italy S-EU, 2019	Tomato (*Solanum lycopersicum*) / H3107	1) 15/05/2019 2) N.A. 3) 22/08/2019 25/08/2019	0.1953 0.1862 0.1876 0.1863 0.1890	1071.3 1021.3 1030.0 1022.5 1037.5	0.018218 0.018218 0.018218 0.018218 0.018218	22/07/2019 30/07/2019 07/08/2019 14/08/2019 22/08/2019	73/81 73/82 74/83 86 88	Tomato fruit	0.165	0.0897	0.0758	ND	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	0	
									<u>0.163</u>	0.0870	0.0763	ND	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	3	
PCR_19_ GOW_LY PES-IT_Z-D04 44015 Portomag-giore (FE) Italy S-EU, 2019	Tomato (*Solanum lycopersicum*) / H3107	1) 30.05.2019 2) N.A 3) 03.09.2019 06/09/2019	0.1758 0.1904 0.1906 0.1817 0.1863	965.0 1045.0 1046.3 997.5 1022.5	0.018218 0.018218 0.018218 0.018218 0.018218	01/08/2019 10/08/2019 18/08/2019 26/08/2019 03/09/2019	73/81 73/83 74/84 86 88	Tomato fruit	0.217	0.111	0.106	ND	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	0	
									<u>0.198</u>	0.100	0.0981	ND	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	3	

* LOQ = 0.010 mg/kg for zoxamide (sum), RH-141452, and 0.005 mg/kg for single isomers.
LOD = 0.003 mg/kg for zoxamide (sum), RH-141452, and 0.0015 mg/kg for single isomers.
Analytical method validated in study BPL-STUDY-18-000085

Max. Storage interval between sampling and analysis: 31 days

ND = not detectable, NR = not relevant

A 2.1.3.2.6 Study 6

Comments of zRMS:	No tomatoes in the intended GAP. Not relevant.

Reference:	**KCA 6.3/21**
Report	Pandolfi, A., 2020: Determination of zoxamide residues and its metabolites in raw agricultural commodity tomato (fruits) following five applications of Zoxium 240 SC (sponsor code GWN-9790 EU) in protected condition (Italy – Southern Europe – 4 trials years 2019)[22] Gowan Crop Protection Ltd., UK Res Agraria s.r.l., Italy, Report No. RA 19 043 BPL GW, GLP, Not published
Guideline(s):	OECD TG 509 (2009)
Deviations:	The mean procedural recovery values for a minor metabolite RH-141288 (R) and RH-141452 (not hydrolysed samples) were higher than the intended analytical method validation range (70-110%) with mean recoveries of 137.2% and 110.7% for RA-141288 (R) and RH-141452, respectively. However, this is an overestimation of the real values, which are nonetheless below the LOQ and – in most cases at or below the limit of detection (LOD).
GLP:	Yes
Acceptability:	Yes

Materials and methods

Test item (Lot/Batch No.)	GWN 9790 EU / Zoxium 240 SC (SIPAL7001)
Active substance content (nominal)	240 g/L Zoxamide (nominal), 242.9 g/L (analysed) (R/S Zoxamide ratio: 50/50)
Expiry date	May 2020

The magnitude of residues of zoxamide (R, S and sum) and RH-141452 in raw commodities (tomatoes) has been determined in 4 decline curve trials (with harvest at 0 and 3 DALA) performed in the greenhouse in 2019.

The field phase has been conducted by Res Agraria S.r.l., Italy, under report no. RA 19 043 BPL GW. The formulated product Zoxium 240 SC was applied five times at a single rate of 0.75 L/ha, corresponding to 180 g/ha of zoxamide, at an interval of 8 days and with the last application 3 days before harvest. One untreated control plot per trial was included. The application was performed with a Knapsack sprayer.

The residues samples were stored under deep frozen conditions between 2:55 and 5:45 hours after sampling and shipped and stored deep frozen at ≤ -18 °C.

The analytical phase has been performed at LabAnalysis in Italy under report no. BPL-STUDY-19-000072 with a method validated according to SANCO/825/00 rev. 8.1 and SANCO/3029/99 rev. 4 under report no. BPL-STUDY-18-000085.

Additional procedural recoveries were determined during the course of this study.

[22] Confirmatory study for zoxamide currently being evaluated by RMS (Latvia).

Results and discussion

The residues of the zoxamide containing a chiral centre were expressed as sum of 2 enantiomers and per single enantiomer (R and S).

The metabolite RH-141452, which is known to form conjugates with matrix molecules (e.g. sugars), was released in an additional hydrolysis step to establish total fractions in addition to the free fractions in the matrices. For RH-141452 the total fractions are considered as worst-case.

The limit of quantification (LOQ) was set at 0.01 mg/kg for zoxamide and RH-141452 and 0.005 mg/kg for (R)-zoxamide and (S)-zoxamide. The limit of determination (LOD) was given as 0.003 mg/kg for zoxamide (sum of enantiomers) and RH-141452, and 0.0015 mg/kg for the single isomers of zoxamide.

No residues were found in the control plot.

In case of residues >> LOQ, they are demonstrating a stable chiral centre of zoxamide and its residues.

A summary of the results is given in the following table.

(Longhi D. 2020)

Table A 35: Summary of the study 6 trials on tomato / greenhouse

Reference:	Pandolfi A. 2020, RA 043 BPL GW		
GLP:	Yes	Sample storage conditions:	at ≤ -18 °C for residues analysis
Crop/crop group:	Tomato / Fruiting vegetables	Analytical method:	BPL-STUDY-18-000085
Indoor/Outdoor:	Indoor	Limit of Quantification (mg/kg):	0.01 mg/kg (sum), to 0.005 mg/kg (single isomers)
Formulation:	SC	Limit of Detection (mg/kg):	0.003 mg/kg (sum), 0.0015 mg/kg (single isomers)
Content of active substance (g/kg or g/l):	240 g/L Zoxamide	Residues calculated as:	Zoxamide (sum), (R)-Zoxamide, (S)-Zoxamide, metabolite RH-141452

Trial No. Location EU zone Year	Commod-ity/ Variety	Date of 1. Sowing or Planting 2. Flowering 3. Harvest	Application rate per treatment			Dates of treatment(s) or no of treatment(s) and last date	Growth stage at last treat-ment or date	Portion analysed	Residues (mg/kg)															PHI (days)	Remarks:
			kg as/hL	Water (L/ha)	kg as/ha				Zoxamide (R)	Zoxamide (S)	Zoxamide (sum)	RH-141288 (R)	RH-141288 (S)	RH-141288 (sum)	(R)-RH-150721	(S)-RH-150721	RH-150721 (sum)	RH-129151 (A)	RH-129151 (B)	RH-1219151 (sum)	RH-24549	RH-141452		
RA 043 BPL IT 01 63828 Campofi-lone (FM) Italy Indoor, 2019	Tomato / Rossano	1) 16/03/2019 2) N.A. 3) 12/07/2019 15/07/2019	0.018	1026.786 1041.667 1046.627 992.063 992.063	0.185 0.188 0.188 0.179 0.179	10/06/2019 18/06/2019 26/06/2019 04/07/2019 12/07/2019	75 79 81 83 89	Tomato fruit	0.141	0.142	0.283	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	ND	0	*
									0.152	0.154	0.307	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	ND	3	
RA 043 BPL IT 02 63065 Ri-patransone (AP) Italy Indoor, 2019	Tomato / Optima	1) 23/03/2019 2) N.A. 3) 12/07/2019 15/07/2019	0.018	950.855 1004.274 1007.479 1014.957 1041.957	0.171 0.181 0.181 0.183 0.183	10/06/2019 18/06/2019 26/06/2019 04/07/2019 12/07/2019	73 75 76 81 89	Tomato fruit	0.159	0.160	0.319	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	ND	0	*
									0.188	0.189	0.377	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	ND	3	

Trial No. Location EU zone Year	Commod-ity/ Variety	Date of 1. Sowing or Planting 2. Flowering 3. Harvest	Application rate per treatment			Dates of treatment(s) or no of treatment(s) and last date	Growth stage at last treat-ment or date	Portion analysed	Residues (mg/kg)															PHI (days)	Remarks:
			kg as/hL	Water (L/ha)	kg as/ha				Zoxamide (R)	Zoxamide (S)	Zoxamide (sum)	RH-141288 (R)	RH-141288 (S)	RH-141288 (sum)	(R)-RH-150721	(S)-RH-150721	RH-150721 (sum)	RH-129151 (A)	RH-129151 (B)	RH-1219151 (sum)	RH-24549	RH-141452		
RA 043 BPL IT 03 63066 Giraottam-mare (AP) Italy Indoor, 2019	Tomato / Mon-tecarlo	1) 04/04/2019 2) N.A. 3) 19/07/2019 22/07/2019	0.018	992.208 1041.558 1019.481 1025.974 974.026	0.179 0.187 0.184 0.185 0.175	17/06/2019 25/06/2019 03/07/2019 11/07/2019 19/07/2019	75 79 82 85 89	Tomato fruit	0.107	0.110	0.218	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	ND	0	*
									0.0732	0.0742	<u>0.147</u>	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	ND	3	
RA 043 BPL IT 04 64014 Martin-sicuro (TE) Italy Indoor, 2019	Tomato / Marglobe	1) 25/03/2019 2) N.A. 3) 12/07/2019 15/07/2019	0.018	1033.303 1040.404 987.879 989.899 1010.101	0.185 0.187 0.178 0.178 0.182	10/06/2019 18/06/2019 26/06/2019 04/07/2019 12/07/2019	72 74 75 81 89	Tomato fruit	0.217	0.218	0.435	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	ND	0	*
									0.170	0.171	<u>0.314</u>	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	ND	3	*

* LOQ = 0.010 mg/kg for zoxamide (sum), RH-141452, and 0.005 mg/kg for single isomers.
LOD = 0.003 mg/kg for zoxamide (sum), RH-141452, and 0.0015 mg/kg for single isomers.
Analytical method validated in study BPL-STUDY-18-000085
Max. Storage interval between sampling and analysis: 21 days

ND = not detectable, NR = not relevant

A 2.1.3.2.7 Study 7

Comments of zRMS:	No tomatoes in the intended CEU GAP. Not relevant.

Reference:	**KCA 6.3/22**
Report	Tetuan, B., 2016: Determination of residues at harvest of zoxamide, cymoxanil and cymoxanil in tomato, following three broadcast applications of GWN-10392, GWN-9823 and IR6141-copper oxychloride-copper hydroxide 5-15-15 WG under greenhouse conditions and determination of residues at harvest of zoxamide and cymoxanil in industry tomato and its processed products (canned tomatoes, puree and juice), following three broadcast applications of GWN-10392, under open field conditions - South Europe - season 2015[23] Gowan Comercio Internacional e Servicos Limitada, Portugal Promovert Crop Services SL, Spain, Report No. 15 F CL GW P/A, GLP, Not published
Guideline(s):	SANCO 7029/Vl/95 - rev. 5 (1997) SANCO 7035/VI/95 - rev. 5 (1997)
Deviations:	Deviation to analytical lab SOP: the weighing of cymoxanil for the preparation of the 1 g/L stock solution was less than 10 mg (9.82 mg). This deviation was regarded to have no impact on the results of the study since the balance allows to weigh less 2 mg. Deviation to the study plan: analyses and an analytical phase-based inspection were performed before the finalisation of a study plan amendment – there was no impact on the results as the first daily sample set was not validated. However, the analytical phase-based inspection performed on the 31st December 2015 was kept as the analytical method was the same for the following samples sets.
GLP:	Yes
Acceptability:	Yes

Materials and methods

Test material (Lot/Batch No.)	GWN-10392 (G005/15)
Active substance content (nominal)	Zoxamide: 225 g/L (nominal), 228.68 g/L (analysed) Benalaxyl-m: 150 g/L (nominal), 143.10 g/L (analysed)
Expiry date	31 July 2017
Test material (Lot/Batch No.)	GWN-9823 (GSGAL4010)
Active substance content (nominal)	Zoxamide: 33% (nominal), 32.0 ± 1% w/w (analysed) Cymoxanil: 33% (nominal), 32.5 ± 0.2% w/w (analysed)
Expiry date	May 2018

[23] Confirmatory study for zoxamide currently being evaluated by RMS (Latvia).

The magnitude of residues of zoxamide, benalaxyl-m and cymoxanil in raw (tomatoes) and processed commodities (canned tomatoes, puree and juice) has been determined in 4 harvest trials, 3 performed in the greenhouse and 1 in the open field under southern European growing conditions in 2015.

The field phase has been conducted by Promovert Crop Services S.L., Spain, under report no. 15 F CL GW P/A. The formulated products GWN-10392 (225 g/L zoxamide + 150 g/L of benalaxyl-m SC) and GWN-9823 (33% w/w zoxamide + 33% w/w cymoxanil WG) were applied three times at a single rate of 0.7 L/ha GWN-10392 (corresponding to nominally 0.1575 kg zoxamide/ha and 0.105 kg benalaxyl-m /ha) and three times at a single rate of 0.45 kg/ha GWN-9823 (corresponding to nominally 0.157 kg zoxamide/ha and 0.157 kg cymoxanil/ha) at an interval of 7-8 (+1) days with the last application 3 days before harvest. One untreated control plot per trial was included. The applications were performed with pressure motor sprayers with a lance (in the greenhouse) or a boom (in the field).

The residues samples were stored under deep frozen conditions within 2.5 hours after sampling and shipped and stored deep frozen at ≤ -18 °C. The processing samples were shipped and stored at ambient temperature until processing.

The processing phase has been performed by Promovert Crop Services S.L., Spain, under report no. 15 F CL GW P/A. Processing of the samples started on the day of sampling or one day after collection of the fruits.

For the production of **preserved tomatoes (canned tomatoes)** the tomatoes were washed and blanched for 45 seconds in boiling water. The peels were removed, and the peeled tomatoes were filled in glass bottles. The rest of the peeled tomatoes was liquated and the raw juice and the wet pomace were separated. 0.02% of $CaCl_2$ was added to raw juice. The raw juice was heated up to 85ºC, then 0.5% of NaCl was added to the raw juice and stirred. Finally, the juice was filled into the glass bottles with the peeled tomatoes (1-1.5 parts of juice plus 2 parts of peeled tomatoes. All together was pasteurized for 60-90 minutes in boiling water.

For the production of **tomato puree** the tomatoes were washed, liquated and separated into raw juice and wet pomace. The raw juice was heated up to 85ºC for 5-10 minutes. 0.5% of common salt was added. Heating was followed-up until reaching 8.00º to 12.00º Brix to obtain the puree. Finally, the puree was filled in glass bottles and pasteurized for 60-90 minutes in boiling water.

For the production of **tomato juice** the tomatoes were washed, liquated and separated into raw juice and wet pomace. The raw juice was heated up to 85ºC for 5-10 minutes. 0.5% of salt was added, the juice was stirred, filled in glass bottles and pasteurized for 60-90 minutes in boiling water.

The analytical phase has been performed at Fredon Pays de la Loire / GIRPA in France under report no. B15G-P2-BCZ-01 with a method validated according to SANCO/825/00 rev. 8.1 and SANCO/3029/99 rev. 4 under report no. BPL-STUDY-18-000085. The analytical method principle was based on the method European Committee for Standardisation (CEN): EN 15662:2009-02. “Foods of plant origin – Determination of pesticide residues using GC-MS and/or LC-MS/MS following acetonitrile extraction/partitioning and clean-up by dispersive SPE – QuEChERS-method” using HPLC-MS/MS.

Results and discussions

The residues of zoxamide, benalaxyl-m and cymoxanil were determined with a limit of quantification (LOQ) of 0.01 mg/kg.
No residues were found in the control plot.
A summary of the results is given in the following table.

(Tetuan B., 2016)

Table A 36: Summary of the study 7 trial on tomatoes / greenhouse and open field (Southern Europe)

Reference:	Tetuan, B. 2016, 15F CL GW P/A		
GLP:	Yes	Sample storage conditions:	at ≤ -18 °C for residues analysis
Crop/crop group:	Tomato / fruiting vegetables	Analytical method:	B15G-P2-BCZ-01
Indoor/Outdoor:	Indoor and outdoor	Limit of Quantification (mg/kg):	0.01 mg/kg
Formulation:	SC for GWN-10392, WG for GWN-9823	Limit of Detection (mg/kg):	--
Content of active substance (g/kg or g/l):	Zoxamide 225 g/L and 33% w/w, benalaxyl-m 150 g/L, cymoxanil 33% w/w	Residues calculated as:	Zoxamide

Trial No./ Location/ EU zone/ Year	Commodity/ Variety	Date of 1.Sowing or planting 2.Flowering 3. Harvest	Application rate per treatment			Dates of treatment or no. of treatments and last date	Growth stage at last treatment or date BBCH	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			kg a.s./ ha * Plot 2 / plot 3	Water (l/ha) Plot 2 / plot 3	g a.s./hl				Zoxamide Plot 2 / plot 3		
15 F CL GW P01 41740 Lebrija (Sevilla) Spain S-EU, 2015	Industry Tomato H-9997	1) 16/04/15 2) 1st flowering mid. May 2015 3) 21/08/15	0.157 / NA 0.161 / NA 0.155 / NA	598 / NA 612 / NA 589 / NA	n.a.	03/08/15 10/08/15 17/08/15	87 88 89	Fruits Juice Preserved puree	0.11 / NA 0.021 / NA <LOQ / NA < LOQ / NA	3 3 3 3	LOQ: 0.01 mg/kg Analytical method B15G-P2-BCZ-01 Max. freezer storage period: 153 days
15 F CL GW P02 41220 Burguillos (Sevilla) Spain Indoor, 2015	Tomato Panekra	1) 15/07/15 2) Beginning August 3) 10/10/15	0.167 / 0.157 0.167 / 0.163 0.162 / 0.151	537 / 528 642 / 659 626 / 610	n.a.	25/09/15 02/10/15 09/10/15	71 72 89	Fruits	0.10 / 0.045 *	3	LOQ: 0.01 mg/kg Analytical method B15G-P2-BCZ-01 Max. freezer storage period: 100 days
15 F CL GW P03 41600 El Arahal (Sevilla) Spain Indoor, 2015	Tomato Matias	1) 10/06/15 2) 09/07/15 3) 1st picking 04/10/15	0.171 / 0.158 0.162 / 0.151 0.154 / 0.144	657 / 636 621 / 607 593 / 579	n.a.	25/09/15 02/10/15 09/10/15	81 82 83	Fruits	0.11 / 0.10 **	3	LOQ: 0.01 mg/kg Analytical method B15G-P2-BCZ-01 Max. freezer storage period: 100 days

LOQ for cymoxanil, cymoxanil and zoxamide: 0.010 mg/kg
NA = not applicable; * geometric mean value = 0.067 mg/kg (n=2); ** geometric mean value = 0.105 mg/kg (n=2)

A 2.1.3.3 Root and tuber crops – potatoes

Table A 37: Comparison of intended and critical EU GAPs

Type of GAP	Number of applications	Application rate per treatment (g/ha)	Interval between application	Growth stage at last application	PHI (days)
cGAP EU (Latvia, 2017; EFSA, 2017)	5	180	8	BBCH 80	7
cGAP EU (Art. 12, EFSA, year)	--	--	--	--	--
Intended cGAP (1-12 for N-EU and 41-47 for S-EU*)	3	148.5	7-10	Not relevant **	7

* Use number(s) in accordance with the list of all intended GAPs in Part B, Section 0

** Last application 28 days before harvest of tubers; harvest data of tubers = BBCH 49; partially erronemously given based on the (visible) developmental stages of the plants/leafs (i.e. BBCH 89)

The following two studies of Tetuan (2011a,b; reports no. 10 F PT GW P/A and 10 F PT GW/B) were considered for the authorisation of Cymoxanil 33% + Zoxamide 33% WG and regarded valid. They are summarised in the following.

A 2.1.3.3.1 Study 1

Comments of zRMS:	Comment of the zRMS Spain during product authorisation: *Two trials in Northern and two trials in Southern Europe are available in potatoes treated with 'Cymoxanil 33% + Zoxamide 33% WG' (Harpon WG, batch XE28160A11, Cymoxanil 32.7% , Zoxamide 33.5%). The 4 trials can be considered as valid according to the intended cGAP. No residues were found on treated and non-treated plant samples (potato tubers) for both active substances (<0.01 mg/kg, i.e.LOQ of the analytical method).* *The method was evaluated in Section 2, and accepted for pre-registration proposes suggesting confirmatory data validation with LOQ = 0.01 mg/Kg for both analytes* (Previously evaluated).

Reference:	**KCA 6.3/23**
Report	Tetuan, B., 2011: Determination of residues at harvest in potatoes following six broadcast applications of Harpon WG under field conditions - Northern Europe, season 2010 Gowan Comercio Internacional & Servicos Ltda, Portugal Promovert, France, Report No. 10 F PT GW P/A, GLP, Not published
Guideline(s):	1607/VI/97 rev. 2, Appendix B 7029/VI/95 rev.5 SANCO/3029/99 rev. 4 SANCO/825/00 rev. 7 ENV /JM/MONO (2007)17
Deviations:	During the preparation of specimens (study specimen reference nos. 10/GWP02/SH-001 and 10/GWP01/SH-001 the two control specimens packaging

	were identified by the same reference. The person who prepared the specimens made a transcription error. However, this deviation was found to have no impact on the study results since no residues were found in the control specimens.
GLP:	Yes
Acceptability:	Yes

and

Reference:	**KCA 6.3/24**
Report	Tetuan, B., 2011: Determination of residues at harvest in potatoes following six broadcast applications of Harpon WG under field conditions - Southern Europe, season 2010 Gowan Comercio Internacional & Servicos Ltda, Portugal Promovert, France, Report No. 10 F PT GW P/B, GLP, Not published
Guideline(s):	1607/VI/97 rev. 2, Appendix B 7029/VI/95 rev.5 SANCO/3029/99 rev. 4 SANCO/825/00 rev. 7 ENV /JM/MONO (2007)17
Deviations:	For trial 10 F PT GW P03 the tests plot labels were not present at the moment of sampling (7 DALA). Test plots were identified at trial settlement, test plot labels removed by someone. However, this deviation was regarded to not change the integrity of the study results.
GLP:	Yes
Acceptability:	Yes

Materials and methods

Test material (Lot/Batch No.):	Cymoxanil 33% + Zoxamide 33% WG / Harpon WG (XE28160A11)
Active substance content (nominal):	33 % w/w zoxamide, 33% w/w cymoxanil (nominal) 33.5 % w/w zoxamide, 32.7 % w/w cymoxanil (analysed)
Expiry date:	June 2011

Two trials in Northern and two trials in Southern Europe are available in potatoes treated with 'Cymoxanil 33% + Zoxamide 33% WG' (Harpon WG, batch XE28160A11, cymoxanil 32.7%, zoxamide 33.5%) at 0.45 kg formulated product/ha, with 6 (Northern Europe) and 5 (Southern Europe) applications at 7 days intervals and with samples collected at 6-7 days after the last application.

After extraction with acetonitrile/2% potassium bicarbonate aqueous solution (80/20, v/v) mixture and clean-up by dispersive solid phase extraction (D-SPE), the determination of cymoxanil and zoxamide was performed by liquid chromatography with detection by tandem mass spectrometry (LC-MS/MS) with a LOQ = 0.01mg/kg for both substances.

The field phases have been performed by BioChem agrar in Germany under the study report nos. 10 F PT GW P/A and 10 F PT GW P/B and by Promovert in Spain under phase repot no. 10 F PT GW P/B, the analytical phase has been performed at GIPRA in France under report no. PROMO/ZOX-CYM/10.01 and

PROMO/ZOX-CYM/10.02 with a method validated according to SANCO/825/00 rev. 7 and SANCO/3029/99 rev. 4 under GIRPA methods GIR/MET/CYMOXANI/04V1 and GIR/MET/ZOX-AMIDE/ 02Vl.

Results and discussion

No residues were found in treated and non-treated plant samples (potato tubers) (<0.01 mg/kg, i.e.LOQ of the analytical method).

(Tetuan B. 2011)

Table A 38: Summary of the study 1 trials on potatoes / Northern EU

Reference:	Tetuan, B., 2011, 10 F PT GW P/A		
GLP:	Yes	Sample storage conditions:	≤ -18°C for residues analysis
Crop/crop group:	Potato / root and tuber crops	Analytical method:	GIPRA method GIR/MET/ZOXAMIDE/02Vl
Indoor/Outdoor:	Outdoor	Limit of Quantification (mg/kg):	0.01 mg/kg
Formulation:	WG	Limit of Detection (mg/kg):	--
Content of active substance (g/kg or g/l):	330 g/kg zoxamide + 330 g/kg cymoxanil	Residues calculated as:	Zoxamide

Trial No./ Location/ EU zone/ Year	Commodity/ Variety	Date of 1.Sowing or planting 2.Flowering 3. Harvest	Application rate per treatment			Dates of treatment or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			kg a.s./ ha (2)	Water (L/ha) (1)	kg a.s./hL						
10F PT GW P01 04668 Motterwitz (Saxony) Germany N-EU, 2011	Potato/Alegria	1) 26/05/2010 2) 10-20/07/2010 3) 06/10/2010	0.1485 0.1485 0.1485 0.1485 0.1485 0.1485	400 400 400 400 400 400	0.037 0.037 0.037 0.037 0.037 0.037	05/08/2010 10/08/2010 16/08/2010 20/08/2010 25/08/2010 30/08/2010	BBCH 81 BBCH 81 BBCH 85 BBCH 85 BBCH 85 BBCH 87	Tubers	< LOQ (< 0.01)	7	LOQ = 0.01 mg/kg Analytical method GIR/MET/ZOXAMIDE/02Vl Max. Storage interval between sampling and analysis: 143 days
10F PT GW P02 18258 Kassow Mecklemburg (West Pomerania) Germany N-EU, 2011	Potato/Albatros	1) 20/04/2010 2) 12-24/07/2010 3) 23/09/2010	0.1485 0.1485 0.1485 0.1485 0.1485 0.1485	400 400 400 400 400 400	0.037 0.037 0.037 0.037 0.037 0.037	04/08/2010 09/08/2010 15/08/2010 20/08/2010 25/08/2010 29/08/2010	BBCH 81 BBCH 81 BBCH 81-85 BBCH 85 BBCH 85 BBCH 85	Tubers	< LOQ (< 0.01)	6	LOQ = 0.01 mg/kg Analytical method GIR/MET/ZOXAMIDE/02Vl Max. Storage interval between sampling and analysis: 145 days

(1): In the study report the nominal volumes were given as range: 380 – 420 L for NEU trials and 410 – 479 L for SEU.

(2): Nominal values. In the study report the rates were given in the range of 0.283 – 0.313 kg as/ha for total of cymoxanil + zoxamide for NEU trials and 0.27 – 0.32 kg as/ha for SEU trials (= as sum of both actives).

Table A 39: Summary of the study 1 trials on potatoes / Southern EU

Reference:	Tetuan, B., 2011, 10 F PT GW P/B		
GLP:	Yes	Sample storage conditions:	≤ -18°C for residues analysis
Crop/crop group:	Potato / root and tuber crops	Analytical method:	PROMO/ZOX-CYM/10.01 (10 F PT GW P/A)
Indoor/Outdoor:	Outdoor	Limit of Quantification (mg/kg):	0.01 mg/kg
Formulation:	WG	Limit of Detection (mg/kg):	--
Content of active substance (g/kg or g/l):	330 g/kg zoxamide + 330 g/kg cymoxanil	Residues calculated as:	Zoxamide

Trial No./ Location/ EU zone/ Year	Commodity/ Variety	Date of 1.Sowing or planting 2.Flowering 3. Harvest	Application rate per treatment			Dates of treatment or no. of treat-ments and last date	Growth stage at last treat-ment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			kg a.s./ ha [2]	Water (L/ha) [1]	kg a.s./hL						
10F PT GW P03 11130 Chiclana de la frontera (Cadiz) Spain S-EU, 2011	Potato/Carlita	1) 23/08/2010 2) 15-30/11/2010 3) 10/12/2010	0.1485 0.1485 0.1485 0.1485 0.1485 0.1485	400 400 400 400 400 400	0.037 0.037 0.037 0.037 0.037 0.037	05/11/2010 10/11/2010 16/11/2010 22/11/2010 26/11/2010	BBCH 40 BBCH 43 BBCH 44 BBCH 45 BBCH 46	Tubers	< LOQ (< 0.01)	7	LOQ = 0.01 mg/kg Analytical method validated in study GIR/MET/ZOX-AMIDE/02Vl Max. Storage interval between sampling and analysis: 55 days
10F PT GW P04 11140 Conil de la frontera (Cadiz) Spain S-EU, 2011	Potato/Spunta	1) 15/09/2010 2) 12-30/11/2010 3) 07/12/2010	0.1485 0.1485 0.1485 0.1485 0.1485 0.1485	400 400 400 400 400 400	0.037 0.037 0.037 0.037 0.037 0.037	05/11/2010 10/11/2010 16/11/2010 22/11/2010 26/11/2010	BBCH 40 BBCH 43 BBCH 44 BBCH 45 BBCH 46	Tubers	< LOQ (< 0.01)	7	LOQ = 0.01 mg/kg Analytical method validated in study GIR/MET/ZOX-AMIDE/02Vl Max. Storage interval between sampling and analysis: 55 days

[1]: In the study report the nominal volumes were given as range: 380 – 420 L for NEU trials and 410 – 479 L for SEU.

[2]: Nominal values. In the study report the rates were given in the range of 0.283 – 0.313 kg as/ha for total of cymoxanil + zoxamide for NEU trials and 0.27 – 0.32 kg as/ha for SEU trials (= as sum of both actives).

Additional / new studies were performed with a zoxamide 240 g/L SC product applied with higher application rates (180 g a.s./ha) and application numbers (i.e. 5) compared to the here intended GAP use (i.e. max. of 3) – and can therefore be considered as a worst-case.
These additional / new studies are submitted for evaluation within this dossier. They have not yet been assessed beforehand.

A 2.1.3.3.2 Study 2

Comments of zRMS:	The study is acceptable. Two harvest trials were conducted in NEU. The objective of the study is to determine the magnitude of the residues of zoxamide and metabolite RH-1452 and RH-1455 in raw potatoes (RAC tubers) and processed fractions after 5 applications of GWN 9790 EU. The application rate was 0.75 L/ha, representing 180 g/ha of zoxamide at each application, starting at 36 or 39 DBH, with 8 ± 1 days interval, last application 7 days before harvest. The analysis was performed on the basis of the analytical method described and validated separately from this study (see B5, BPL-STUDY-18-000085). The analytical method was validated in potato following SANCO/825/00 rev.8.1 and SANCO/3029/99 rev.4. During the study analysis acceptance criteria for method validations were met, with average recoveries ranging from 70% to 110%. The LOQ for zoxamide and metabolites RH-141452 and RH-141455 was 0.010 mg/kg. No residue of zoxamide and its metabolites RH-141452 and RH-141455 were found above the LOQ in all the specimens (RAC tubers, potato flakes and French fries).

Reference:	**KCA 6.3/25**
Report:	Terranegra, A., 2020: Magnitude of residue of zoxamide and metabolite RH-1452 and RH-1455 in potatoes (RAC tubers) and processed fractions, following 5 applications of GWN 9790 EU in two trials (2 HS), Northern Europe (France and Poland) – 2017 – amended Final Report[24] GOWAN Crop Protection Ltd., UK Staphyt Italia S.r.l., Italy, Report No. ATA-18-30694, GLP, Not published
Guideline(s):	OECD TG 509 (2009) OECD TG 508 (2008) SANCO 7029/VI/95 rev. 5 (1997) SANCO 7035/VI/95 rev. 5 (1997)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Test item (Lot/Batch No.):	GWN 9790 EU / Zoxium 240 SC (SB 2401)
Active substance content:	240 g/L Zoxamide (nominal), 232g/L (analysed) (R/S Zoxamide ratio: 50/50)

[24] Confirmatory study for zoxamide currently being evaluated by RMS (Latvia).

Expiry date: April 2018

The magnitude of residues of zoxamide and its metabolites RH-1452 (synonym to RH-141455) and RH-1455 (synonym to RH-141452) in raw agricultural commodity specimens of potatoes (RAC tubers) and processed fractions has been determined in two harvest trials performed in Northern Europe (France and Poland) in 2017. In addition, the metabolite RH-150721 has been determined in potato processed commodities (potato flakes and fried potatoes). Two plots were established per trial: plot 1 (control) was left untreated, plot 2 was treated five times with each 0.75 L/ha GWN 9790 EU at a 7-8 day's interval with the last application 7 days before harvest (one exception: the last application in trial ATA-18-30694 PL02 has been performed after 11 days interval). The test item has been applied with boom sprayers to reflect common agricultural practice.

The field phase has been performed by Staphyt in France and Germany under phase report no. ATA-18-30694, the processing phase has been performed by Staphyt in France under phase report no. ATA-18-30694, the analytical phase has been performed at Biotecnologie BT in Italy under report no. BPL-STUDY-19-0000065 with a method validated according to SANCO/825/00 rev. 8.1 and SANCO/3029/99 rev. 4 under report no. BPL-STUDY-18-000085.

The raw agricultural commodities (potato tubers) for processing were collected and stored under ambient conditions before they were shipped under cool conditions for processing to potato flakes and French fries at Staphyt in France. All other potato tuber samples were frozen down within 2:10 – 3:10 hours and kept frozen until analysis.

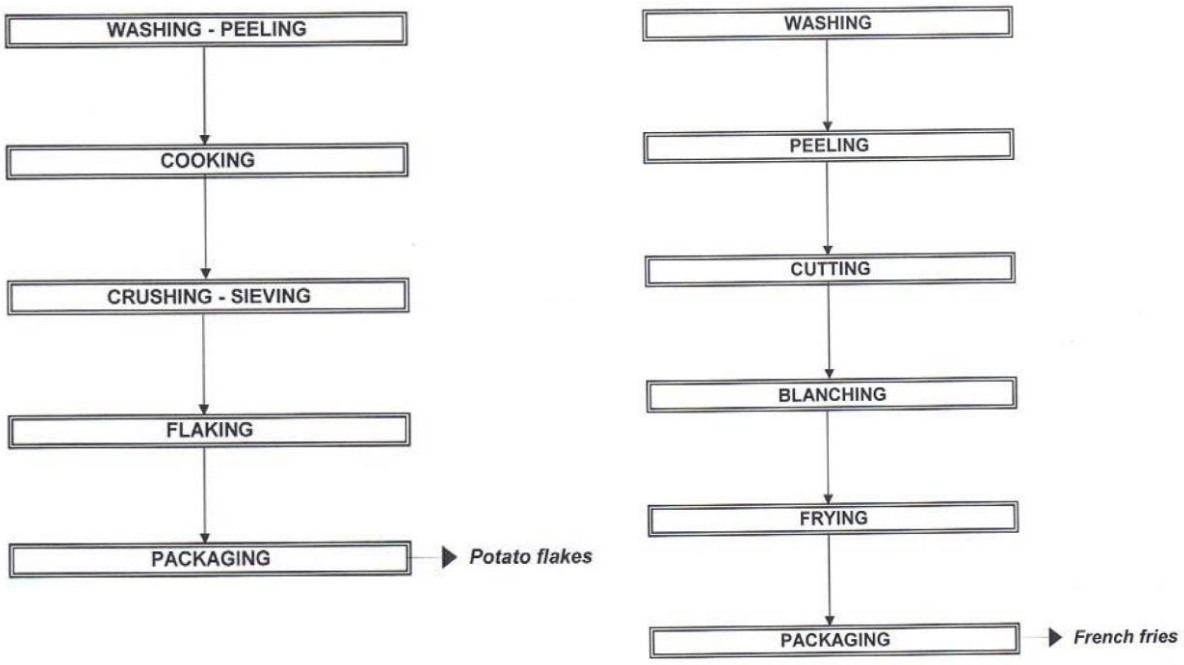


Figure A 8: Processing of potato tubers

The metabolites RH-141452 and RH-141455, which were known to form conjugates with matrix molecules (e.g., sugars), were released in an additional hydrolysis step to establish total fractions in addition to the free fractions in the matrices.

Results and discussion

There were no residues detectable in the untreated control plots.

The residues of zoxamide and its metabolite containing a chiral centre (RH-150721) were expressed as sum

of 2 enantiomers and per single enantiomer (R and S). The sum of both is depicted in the following table.

Total fractions of RH-141452 and RH-141455 are summarised.

The limit of quantification (LOQ) was set at 0.010 mg/kg for zoxamide and RH-150721 (sum of enantiomers), RH-141452 and RH-141455, and 0.005 mg/kg for single isomers. The limit of determination (LOD) is given as 0.003 mg/kg for zoxamide and RH-150721 (sum of enantiomers), RH-141452 and RH-141455, and 0.0015 mg/kg for single isomers.

As a result, no residue of zoxamide and its metabolites were found above LOD in all the specimens (RAC specimens: potato tuber, processing specimens: potato flakes and French fries). A summary of the results is given in the following table.

(Terranegra A. 2020)

Table A 40: Summary of the study 2 trials on potatoes / Northern EU

Reference:	Terranegra A. 2020, ATA-18-30694		
GLP:	Yes	Sample storage conditions:	≤ -18°C for residues analysis
Crop/crop group:	Potatoes / root and tuber crops	Analytical method:	BPL-STUDY-18-000085
Indoor/Outdoor:	Outdoor	Limit of Quantification (mg/kg):	0.01 mg/kg
Formulation:	SC	Limit of Detection (mg/kg):	0.003 mg/kg
Content of active substance (g/kg or g/l):	240 g/L zoxamide	Residues calculated as:	Zoxamide (sum), (R)-Zoxamide, (S)-Zoxamide, RH-150721 (sum), (R)-RH-150721, (S)-RH-150721, RH-141452, RH-141455

Trial No. Location EU zone Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest	Application rate per treatment			Dates of treatment or no. of treatments and last date	Growth stage at last treatment or date **	Portion analysed	Residues (mg/kg)				PHI (days)	Remarks
			kg a.s./ha	Water (l/ha)	kg a.s./hL				Zoxamide	RH-150721	RH-141452 *	RH-141455 *		
ATA-18-30694 FR01 59400 Moeuvres Hauts de France France N-EU, 2017	Potato / Marabelle	1) 19/04/2017 2) 15/06/2017 – 04/07/2017 3) 26/09/2017	0.177 0.177 0.174 0.181 0.180	0.763 0.738 0.750 0.781 0.775	0.087 0.087 0.087 0.087 0.087	25/07/2017 01/08/2017 09/08/2017 16/08/2017 23/08/2017	45 47 48 48 49	Potato tubers	ND (< 0.003)	NR	ND (< 0.003)	ND (< 0.003)	7	LOQ: 0.01 mg/kg LOD: 0.003 mg/kg Analytical method validated in study BPL-STUDY-18-000085. Max. freezer storage period: 638 days
								Potato flakes	ND (< 0.003)	ND (< 0.003)	ND (< 0.003)	ND (< 0.003)		
								French fries	ND (< 0.003)	ND (< 0.003)	ND (< 0.003)	ND (< 0.003)		
ATA-18-30694 PL02 21-200 Przewłoka Lubelskie Poland N-EU, 2017	Potato / Harpin	1) 01/05/2017 2) 04/07/2017 – 01/08/2017 3) 23/09/2017	0.169 0.172 0.173 0.171 0.177	0.730 0.740 0.745 0.735 0.765	0.058 0.058 0.058 0.058 0.058	10/08/2017 18/08/2017 26/08/2017 02/09/2017 11/09/2017	45 46 47 48 49	Potato tubers	ND (< 0.003)	NR	ND (< 0.003)	ND (< 0.003)	7	LOQ: 0.01 mg/kg LOD: 0.003 mg/kg Analytical method validated in study BPL-STUDY-18-000085. Max. freezer storage period: 619 days
								Potato flakes	ND (< 0.003)	ND (< 0.003)	ND (< 0.003)	ND (< 0.003)		
								French fries	ND (< 0.003)	ND (< 0.003)	ND (< 0.003)	ND (< 0.003)		

* Total fraction
** Potato tuber developmental stage
ND = not detectable, NR = not relevant

A 2.1.3.3.3 Study 3

Comments of zRMS:	Not relevant.

Reference:	**KCA 6.3/26**
Report	Pandolfi, A., 2020: Determination of the residues of zoxamide (R), (S) and sum and its metabolites in raw agricultural commodity potato (tubers) and its processed fractions (chips, baked/cooked, fried and flakes) following five applications of Zoxium 240 SC (sponsor code GWN-9790 EU) in open field condition (Italy - Southern Europe - 2 trials year 2018)[25] Gowan Grop Protection Ltd., UK Res Agraria S.r.l., Italy, Report No. RA 18 051 BPL GW, GLP, Not published
Guideline(s):	OECD 13 - ENV/MC/CHEM (98) OECD ENV/JM/MONO (2002) SANCO/825/00 rev. 8.1 (2010) SANCO/3029/99 rev. 4 (2000).
Deviations:	No
GLP:	Yes

Materials and methods

Test item (Lot/Batch No.):	GWN 9790 EU / Zoxium 240 SC (SIPAL7001)
Active substance content:	240 g/L Zoxamide (nominal), 242.9 g/L (analysed) (R/S Zoxamide ratio: 50.0/50.0)
Expiry date:	May 2020

The magnitude of residues of zoxamide and its metabolites RH-141455 and RH-141452 in raw agricultural commodity specimens of potatoes (RAC tubers) and processed fractions has been determined in one harvest (RA 18 051 BPL IT 01) and one decline trial (RA 18 051 BPL IT 02) performed in Southern Europe (Italy) in 2018. In addition, the metabolite RH-150721 has been analysed in potato processed commodities (potato chips, baked/cooked potato, fried potato and potato flakes). Two plots were established per trial: plot 1 (control) was left untreated, plot 2 was treated five times with each 0.75 L/ha GWN 9790 EU (180 g a.i./ha of zoxamide) at a 7-8 day's interval with the last application either 7 or 0, 1, 3, 5 and 7 days before harvest. The test item has been applied with Knapsack sprayers.

The field and processing phase has been performed by RES AGRARIA S.r.l. in Italy under phase report no. RA 18 051 BPL GW, the analytical phase has been performed at LabAnalysis s.r.l. in Italy under report no. BPL-STUDY-19-000025 with a method validated according to SANCO/825/00 rev. 8.1 and SANCO/3029/99 rev. 4 under report no. BPL-STUDY-18-000085.

Part of the samples of the raw agricultural commodities (potato tubers) were processed to potato chips, baked/cooked potato, fried potato and potato flakes. The processing started within the same day of the sampling. All other potato tuber samples were frozen down within 1:20 – 2:55 hours and kept frozen at ≤ -18 °C until analysis.

The following processing procedures were applied:

[25] Confirmatory study for zoxamide currently being evaluated by RMS (Latvia).

Chips: The potatoes were peeled, cut into very thin slices and fried in hot oil at 130°C until they were golden, they were extracted from the oil and dried using absorbent paper.

Baked/cooked potatoes: The potatoes were peeled and divided in half, placed on a plate and cooked in the microwave for 16 minutes at 800 watts.

Fried potatoes: The potatoes were peeled, cut into sticks and fried in a pan in hot oil at 130°C for 20 minutes – until they were golden, they were extracted from the oil and dried using absorbent paper.

Flakes: The potatoes were peeled by knife. They were placed in a pot and filled the pot with cold water until the tubers are completely immersed. The potatoes were cooked for 8 minutes after the water reached boiling point by Induction Plate, keeping the boiling. Then the water was drained and let the potatoes cool. Finally, they were put in the drier for 25 hours in a coarse slice.

The cold samples were introduced in double polythene bags, weighed, labeled and frozen.

All specimens (processed and unprocessed) were homogenised frozen in a vegetable grinder with dry ice before shipment of homogeneous sub-samples at –18°C or lower for analysis. The residues content was determined at LabAnalysis S.r.l. in Italy under report no. BPL-STUDY-19-000025 with a method validated according to SANCO/825/00 rev. 8.1 and SANCO/3029/99 rev. 4 under report no. BPL-STUDY-18-000085.

The metabolites RH-141452 and RH-141455, which were known to form conjugates with matrix molecules (e.g. sugars), were released in an additional hydrolysis step to establish total fractions in addition to the free fractions in the matrices.

Results and discussion

There were no residues detectable in the untreated control plots.

The residues of zoxamide and its metabolite containing a chiral centre (RH-150721) were expressed as sum of 2 enantiomers and per single enantiomer (R and S).

Total fractions of RH-141452 and RH-141455 are summarised.

The limit of quantification (LOQ) was set at 0.010 mg/kg for zoxamide and RH-150721 (sum of enantiomers), RH-141452 and RH-141455, and 0.005 mg/kg for single isomers. The limit of determination (LOD) is given as 0.003 mg/kg for zoxamide and RH-150721 (sum of enantiomers), RH-141452 and RH-141455, and 0.0015 mg/kg for single isomers.

As a result, no residue of zoxamide and its metabolites were found above LOQ in all the specimens (RAC specimens: potato tuber, processing specimens: potato flakes and French fries) after harvest at the intended PHI of 7 days and beforehand (i.e. last application 1, 3 and 5 days before harvest). A summary of the results is given in the following table.

(Pandolfi A. 2020)

Table A 41: Summary of the study 3 trials on potatoes / Southern EU

Reference:	Pandolfi A., 2020, RA 18 051 BPLGW		
GLP:	Yes	Sample storage conditions:	≤ -18 °C for residues analysis
Crop/crop group:	Potatoes / root and tuber crops	Analytical method:	BPL-STUDY-18-000085
Indoor/Outdoor:	Outdoor	Limit of Quantification (mg/kg):	0.01 mg/kg (sum), 0.005 mg/kg (for each isomer)
Formulation:	SC	Limit of Detection (mg/kg):	0.003 mg/kg (sum), 0.0015 mg/kg (for each isomer)
Content of active substance (g/kg or g/l):	240 g/L zoxamide	Residues calculated as:	Zoxamide (sum), (R)-Zoxamide, (S)-Zoxamide, RH-150721 (sum), (R)-RH-150721, (S)-RH-150721, RH-141452, RH-141455

Report No. Location (region)	Commodity/ Variety (a)	Date of 1. Sowing 2. Flowering 3. Harvest	Application rate per treatment			Dates of treatment(s) or no of treatment(s) and last date	Growth stage at last treatment or date ***	Portion Analysed	Residues (mg/kg)								PHI (days)	Remarks:
			kg as/ha	Water (L/ha)	kg as/hL				Zoxamide (R)	Zoxamide (S)	Zoxamide (sum R + S)	(R)-RH-150721	(S)-RH-150721	RH-150721 (sum)	RH-141452 **	RH-141455 **		
RA 18 051 BPL IT 01 64026 Roseto degli Abruzzi Abruzzo Italy S-EU, 2018	Potato / Montreal	1) 15/03/2018 2) Middle - End of June 3) Beginning-middle of August	0.177 0.172 0.177 0.178 0.173	981.667 955.000 981.667 990.000 959.667	0.018	02/07/2018 09/07/2018 17/07/2018 25/07/2018 02/08/2018	43 43 45 47 47	Potato tubers	ND	ND	ND	NR	NR	NR	ND	ND	7	*
								Potato chips	ND	ND	ND	ND	ND	ND	ND	ND		
								Baked/ cooked potatoes	ND	ND	ND	ND	ND	ND	ND	ND		
								Fried potatoes	ND	ND	ND	ND	ND	ND	ND	ND		
								Potato flakes	ND	ND	ND	ND	ND	ND	ND	ND		
RA 18 051 BPL IT 02 64013 Corropoli Abruzzo Italy S-EU, 2018	Potato / Desirée	1) 27/02/2018 2) Beginning - Middle of June 3) Beginning - Middle of July	0.173 0.174 0.184 0.178 0.180	962.000 966.667 1020.167 991.500 997.833	0.018	04/06/2018 12/06/2018 20/06/2018 28/06/2018 06/07/2018	42 43 45 47 48	Potato tubers	<LOQ (0.00247)	< LOQ (0.00293)	< LOQ (0.00540)	NR	NR	NR	ND	ND	0	*
									ND	ND	ND	NR	NR	NR	ND	ND	1	
									ND	ND	ND	NR	NR	NR	ND	ND	3	
									ND	ND	ND	NR	NR	NR	ND	ND	5	
									ND	ND	ND	NR	NR	NR	ND	ND	7	
								Potato chips	ND	ND	ND	ND	ND	ND	ND	ND		
								Baked/ cooked potatoes	ND	ND	ND	ND	ND	ND	ND	ND		

								Fried pota-toes	ND	ND	ND	ND	ND	ND	ND	ND		
								Potato flakes	ND	ND	ND	ND	ND	ND	ND	ND		

* LOQ = 0.010 mg/kg for zoxamide and RH-150721 (sum of enantiomers), RH-141452 and RH-141455, and 0.005 mg/kg for single isomers.
LOD = 0.003 mg/kg for zoxamide and RH-150721 (sum of enantiomers), RH-141452 and RH-141455, and 0.0015 mg/kg for single isomers.
Analytical method validated in study BPL-STUDY-18-000085
Max. Storage interval between sampling and analysis: 600 – 634 days
** Total fraction
*** Potato tuber developmental stage
ND = not detectable, NR = not relevant

A 2.1.3.4 Lettuce

The disappearance of zoxamide was studied by Luciani in 2012 (reports no. AGRI 013/12 GLP DEC and AGRI 014/12 GLP DEC) in eight residues trials on salad performed each in the open field in Southern Europe (Italy) and in the greenhouse. The total 16 trials on open head salads (lettuce, rocket salad, endive and escarole) were already evaluated and regarded valid by EFSA (2016)[26] for the modification of maximum residue levels (MRLs) of zoxamide in the crop groups 'lettuces and salad plants', 'spinaches and similar leaves' and 'herbs and edible flowers'. The experimental results are reported since they were taken as input data for CAKE 3.4 calculations to generate substance specific DT_{50} values for zoxamide on/in plants after the last application of zoxamide (see dRR Part B Section 8).

A 2.1.3.4.1 Study 1

Comments of zRMS:	The study is acceptable. No lettuces in the intended GAP and the presented 4 trials are from SEU, however they were used to generate substance specific DT50 values for fate modelling and worker exposure. Therefore, the study was evaluated. The method used was the validated in lettuce LC-MS/MS method. Two transitions were monitored (confirmatory method). The recoveries were within the required range.

Reference:	**KCA 6.3/27**
Report	Luciani, G.P., 2012: Determination of zoxamide and dimethomorph residues after two application of Zoxium 240 SC and GWN 9963 on lettuce, rocket salad and endive under field conditions – Italian trials, year 2012[27] Gowan Italia Spa., Italy Agriparadigma Srl, Italy, Report No AGRI 013/12 GLP DEC, GLP, Not published
Guideline(s):	OECD TG 509 (2009) SANCO 7029/Vl/95 - rev. 5 (1997)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Test material (Lot/Batch No.)	Zoxium 240 SC (OD0601)
Active substance content (nominal)	240 g/L zoxamide (nominal), 241.5 g/L (analysed)
Expiry date	November 2014
Test material (Lot/Batch No.)	GWN-9963 (12020301)
Active substance content (nominal)	180 g/L zoxamide (nominal), 179.1 g/L (analysed) 180 g/L dimethomorph (nominal), 177.6 g/L (analysed)

[26] EFSA (2016): Reasoned opinion on the modification of the existing maximum residue levels for zoxamide in various leafy crops. EFSA Journal 2016;14 (7): 4527, 13 pp.

[27] Confirmatory study for zoxamide currently being evaluated by RMS (Latvia).

Expiry date	January 2015

Materials and methods

In a study report by Luciani G.P. (2012; report no. AGRI 013/12 GLP DEC) in total four decline trials on lettuce, rocket salad and endive were performed in 2012 in double in the open field at four different locations in Italy, Southern Europe. In these trials, zoxamide was applied twice with a Knapsack sprayer at nominally 180 g/ha in a water volume of 500 L/ha with either Zoxium 240 SC (an SC formulation containing nominally 240 g/L zoxamide) or GWN-9963 (SC formulation containing nominally 180 g/L of each zoxamide and dimethomorph) at an interval of 8 ± 1 (i.e. 8) days during crop growth stages BBCH 45–46. One control plot per trial was left untreated.

The residues samples were stored under deep frozen conditions between 3 and 6:48 hours after sampling and shipped and stored deep frozen at ≤ -18 °C. Whole plants were analysed 0, 3, 7, 10 and 14 days after the last application with a method validated according to SANCO/825/00 rev. 8.1 (2010) and proved to be fit for purpose during EU MRL evaluation (EFSA, 2016). The Limit of Quantification (LOQ) for zoxamide was 0.01 mg/kg on lettuce.

Samples were extracted with acetonitrile, after addition of magnesium sulfate, sodium chloride and buffering citrate salts, the mixture was shaken intensively and centrifuged for phase separation. The final extracts were directly employed for LC analysis. (Dimethomorph and) zoxamide residues were quantified by high performance liquid chromatography coupled to a triple quadrupole mass spectrometer operating in Multiple Reaction Monitoring (MRM) mode.

The field phase has been performed by Soc. Coop. Res Agraria arl, Italy, under report no. R 12 058BPL. The analytical phase has been performed by Agriparadigma S.r.l, Italy, under report no AGRI 013/12 GLP DEC with a method Agri BPL 040 rev. 6.

Results and discussion

The limit of quantification (LOQ) for zoxamide was 0.01 mg/kg on lettuce and similar.

In lettuce, rocket salad and endive the residues of zoxamide were below the method limit of quantification in all untreated specimens, while the treated samples had detectable residues. Results of the residue studies are summarised in the following table.

(Luciani G.P. 2012)

Table A 42: Summary of the study 1 trials on lettuce / Southern EU

Reference:	Luciani, P.G., 2012, AGRI 013/12 GLP DEC		
GLP:	Yes	Sample storage conditions:	at ≤ -18 °C for residues analysis
Crop/crop group:	Lettuce	Analytical method:	Agri BPL 040 Rev.6
Indoor/Outdoor:	Outdoor	Limit of Quantification (mg/kg):	0.01 mg/kg
Formulation:	SC	Limit of Detection (mg/kg):	--
Content of active substance (g/kg or g/l):	240 g/L zoxamide 180 g/L zoxamide + 180 g/L dimethomorph	Residues calculated as:	Zoxamide

Trial No./ Location/ EU zone/ Year	Commodity/ Variety	Date of 1.Sowing or planting 2.Flowering 3. Harvest	Application rate per treatment			Dates of treatment or no. of treatments and last date	Growth stage at last treatment or date (BBCH)	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			g a.s./ ha	Water (l/ha)	g a.s./hl				Zoxamide		
RA 12 058BPL IT 01 63010 Campofilone Italy S-EU, 2012	Lettuce Trocadero	1) 25/09/2012 2) - 3) 18/12/2012	183 177	510 492	-	16/11/2012 24/11/2012	45 46	Leaf	8.24 5.05 4.44 2.18 0.78	0 3 7 10 14	LOQ = 0.01 mg/kg Analytical method Agri BPL 040 Rev.6 Max. Storage interval between sampling and analysis: 5-54 days
			174 174	483 520	-	16/11/2012 24/11/2012	45 46	Leaf	8.72 6.74 3.49 2.28 1.03	0 3 7 10 14	
RA 12 058BPL IT 02 64027 Sant'Omero Italy	Lettuce Trocadero	1) 05/10/2012 2) - 3) 08/12/2012	180 179	499 498	-	26/11/2012 04/12/2012	45 46	Leaf	12.07 6.79 4.66 2.54 1.02	0 3 7 10 14	LOQ = 0.01 mg/kg Analytical method Agri BPL 040 Rev.6

Trial No./ Location/ EU zone/ Year	Commodity/ Variety	Date of 1.Sowing or planting 2.Flowering 3. Harvest	Application rate per treatment			Dates of treatment or no. of treatments and last date	Growth stage at last treatment or date (BBCH)	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			g a.s./ ha	Water (l/ha)	g a.s./hl				Zoxamide		
S-EU, 2012			174 183	482 507	-	26/11/2012 04/12/2012	45 46	Leaf	9.53 4.20 3.99 3.36 2.21	0 3 7 10 14	Max. Storage interval between sampling and analysis: 5-54 days
RA 12 058BPL IT 03 64011 Alba Adriatica Italy S-EU, 2012	Rocket salad Selvatica	1) 17/10/2012 2) - 3) 18/12/2012	184 182	510 477	-	26/11/2012 04/11/2012	45 46	Leaf	21.26 8.86 7.45 7.41 5.50	0 3 7 10 14	LOQ = 0.01 mg/kg Analytical method Agri BPL 040 Rev.6 Max. Storage interval between sampling and analysis: 5-54 days
			175 176	487 490	-	26/11/2012 04/11/2012	45 46	Leaf	23.34 16.77 9.46 8.62 6.11	0 3 7 10 14	
RA 12 058BPL IT 04 63061 Massignano Italy S-EU, 2012	Endive Quintana	1) 27/09/2012 2) - 3) 08/12/2012	173 176	481 490	-	16/11/2012 24/11/2012	45 46	Leaf	5.65 5.35 3.18 2.33 2.05	0 3 7 10 14	LOQ = 0.01 mg/kg Analytical method Agri BPL 040 Rev.6 Max. Storage interval between sampling and analysis: 5-54 days
			182 179	505 496	-	16/11/2012 24/11/2012	45 46	Leaf	5.02 3.97 2.91 2.71 2.28	0 3 7 10 14	

A 2.1.3.4.2 Study 2

Comments of zRMS:	The study is acceptable. No lettuces in the intended GAP and the presented 4 trials are from SEU, however they were used to generate substance specific DT50 values for fate modelling and worker exposure. Therefore, the study was evaluated. The method used was the validated in lettuce LC-MS/MS method. Two transitions were monitored (confirmatory method). The recoveries were within the required range.

Reference:	**KCA 6.3/28**
Report	Luciani G.P., 2012: Determination of zoxamide and dimethomorph residues after two application of Zoxium 240 SC and GWN 9963 on lettuce and rocket – Italian trial, year 2012[28] Gowan Italia Spa, Italy AgriParadigma Srl, Italy, Report No. AGRI 014/12 GLP DEC, GLP, Not published
Guideline(s):	OECD TG 509 (2009) SANCO 7029/Vl/95 - rev. 5 (1997)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Test material (Lot/Batch No.)	Zoxium 240 SC (OD0601)
Active substance content (nominal)	240 g/L zoxamide (nominal), 241.5 g/L (analysed)
Expiry date	November 2014
Test material (Lot/Batch No.)	GWN-9963 (12020301)
Active substance content (nominal)	180 g/L zoxamide (nominal), 179.1 g/L (analysed) 180 g/L dimethomorph (nominal), 177.6 g/L (analysed)
Expiry date	January 2015

In a study report by Luciani G.P. (2012; report no. AGRI 014/12 GLP DEC) in total four decline trials on lettuce, rocket salad and escarole were performed in 2012 in double under greenhouse conditions. In these trials, zoxamide was applied twice with a Knapsack sprayer at nominally 180 g/ha in a water volume with either Zoxium 240 SC (an SC formulation containing nominally 240 g/L zoxamide) or GWN-9963 (SC formulation containing nominally 180 g/L of each zoxamide and dimethomorph) at an interval of 8 ± 1 (i.e. 9) days during crop growth stage BBCH 14-41. One control plot per trial was left untreated.

The residues samples were stored under deep frozen conditions in < 24 hours after sampling and shipped and stored deep frozen at ≤ -18°C until extraction and analysis. The whole plants were analysed 0, 3, 7, 10 and 14 days after the last application with a method validated according to SANCO/825/00 rev. 8.1 (2010)

[28] Confirmatory study for zoxamide currently being evaluated by RMS (Latvia).

and proved to be fit for purpose during EU MRL evaluation (EFSA, 2016). The Limit of Quantification (LOQ) for zoxamide was 0.01 mg/kg on lettuce.

Samples were extracted with acetonitrile, after addition of magnesium sulfate, sodium chloride and buffering citrate salts, the mixture was shaken intensively and centrifuged for phase separation. The final extracts were directly employed for LC determinative analysis. (Dimethomorph and) zoxamide residues were quantified by high performance liquid chromatography coupled to a triple quadrupole mass spectrometer operating in Multiple Reaction Monitoring (MRM).

The field phase has been performed by Agrigeos srl, Italy, under report no. R03AG12. The analytical phase has been performed by Agriparadigma S.r.l, Italy, Report No AGRI 014/12 GLP DEC with a method Agri BPL 040 rev. 6

Results and discussions

The Limit of Quantification (LOQ) for zoxamide was 0.01 mg/kg on lettuce and similar.

In lettuce, rocket salad and escarole the residues of zoxamide were below the method limit of quantification in all the untreated specimens, while the treated samples had detectable residues. Results of the residue studies are summarized in tables below.

(Luciani G.P. 2012)

Table A 43: Summary of the study 2 trials on lettuce / Greenhouse

Reference:	Luciani P.G. 2012, AGRI 014/12 GLP DEC		
GLP:	Yes	Sample storage conditions:	at ≤ -18 °C for residues analysis
Crop/crop group:	Lettuce	Analytical method:	Agri BPL 040 Rev.6
Indoor/Outdoor:	Indoor	Limit of Quantification (mg/kg):	0.01 mg/kg
Formulation:	SC	Limit of Detection (mg/kg):	--
Content of active substance (g/kg or g/l):	240 g/L zoxamide 180 g/L zoxamide + 180 g/L dimethomorph	Residues calculated as:	Zoxamide

Trial No./ Location/ EU zone/ Year	Commodity/ Variety	Date of 1.Sowing or planting 2.Flowering 3. Harvest	Application rate per treatment			Dates of treatment or no. of treatments and last date	Growth stage at last treatment or date (BBCH)	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			g a.s./ ha	Water (l/ha)	g a.s./hl				Zoxamide		
R03AG12-01 97019 Ragusa Italy Indoor, 2012	Lettuce Maximus	1) 16/10/2012 2) - 3) Nov-Dec 2012	198 196	500	-	14/11/2012 23/112012	14-18 18-33	Leaf	38.69 15.85 5.54 4.81 1.34	0 3 7 10 14	LOQ = 0.01 mg/kg Analytical method Agri BPL 040 Rev.6 Max. Storage interval between sampling and analysis: 91 days
			191 192	500	-	14/11/2012 23/11/2012	14-18 18-33	Leaf	35.90 29.99 6.40 5.71 0.88	0 3 7 10 14	
R03AG12-02 97019 Ragusa Italy Indoor, 2012	Lettuce Fabietto	1) 16/10/2012 2) - 3) Nov-Dec 2012	196 198	500	-	14/11/2012 23/11/2012	14-18 18-34	Leaf	36.87 18.40 6.43 5.06 4.39	0 3 7 10 14	LOQ = 0.01 mg/kg Analytical method Agri BPL 040 Rev.6 Max. Storage interval between sampling and analysis: 91 days

Trial No./ Location/ EU zone/ Year	Commodity/ Variety	Date of 1.Sowing or planting 2.Flowering 3. Harvest	Application rate per treatment			Dates of treatment or no. of treatments and last date	Growth stage at last treatment or date (BBCH)	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
			g a.s./ ha	Water (l/ha)	g a.s./hl				Zoxamide		
			191 191	500	-	14/11/2012 23/11/2012	14-18 18-34	Leaf	39.84 32.63 7.55 5.94 2.38	0 3 7 10 14	Max. Storage interval between sampling and analysis: 91days
R03AG12-03 97019 Ragusa Italy Indoor, 2012	Rocket Broadleaf	1) 16/10/2012 2) - 3) Nov-Dec 2012	198 196	500	-	14/11/2012 23/11/2012	14-18 33-35	Leaf	27.13 21.49 10.73 5.48 5.01	0 3 7 10 14	LOQ = 0.01 mg/kg Analytical method Agri BPL 040 Rev.6 Max. Storage interval between sampling and analysis: 91 days
			192 192	500	-	14/11/2012 23/11/2012	14-18 33-35	Leaf	30.43 15.00 5.82 4.60 3.90	0 3 7 10 14	
R03AG12-04 97019 Ragusa Italy Indoor, 2012	Escarole Arlonia	1) 16/10/2012 2) - 3) Nov-Dec 2012	200 198	500	-	14/11/2012 23/11/2012	14-18 35-41	Leaf	47.01 37.59 6.16 4.96 4.45	0 3 7 10 14	LOQ = 0.01 mg/kg Analytical method Agri BPL 040 Rev.6 Max. Storage interval between sampling and analysis: 91 days
			191 191	500	-	14/11/2012 23/11/2012	14-18 33-5-41	Leaf	29.14 18.82 16.24 4.95 2.44	0 3 7 10 14	

A 2.1.4 Magnitude of residues in livestock

A 2.1.4.1 Livestock feeding studies

No new data / not relevant.

A 2.1.5 Magnitude of residues in processed commodities (Industrial Processing and/or Household Preparation)

A 2.1.5.1 Distribution of the residue in peel/pulp

Not relevant.

A 2.1.5.2 Processing studies on a core set of representative processes

Out of the new presented supervised residue trials provided with this submission, processing factors (and the related median values) have been calculated. Only data available at the relevant PHI values were taken into account. As far as results at least 10-fold above the analytical method Limit of Quantification (LOQ) were available in the commodity relevant for processing and distinct values were determined in the processed commodity, they have been taken forward for the calculation of processing factors.

Table A 44: Data from processing studies with zoxamide

Commodity	Processing factor	Reference / study no.	Median
Raisns	1.11	BPL-STUDY-19-000058	
	1.731	18097-03R	
			1.4205 (n=2)
Limpid juice before pasteurisation	0.032	AB2-18-35355	
	0.042	AB2-18-35355	
			0.037 (n=2)
Limpid juice after Pasteurisation	0.005	AB2-18-35355	
	0.0025	AB2-18-35355	
	0.019	BPL-STUDY-19-000041	
	0.083	BPL-STUDY-19-000051	
	0.405	BPL-STUDY-19-000051	
	0.063	19200-01R	
			0.041 (n=6)
Must	1.064	AB2-18-35355	
	0.385	AB2-18-35355	
	1.09	19200-01R	
	0.846	19200-01R	
	0.054	RAU-049-15	
			0.846 (n=5)
Young wine	0.074	AB2-18-35355	
	0.148	AB2-18-35355	
	0.034	BPL-STUDY-19-000041	
	0.006	BPL-STUDY-19-000051	
	0.019	BPL-STUDY-19-000051	
	0.026	19200-01R	

	0.196	19200-01R	
			0.034 (n=7)
Bottled wine	0.048	AB2-18-35355	
	0.123	AB2-18-35355	
	0.033	BPL-STUDY-19-000041	
	0.021	19200-01R	
	0.165	19200-01R	
			0.048 (n=5)

A 2.1.6 Magnitude of residues in representative succeeding crops

Considering the available data dealing with nature of residues (see 7.2.2.2), no study dealing with magnitude of residues in succeeding crops is needed.

A 2.1.7 Other/Special Studies

A study to evaluate the MRL of zoxamide in honey is presented in the following.

A 2.1.7.1 Study 1

Comments of zRMS:	The study is acceptable. 4 trials (2 NEU, 2 SEU) were performed. The objective of this study was to determine residues of Zoxamide in honey made of Phacelia flowers after application of GWN-9790EU at a worst-case use pattern of 3 x 180 g a.s./ha in field tunnels as far as possible (BBCH 61-65) to the flowering crop. The employed LC-MS/MS method can be regarded as highly specific. The method was fully validated according to SANCO/3029/99 rev. 4 and SANCO/825/00 guideline for (R)- and (S)-Zoxamide, and Zoxamide (sum). The limit of quantification and limit of detection were set to 0.01 mg/kg and 0.003 mg/kg, respectively for Zoxamide. The validity of the analytical method was proven by analysis of fortified samples (high and low concentration), five replicates each, and two validation blank samples. The recoveries of the validation samples were within the range of 70-110% with an RSD of below 20%. The highest Zoxamide residues in Phacelia honey amounted 0.0784 mg/kg however, mostly were <LOQ.

Reference:	**KCA 6.10/01**
Report	Poráczki, K., 2020: Magnitude of residues of Zoxamide in Phacelia (*Phacelia tanacetifolia* BENTH.) honey after three applications of GWN-9790EU under semi-field conditions in Northern and Southern Europe[29] Gowan Crop Protection Ltd, UK BioChem agrar, Germany, Report No. 19 48 BTR 0003, 19 35 CRB 0163, GLP, Not published
Guideline(s):	SANTE/11956/2016 rev. 9 (2018)
Deviations:	In trial 19BTR0003_T3 the following specimen could not be generated as intended (no honey available) : 19BTR0003_06-T-A1

[29] Confirmatory study for zoxamide currently being evaluated by RMS (Latvia).

	19BTR0003_06-T-A2 19BTR0003_06-T-R1 19BTR0003_06-T-R2 19BTR0003_06-T-PD Trial 3 was therefore repeated
GLP:	Yes
Acceptability:	Yes

Materials and methods

Test material (Lot/Batch No.)	Zoxium 240 SC / GWN-9790EU (18011201-72-52)
Purity	240g/L Zoxamide (nominal), 21.49% w/w (analysed) (R/S Zoxamide ratio: 50/50)
Expiry date	13 January 2022

The inadvertent residues of zoxamide in Phacelia (*Phacelia tanacetifolia* BENTH.) honey after application of GWN-9790EU (synonym Zoxium 240 SC; a 240 g/L zoxamide SC formulation) at a worst-case use pattern of 3 x 180 g a.s./ha with a minimum interval of 7(+1) days under semi-field conditions (in field tunnels) were determined.

During the growing season in 2020 four separate field trials were conducted at various places in Southern Spain and Eastern Germany. Trials 19BTR0003_T1 and 19BTR0003_T2 (subsequently named T1 and T2) were performed in the area of Eastern Germany (Leipzig and Lossatal; Saxony; Germany), respectively. Trials 19BTR0003_T3 and 19BTR0003_T4 (subsequently named T3 and T4) were performed in the area of Southern Spain (Utrera & Adriano; Andalusia; Spain). Two plots (tunnel) with one beehive each were set up per trial. Each tunnel covered an area of 216 m² (192.5 m² effective crop size).

GWN-9790EU was applied three times at a rate equivalent to 180 g a.i./ha of zoxamide (0.75 L product/ha; based on nominal content). The initial spray volume amounted 400 L/ha and the three applications were carried during the flowering of *Phacelia* at crop growth stages BBCH 61-65 (at the last application).

Two assessments of the colony condition were performed per plot, once before the last application and once directly before sampling. The bee hives were set up in the tunnels after the last application and remained in the tunnels until the honey showed a water content < 20%. Approximately 9 to 14 days after the placement of the colonies, honey combs were removed from the bee hives and gathered in the laboratory using a honey extractor.

After extraction of the honey samples from the combs, the respective specimen containers were stored at the test facility under deep-frozen conditions (≤ -18 °C) until analysis. Alternatively, specimen were stored under deep-frozen (≤ -18 °C) conditions until the transport on dry ice to the test facility.

Honey sampled for determination of *Phacelia* pollen content (for honey variety confirmation) was stored under deep-frozen (≤ -18 °C) conditions until the transport to the Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit, Institut für Bienenkunde, Germany, for analysis.

The specimens were extracted according to the multi-residue method QuEChERS and analysed by high performance liquid chromatography (HPLC) on a chiral column with mass-spectrometric (MS-MS) detection at a limit of quantification of 0.01 mg/kg for zoxamide (racemate). This method is regarded as highly specific. Zoxamide (racemate) concentrations were determined as sum of both isomer peaks. Reference standards of the two single isomers of the active substance zoxamide were used to distinct between the (R)- and the (S)-isomer peaks. The method was fully validated according to SANCO/3029/99 rev. 4 (2000) and

SANCO/825/00 (2010) guideline for each (R)- and (S)-zoxamide and zoxamide (sum) within the analytical phase report 19 35 CRB 0163.

Results and discussions

The residue levels in honey in four independent trials, performed under Southern European (Spain) and Northern European (Germany) growing conditions during the 2020 growing season were determined after application of GWN-9790EU (a 240 g/L zoxamide SC formulation) at a worst-case use pattern of 3 x 180 g a.s./ha with a minimum interval of 7(+1) days under semi-field conditions (in field tunnels). Applications took place over the full flowering phase of the crop.

The tunnel study design ensured that the bee colonies only had access to the GWN-9790EU treated crop. Pollen analysis confirmed that the bees gathered nectar mainly from the treated *Phacelia*.

The honey samples have been measured with a highly specific HPLC-MS/MS method fully validated according to SANCO/3029/99 rev. 4 (2000) and SANCO/825/00 (2010) guideline for Zoxamide with a limit of quantification (LOQ) of 0.01 mg/kg and a limit of detection of 0.003 mg/kg, respectively.

The maximum freezer storage period for honey samples of the field part was 83 days.

The stability of honey samples in the freezer was demonstrated by recovery experiments over a storage period of at least 85 days. For this, samples for storage stability investigations were prepared, one set of samples was analysed directly, the other set was stored at < -18°C for 85 days and was analysed thereafter along with freshly prepared samples of the same concentration level (0.10 mg/kg of zoxamide). Details of sampling, storage, extraction and analysis are given in the analytical phase report in appendix 4, the results are summarised in the following table.

Table A 45: Freezer storage stability results for zoxamide in honey

Sample name	Storage period (days)	Nominal conc. of zoxamide [mg/kg]	Analysed conc. of zoxamide [mg/kg]	Recovery [%]	Mean Recovery [%]
19CRB0163-StoSta-01	0	0.100	0.090	90	91
19CRB0163-StoSta-02			0.092	92	
19CRB0163-StoSta-C		0.00	<30% LOQ	-	
19CRB0163-StoSta-01-t1	85	0.10	0.092	91	91
19CRB0163-StoSta-02-t1			0.091	91	
19CRB0163-StoSta-C-t1		0.00	<30% LOQ	-	

As a result, no residues of Zoxamide were detected (<LOD; 0.003 mg/kg) in untreated (Control) honey from all four trials.

The highest zoxamide residues in *Phacelia* honey analysed over all trials were found in Trial T1. Here, residues amounted to 0.0784 mg/kg. In comparison to that, zoxamide residues were not detectable (< LOD of 0.003 mg/kg) in Trial T4. A summary of the results is given in the following table.

Table A 46: Summarized results of the residue analysis

EU zone / country	Trial No.	Treatment group	Residues of Zoxamide (mg/kg)
Northern EU Germany 04319 Leipzig	T1	Control	< LOD
		Test item	0.0784
Northern EU Germany 04808 Lossatal	T2	Control	< LOD
		Test item	< LOQ
Southern EU	T3	Control	< LOD

Spain 41710 Utrera		Test item	< LOQ
Southern EU Spain 41728 Adriano (Dos Hermanas)	T4	Control	< LOD
		Test item	< LOD

LOQ: 0.010 mg/kg of zoxamide (sum)
LOD: 0.003 mg/kg of zoxamide (sum)

The ratio of *r*-zoxamide to *s*-zoxamide in the field specimens was in the range of 0.90 to 0.92[2], thus confirming the stability of the chiral centre of zoxamide

The maximum storage period of the honey specimens was 83 day (in the freezer). The freezer storage stability of zoxamide in honey (at ≤ -18 °C) was determined by recovery experiments during the course of this study for a period of 85 days.

Conclusions

The residue levels in honey in four independent trials, performed under Southern European (Spain) and Northern European (Germany) growing conditions during the 2020 growing season were determined after application of GWN-9790EU (a 240 g/L zoxamide SC formulation) at a worst-case use pattern of 3 x 180 g a.s./ha with a minimum interval of 7(+1) days under semi-field conditions (in field tunnels). Applications took place over the full flowering phase of the crop. A tunnel study design ensured that the bee colonies only had access to the GWN-9790EU treated crop. Pollen analysis confirmed that the bees gathered nectar mainly from the treated Phacelia.

The honey samples were measured with a highly specific HPLC-MS/MS method fully validated according to SANCO/3029/99 rev. 4 (2000) and SANCO/825/00 (2010) guideline for the determination of zoxamide (R, S and sum) with a limit of quantification (LOQ) of 0.01 mg/kg and a limit of detection of 0.003 mg/kg.

As a result of the study, no residues of zoxamide were detected (<LOD; 0.003 mg/kg) in untreated (control) honey from all four trials. The zoxamide residues in *Phacelia* honey from the four treated trials were 0.0784 mg/kg, not detectable (< LOD of 0.003 mg/kg), and 2x <LOQ (< 0.01 mg/kg).

(Poráczki K. 2020)

Below 4 residue studies (7, 8, 9, 10) from the Section 8 (Environmental Fate) previously not submitted here are presented, that should be assessed in this section.

A 2.1.7.2 Study 7 – Residue degradation of zoxamide in mono- and dicotyledonae plants under northern European growing conditions

Substance specific DT50 values for residues dissipation of zoxamide were taken into account for refined PEC SW calculations. These values were obtained for salad plants in supervised residue studies (for MRL evaluation) of Luciani (2012) in reports no. AGRI 013/12 GLP DEC and AGRI 014/12 GLP DEC, summarised in Part B Section 7. The residue data were kinetically re-evaluated by Klein et al. (2020; report no. GOW1020-1), the results of the kinetic evaluation are summarised in the following. In addition, the dissipation of zoxamide on/in surrogate dicotyledonae (i.e. sugar beet leaves) and monocotyledonae (i.e. cereals) plants has been studied by Appeltauer (2020a,b,c,d) in the field under Northern and Southern European growing conditions, inclusive a kinetic evaluation of the degradation data. These studies are summarised in the following. All available dissipation data of zoxamide on/in plants were kinetically evaluated by Klein & Mendel-Kreusel (2020) in report no. GOW1120-1.

Comments of zRMS:	The study is accepted (acc. SANCO/3029/99 rev. 4). The data presented in this report demonstrate that the used method permits the determination of residues of Zoxamide in sugar beet leaves (representing the feed item group “non-grass herbs”) and in wheat plants (representing the feed item group “grass and cereaIs”) (without roots) with accuracy, precision and repeatability. The method is based on QuEChERS multi-residue method, validated by RICHTER (2014) according to SANCO/825/00 rev. 8.1 for the determination of Zoxamide in various crop commodities. This method was validated under the laboratory conditions of CIP for the determination of residues of Zoxamide in sugar beet leaves and wheat plants according to guideline SANCO/3029/99 rev. 4. For this purpose, recovery experiments were performed by fortifying control (untreated) specimens. The method employed extraction with acetonitrile/water, phase separation by addition of buffer salt mixture; Dispersive SPE clean up, followed by filtration through a 0.45 pm single-use syringe filter and dilution with acidified acetonitrile/water; final determination with HPLC - MS/MS detection. The LOQ is 0.01 mg/kg, the LOD is defined as 30% of the LOQ (i.e. 0.003 mg/kg). The RSD (precision) for the quantification ion mass transition per fortification level ranges from 1.4 % to 4.8 % for Zoxamide in sugar beet leaves and wheat plant. These values are within the guideline requirements for relative standard deviations (≤ 20%). The calibration graphs (linearity) for Zoxamide are linear within the range of 0.1 µg/L to 250 µg/L with correlation coefficients of ≥ 0.9998 (matrix-matched standard solutions). Regarding specificity typical retention time with no significant interferences (< 30%); analysis of control specimens used for recoveries yielded no residues of Zoxamide above 30% of the LOQ, indicating that no interferences were present. Zoxamide parent compound was detected with two ion mass transitions (SRM 336 —> 187 for quantification, SRM 338 —> 189 for confirmation). Recoveries were analysed at the LOQ of 0.01 mg/kg and at a higher level of 50 mg/kg (sugar beet leaves) or100 mg/kg (wheat plant) to cover the estimated residues found in field samples. The method validation part of this study was performed together with the analytical part of study S16-05376 (CIP phase ID 17E10095-02-RAVE). The following recoveries were obtained with HPLC-MS/MS for Zoxamide:

Matrix	Forti-fication Level [mg/kg]	Recoveries Single Values [%]	Mean [%]	RSD [%]	No. of Analyses	Overall recovery Mean [%]	Overall recovery RSD [%]
Zoxamide SRM 336 → 187 (quantification)							
sugar beet leaves	0.01	96 / 95 / 102 / 102 / 95 / 106 / 98	99	4.3	7	104	5.4
	50	110 / 108 / 109 / 109 / 108 / 105 / 108	108	1.5	7		
Zoxamide SRM 338 → 189 (confirmation)							
sugar beet leaves	0.01	106 / 105 / 102 / 107 / 104 / 98 / 104	104	2.9	7	105	3.0
	50	109 / 109 / 108 / 109 / 107 / 102 / 105	107	2.5	7		
Zoxamide SRM 336 → 187 (quantification)							
wheat plant	0.01	96 / 100 / 104 / 92 / 98 / 102 / 92	98	4.8	7	103	6.1
	100	106 / 108 / 109 / 110 / 109 / 106 / 108	108	1.4	7		
Zoxamide SRM 338 → 189 (confirmation)							
wheat plant	0.01	88 / 94 / 99 / 94 / 93 / 94 / 84	92	5.3	7	100	8.7
	100	110 / 107 / 109 / 108 / 109 / 105 / 105	108	1.8	7		

RSD = Relative Reaction Monitoring Standard Deviation, SRM: Single

All mean recoveries obtained by HPLC-MS/MS for Zoxamide at all fortification levels comply with the standard acceptance criteria of SANCO/3029/99, which demand that the mean recovery at each fortification level should be in the range of 70 — 110%.

From field trials in the sugar beet leaves specimens residues of Zoxamide were at maximum in the range of 6 - 8 mg/kg and declined to values between 0.1 and 0.7 mg/kg within 16 days after the last application of Zoxium 240 EC.

In the wheat plant specimens, residues of Zoxamide were at maximum around 10 mg/kg and declined to values between 0.2 and 0.8 mg/kg within 16 days after last application of Zoxium 240 EC. Residues of Zoxamide in untreated sugar beet leaves and wheat plants (without roots) were below the limit of detection (< 0.003 mg/kg) in all untreated specimens.the residues are summarised in the following tables:

Trial S16-05375-01:

Sample No. L16-05375-	Laboratory sample No.	Timing	Treat-ment	Matrix	Residue Zoxamide (mg/kg)
01-C-sbl-S01-A	5375-01-01	5 ± 5 DBA1	C	sugar beet leaves	< LOD
01-C-w-S01-A	5375-01-02	5 ± 5 DBA1	C	wheat plant	< LOD
01-T-sbl-S02-A	5375-01-03	2 ± 2 HBA2	T	sugar beet leaves	0.53
01-T-sbl-S03-A	5375-01-04	3 ± 1 HAA2	T	sugar beet leaves	6.84
01-T-sbl-S04-A	5375-01-05	24 ± 1 HAA2	T	sugar beet leaves	5.30
01-T-sbl-S05-A	5375-01-06	48 ± 1 HAA2	T	sugar beet leaves	2.00
01-T-sbl-S06-A	5375-01-07	4 ± 1 DAA2	T	sugar beet leaves	1.90
01-T-sbl-S07-A	5375-01-08	6 ± 1 DAA2	T	sugar beet leaves	1.76
01-T-sbl-S08-A	5375-01-09	8 ± 1 DAA2	T	sugar beet leaves	0.71
01-T-sbl-S09-A	5375-01-10	16 ± 1 DAA2	T	sugar beet leaves	0.22
01-T-w-S02-A	5375-01-11	2 ± 2 HBA2	T	wheat plant	1.80
01-T-w-S03-A	5375-01-12	3 ± 1 HAA2	T	wheat plant	10.1
01-T-w-S04-A	5375-01-13	24 ± 1 HAA2	T	wheat plant	8.26
01-T-w-S05-A	5375-01-14	48 ± 1 HAA2	T	wheat plant	4.60
01-T-w-S06-A	5375-01-15	4 ± 1 DAA2	T	wheat plant	3.55
01-T-w-S07-A	5375-01-16	6 ± 1 DAA2	T	wheat plant	2.26
01-T-w-S08-A	5375-01-17	8 ± 1 DAA2	T	wheat plant	2.64
01-T-w-S09-A	5375-01-18	16 ± 1 DAA2	T	wheat plant	0.27

C: control sample, T: treated sample, HBA/DBA: hours/days before application, HAA/DAA: hours/days after application< LOD: below the limit of detection (0.003mg/kg); LOQ: limit of quantification (0.01mg/kg)

Trial S16-05375-02:

Sample No. L16-05375-	Laboratory sample No.	Timing	Treat-ment	Matrix	Residue Zoxamide (mg/kg)
02-C-sbl-S01-A	5375-02-01	5 ± 5 DBA1	C	sugar beet leaves	< LOD
02-C-w-S01-A	5375-02-02	5 ± 5 DBA1	C	wheat plant	< LOD
02-T-sbl-S02-A	5375-02-03	2 ± 2 HBA2	T	sugar beet leaves	0.54
02-T-sbl-S03-A	5375-02-04	3 ± 1 HAA2	T	sugar beet leaves	7.60
02-T-sbl-S04-A	5375-02-05	24 ± 1 HAA2	T	sugar beet leaves	3.96
02-T-sbl-S05-A	5375-02-06	48 ± 1 HAA2	T	sugar beet leaves	3.08
02-T-sbl-S06-A	5375-02-07	4 ± 1 DAA2	T	sugar beet leaves	1.58
02-T-sbl-S07-A	5375-02-08	6 ± 1 DAA2	T	sugar beet leaves	1.77
02-T-sbl-S08-A	5375-02-09	8 ± 1 DAA2	T	sugar beet leaves	0.83
02-T-sbl-S09-A	5375-02-10	16 ± 1 DAA2	T	sugar beet leaves	0.66
02-T-w-S02-A	5375-02-11	2 ± 2 HBA2	T	wheat plant	1.88
02-T-w-S03-A	5375-02-12	3 ± 1 HAA2	T	wheat plant	9.17
02-T-w-S04-A	5375-02-13	24 ± 1 HAA2	T	wheat plant	7.70
02-T-w-S05-A	5375-02-14	48 ± 1 HAA2	T	wheat plant	8.24
02-T-w-S06-A	5375-02-15	4 ± 1 DAA2	T	wheat plant	5.91
02-T-w-S07-A	5375-02-16	6 ± 1 DAA2	T	wheat plant	3.02
02-T-w-S08-A	5375-02-17	8 ± 1 DAA2	T	wheat plant	2.22
02-T-w-S09-A	5375-02-18	16 ± 1 DAA2	T	wheat plant	0.72

C: control sample, T: treated sample, HBA/DBA: hours/days before application, HAA/DAA: hours/days after application, < LOD: below the limit of detection (0.003mg/kg); LOQ: limit of quantification (0.01mg/kg)

Trial S16-05375-03:

Sample No. L16-05375-	Laboratory sample No.	Timing	Treat-ment	Matrix	Residue Zoxamide (mg/kg)
03-C-sbl-S01-A	5375-03-01	5 ± 5 DBA1	C	sugar beet leaves	< LOD
03-C-w-S01-A	5375-03-02	5 ± 5 DBA1	C	wheat plant	< LOD
03-T-sbl-S02-A	5375-03-03	2 ± 2 HBA2	T	sugar beet leaves	0.74
03-T-sbl-S03-A	5375-03-04	3 ± 1 HAA2	T	sugar beet leaves	5.44
03-T-sbl-S04-A	5375-03-05	24 ± 1 HAA2	T	sugar beet leaves	6.70
03-T-sbl-S05-A	5375-03-06	48 ± 1 HAA2	T	sugar beet leaves	5.50
03-T-sbl-S06-A	5375-03-07	4 ± 1 DAA2	T	sugar beet leaves	2.44
03-T-sbl-S07-A	5375-03-08	6 ± 1 DAA2	T	sugar beet leaves	1.27
03-T-sbl-S08-A	5375-03-09	8 ± 1 DAA2	T	sugar beet leaves	0.62
03-T-sbl-S09-A	5375-03-10	16 ± 1 DAA2	T	sugar beet leaves	0.16
03-T-w-S02-A	5375-03-11	2 ± 2 HBA2	T	wheat plant	1.25
03-T-w-S03-A	5375-03-12	3 ± 1 HAA2	T	wheat plant	9.74
03-T-w-S04-A	5375-03-13	24 ± 1 HAA2	T	wheat plant	6.77
03-T-w-S05-A	5375-03-14	48 ± 1 HAA2	T	wheat plant	6.30
03-T-w-S06-A	5375-03-15	4 ± 1 DAA2	T	wheat plant	4.71
03-T-w-S07-A	5375-03-16	6 ± 1 DAA2	T	wheat plant	3.30
03-T-w-S08-A	5375-03-17	8 ± 1 DAA2	T	wheat plant	1.86
03-T-w-S09-A	5375-03-18	16 ± 1 DAA2	T	wheat plant	0.28

C: control sample, T: treated sample, HBA/DBA: hours/days before application, HAA/DAA: hours/days after application, < LOD: below the limit of detection (0.003mg/kg); LOQ: limit of quantification (0.01mg/kg)

Reference: KCP 9.2.5/02

Report: Appeltauer, A., 2020: Determination of residues of zoxamide on/in typical feed items of herbivorous birds and mammals after two applications of Zoxium 240 SC on sugar beet and wheat in Germany 2017
Gowan Crop Protection Ltd., UK
Eurofins GmbH, Germany, Report No. S16-05375, GLP, Not published

Guideline(s): SANCO/4145/2000
EFSA Guidance document on Risk Assessment for Birds and Mammals (2009)
SANCO/3029/99 rev. 4 (2000)
FOCUS (2014)

Deviations:	For all three trials the growth stage of wheat (BBCH) at application A2 was higher (>19) than requested in the study plan. The reason was a different growth development of the two crops (sugar beet and wheat). There was no impact on study.
GLP:	Yes
Acceptability:	Yes

The objective of this study was to determine the residue decline of zoxamide on/in feed items of herbivorous birds and mammals under representative growing conditions in Northern Europe (Germany) in the field: In sugar beet leaves (as surrogate dicotyledonae, representative for the feed item group "non-grass herbs") and in wheat green mass above soil (as surrogate monocotyledonae, representative for the feed item group "grass and cereals"). The residues and degradation kinetics of the active ingredient were investigated after two applications of Zoxium 240 SC (240 g/L zoxamide, SC) at application rates for zoxamide of 180 g a.s./ha with an interval of 7 days (BBCH 14-24 for sugar beet and wheat). The early application timing of zoxamide to both plant groups is representative for the more sensitive phase for wild birds and mammals.
The study consisted of three field trials, S16-05375-01 to -03 and one residue analysis trial, S16-05375-L1. The field parts were carried out in three fields located in near Stutensee (trial -01), Pforzheim (trial -02) and near Brackenheim (trial -03), in Baden Württemberg, Germany The field sites of all trials covered an area of 300 m² sugar beet and 300 m² summer wheat.
Sugar beet plants were planted on 05 April 2017 in trial -01, on 25 April 2017 in trial -02 and on 22 April 2017 in trial -03. Wheat was sown on 05 May 2017 in trial -01, on 17 May 2017 in trial -02 and on 19 May 2017 in trial -03. Samples were taken before each application and up to 16 days after the last application. One sampling before the 1st application served as control.
For the two specimen types, the sampling schedule was as follows: In trial -01, first sampling before the 1st application (control), 0 to 1 hour before the 2nd application, 3, 24, 48 hours after the 2nd application, and 4, 6, 8 and finally 16 days after the 2nd application. In trial -02, first sampling before the 1st application (control), 0 to 1 hour before the 2nd application, 3, 24, 48 hours after the 2nd application, and 4, 6, 8 and finally 15 days after the 2nd application. In trial -03, first sampling before the 1st application (control), 1 hour before the 2nd application, 3, 24, 48 hours after the 2nd application, and 4, 6, 8 and finally 16 days after the 2nd application.
The samples were weighed in the field and frozen immediately on dry ice. Retained samples were taken for all matrices. All residues samples were stored in the freezer within 6 hours after collection in the field. The samples were stored and shipped frozen. The maximum freezer storage time of samples was 88 days for sugar beet leaves and 90 days for wheat green mass. At the testing facility and test site the samples were stored deep frozen at ≤ -18°C for a maximum of 90 days until extraction and residue analysis. Residue analysis took place within 7 days after extraction.
The residues of the active ingredients on/in sugar beet leaves and wheat green mass were analysed with fully validated analytical methods according to SANCO/3029/99 rev. 4. The method for the determination of zoxamide in sugar beet leaves and wheat green mass was validated in this study together with the analytical part of study under EAS study number S16-05376 and CIP Phase ID 17E10095-01-RAVE, according to SANCO Guideline 3029/99 rev. 4. Specimens were extracted (in analogy to the QuEChERS multi residue method) with acetonitrile/water, phase separation was done by addition of buffer salt mixture. The final analysis was conducted with highly specific HPLC with MS/MS detection. Recoveries in the fortified samples were within the acceptable range of 70 - 110 %, therefore the stability of the analyte during storage of the final sample extracts is sufficiently proven.
The degradation kinetics of the active substances were analysed according to the recommendations of the EFSA Guidance document on Risk Assessment for Birds and Mammals (2009) and the Guidance document on Estimating Persistence and Degradation Kinetics from Environmental Studies on Pesticides in EU Registration (FOCUS 2014). The calculation of the DT50 values and DT90 values as well as the fitting of the kinetic degradation models was done using the computer software KinGU II (version 2.2012). Four different kinetic degradation models were taken into account: single first-order, first order multi-compartment (GUSTAFSON & HOLDEN, 1990), hockey stick (bi-phasic) and double first-order (bi-exponential) kinetics. The operating system was Microsoft Windows 7 Professional.

Materials and methods
Materials

1. Test material	ZOXIUM 240 SC
Lot/batch:	SB 2401
Active substance content:	240 g/L zoxamide (nominal), 232 g/L zoxamide (analysed)
Expiry date:	April 2018

Methods
Experimental conditions
The study comprised three sugar beet and wheat fields, one per trial. All trials were treated with two applications of Zoxium 240 SC at a nominal rate of 180 g zoxamide/ha. The field sites were located near Stutensee (trial -01), Pforzheim (trial -02) and near Brackenheim (trial -03), in Baden Württemberg, Germany. The agricultural practices and sugar beet / wheat varieties were in accordance with the local farming practices.
Each trial was designed to produce a single sample for each food type at each sampling date (i.e. to provide an assessment of the average residue level as well as to ensure that sufficient material was collected for the actual residue analysis). To minimise edge effects from neighbouring fields, sampling was not carried out at the outer 50 cm of the plot.
During the study, weather data obtained from portable data loggers on the field sites and from weather stations in the vicinity of the field sites including precipitation and air temperature, were taken. During applications and samplings, the climatic conditions (GLP data) were measured at the field site with a portable thermo-hygrometer, a soil thermometer and a portable anemometer. Additional data for the long-term average were taken from official weather stations (non-GLP data).
No other formulations containing zoxamide were applied during the trial period onto the plots.
Sampling
Samples of different food items for birds and mammals were collected for residue analysis. Two categories of potential bird and mammalian food items were considered:
1. Sugar beet green mass / leaves
2. Wheat green mass / leaves

For each trial 9 samplings per category were carried out. The first sampling took place before the 1st application and was used as control sample.
For the two specimen types, the sampling schedule was as follows: In trial -01, first sampling before the 1st application (control), 0 to 1 hour before the 2nd application, 3, 24, 48 hours after the 2nd application, and 4, 6, 8 and finally 16 days after the 2nd application. In trial -02, first sampling before the 1st application (control), 0 to 1 hour before the 2nd application, 3, 24, 48 hours after the 2nd application, and 4, 6, 8 and finally 15 days after the 2nd application. In trial -03, first sampling before the 1st application (control), 1 hour before the 2nd application, 3, 24, 48 hours after the 2nd application, and 4, 6, 8 and finally 16 days after the 2nd application.
The samples were sampled randomly on 12 locations per trial. There was at least 50 g plant material taken per field at each sampling occasion. Samplings were done by hand or with scissors. Samples were taken with a minimum distance of 0.5 m to the border of the plot. Samples were taken with a minimum distance of 0.5 m to the border of the plot. The samples of all locations of one field were put together to one pooled sample.
The samples were weighed in the field and frozen immediately on dry ice. Retained samples were taken for all matrices. At the testing facility and test site the samples were stored deep frozen at ≤ -18°C for a maximum of 90 days until extraction and residue analysis.
Description of the analytical procedure
The data presented in this report demonstrate that the used method permits the determination of residues of zoxamide in sugar beet leaves (representing the feed item group “non-grass herbs”) and in wheat plants (representing the feed item group “grass and cereals”) (without roots) with accuracy, precision and repeatability. The method was based on QuEChERS multi-residue method, validated by RICHTER (2014) according to SANCO/825/00 rev. 8.1 for the determination of zoxamide in various crop commodities. This method was validated under the laboratory conditions of CIP for the determination of residues of zoxamide in sugar beet leaves and wheat plants according to guideline SANCO/3029/99 rev. 4. For this purpose, recovery experiments were performed by fortifying control (untreated) specimens.

10 g (± 0.1 g) of sugar beet leaves and wheat plant specimens were weighed into 50 mL single use centrifuge tubes. Recovery samples were fortified at this step. 10 mL acetonitrile were added and the samples were homogenised for at least 2 min using a vortex mixer. Thereafter, QuEChERS EN15662 salt-mixture (1 g sodium citrate, 0.5 g sodium hydrogencitrate sesquihydrate, 4 g magnesium sulphate, 1 g sodium chloride) was added, thoroughly shaken and mixed again on a vortex mixer for at least 1 min. The samples were centrifuged at 4000 min-1 for at least 5 minutes. An aliquot of 1 mL of the supernatant was transferred into a tube prepared with 25 mg PSA (primary-secondary amino phase) and 150 mg anhydrous magnesia sulphate and mixed on a Vortex mixer for 1 min. The extract was filtered through a single–use syringe filter (0.45 µm) into an autosampler vial (1.8 mL). 0.5 mL of this solution were transferred into a second vial, 5 µL of acetonitrile + 5 % formic acid were added, the vial capped and thoroughly shaken. 50 µL of this sample extract were then diluted with 950 µL acetonitrile/water (20:80, v/v) plus 0.1 % formic acid. If necessary, these final extracts were diluted further with final extract of untreated samples to achieve final concentrations falling within the calibrated concentration range of the detection system.
For detailed information on the analytical method validation, please refer to Part B, Section 5.
Calculation of initial concentration (C0) and DT50/DT90 values
The degradation time of zoxamide was calculated, including information about the kinetics of the decay according to the recommendations of the guidance document on estimating persistence and degradation kinetics from environmental studies on pesticides in EU registration (FOCUS 2014).
The calculation of the DT50 values and DT90 values as well as the fitting of the kinetic degradation models was done using the computer software KinGU II (version 2.2012). The fitting of the analysed data was calculated for four kinetic degradation models – single first order kinetic (simple first order), first order multi compartment kinetic (GUSTAFSON & HOLDEN, 1990), hockey stick kinetic (bi-phasic) and double first order kinetic (bi-exponential). The operating system was Microsoft Windows 7 Professional. The significance of the used models was determined, considering the significance of the parameters k, k1 and k2, which was part of the results obtained from the calculation with KinGU II.
For both commodities, the analysed residues after the last application (2nd application), i.e. starting from three hours after the last application to 16/15/16 days after the last application were chosen to establish degradation kinetics for the single trials. For the two commodities timings were calculated separately from the end of application until the samples were put on dry ice (i.e. time degradation of residues stops). Times were rounded to full hours.
Results and discussions
Weather conditions
The climatic conditions during trial -01 compared to the long-term average (1961-1990) revealed higher average temperatures for May and June 2017. During the trial period the rainfall recorded at the field site was 48.8 mm.
The climatic conditions during trial -02 compared to the long-term average (1981-2010) revealed higher average temperatures for June 2017. During the trial period the rainfall recorded at the field site was 14.8 mm.
The climatic conditions during trial -03 compared to the long-term average (1971-2000) revealed higher average temperatures for June 2017. During the trial period the rainfall recorded near the field site (~ 8.8 km) was 44.0 mm.
Zoxamide residues
In the control samples, taken directly before the 1st application, concentrations analysed were below the LOD (0.003 mg/kg) in all trials.
Zoxamide in/on sugar beet leaves
Zoxamide concentrations in sugar beet leaves of the three trials were 0.53 mg/kg (trial -01), 0.54 mg/kg (trial -02), and 0.74 mg/kg (trial -03) shortly before the 2nd application (0-1 HBA2). The highest concentrations were analysed three hours after the 2nd application in trial -01 and -02 and 24 hours after the 2nd application in trial -03 (trial -01: 6.84 mg/kg; trial -02: 7.60 mg/kg, trial -03: 6.70 mg/kg). At the last sampling 16/15/16DAA2 concentrations for the field trials were 0.22 mg/kg (trial -01), 0.66 mg/kg (trial -02) and 0.16 mg/kg (trial -03). A summary of the residue levels found in sugar beet leaf samples is shown in the following table.
Table A 2-1: Zoxamide residue levels determined in/on sugar beet leaves (individual values of all field sites) after 2 applications of Zoxium 240 SC

Timing (trial -01/-02/-03)	Trial		
	-01	-02	-03
	[mg/kg]	[mg/kg]	[mg/kg]
0/1/0DBA1	<LOD	<LOD	<LOD
0/0/1HBA2	0.53	0.54	0.74
3/3/3HAA2	6.84	7.60	5.44
24/24/24HAA2	5.30	3.96	6.70
48/48/48HAA2	2.00	3.08	5.50
4/4/4DAA2	1.90	1.58	2.44
6/6/6DAA2	1.76	1.77	1.27
8/8/8DAA2	0.71	0.83	0.62
16/15/16DAA2	0.22	0.66	0.16

DBA: days before application, DAA: days after application,
HAA: hours after application, HBA: hours before application,
LOD: level of detection (0.003 mg/kg)

In trial -01 none of the models achieved the critical values < 15 for χ^2-error. In trial -02 the calculation of degradation rates for zoxamide in sugar beet leaves achieved critical values < 15 for χ^2-error for the FOMC and DFOP model. In trial -03 the calculation of degradation rates for zoxamide in sugar beet leaves achieved critical values < 15 for χ^2-error for the HS model. The determination coefficient of r2 > 0.85 was achieved for all trials for all models. In trial -01 the FOMC and DFOP models showed the highest r2 with 0.94. However, the DFOP model was not significant at p(k)<0.05. In trial -02 the FOMC and DFOP models also achieved the highest r2 with 0.99. Here, too, the DFOP model was not significant at p(k)<0.05. Therefore, for both trials (-01 and -02) the SFO model was used for calculation of degradation rates of zoxamide in sugar beet leaves. In trial -03 the HS model reached the highest r^2 with 0.98 and one of the two constants was significant at p(k)<0.05. The results of the calculation showed DT50 values between 1.81 and 3.55 days and DT90 values between 6.02 and 7.79 days, respectively for the degradation of zoxamide in sugar beet leaves. The calculated χ^2-errors for the three trials were 20.35 (SFO), 19.55 (SFO) and 10.34 (HS) for trials -01, -02 and -03, respectively. The calculated r^2 for the three trials were 0.92 (SFO), 0.93 (SFO) and 0.98 (HS) for trials -01, -02 and -03, respectively.

Zoxamide in/on wheat green mass

Zoxamide concentrations in wheat green mass were 1.80 mg/kg (trial -01), 1.88 mg/kg (trial -02) and 1.25 mg/kg (trial -03) shortly before 2nd application (0-1 HBA2). The highest concentrations were analysed three hours after the 2nd application in trial -01, -02 and -03 (trial -01: 10.1 mg/kg, trial -02: 9.17 mg/kg, trial -03: 9.74 mg/kg). At the last sampling 16/15/16DAA2 concentrations for the field trials were 0.27 mg/kg (trial -01), 0.72 mg/kg (trial -02) and 0.28 mg/kg (trial -03). A summary of the residue levels found in wheat green mass samples is shown in the following table.

Table A 2-2: Zoxamide residue levels determined in/on wheat green mass (individual values of all field sites) after 2 applications of Zoxium 240 SC

Timing (trial -01/-02/-03)	Trial		
	-01	-02	-03
	[mg/kg]	[mg/kg]	[mg/kg]
0/1/0DBA1	<LOD	<LOD	<LOD
0/0/1HBA2	1.80	1.88	1.25
3/3/3HAA2	10.1	9.17	9.74
24/24/24HAA2	8.26	7.70	6.77
48/48/48HAA2	4.60	8.24	6.30
4/4/4DAA2	3.55	5.91	4.71
6/6/6DAA2	2.26	3.02	3.30
8/8/8DAA2	2.64	2.22	1.86
16/15/16DAA2	0.27	0.72	0.28

DBA: days before application, DAA: days after application,

HAA: hours after application, HBA: hours before application,
LOD: level of detection (0.003 mg/kg)
In trials -01, -02 and -03 the calculation of degradation rates for zoxamide in wheat green mass achieved critical values < 15 for χ^2-error for all models, with one exception. In trial -01 the χ^2-error was > 15 for the HS model. All models showed a determination coefficient of r2 > 0.85. As the t-test of the SFO model for all trials was significant (p(k)<0.05) the SFO model was used to calculate the degradation rates of the trials. The results of the calculation showed DT50 values between 2.74 and 4.31 days and DT90 values between 9.10 and 14.32 days, respectively for the degradation of zoxamide in wheat green mass. The calculated χ^2-errors for the three trials were 13.65 (SFO), 10.22 (SFO) and 8.04 (SFO) for trials -01, -02 and -03, respectively. The calculated r^2 for the three trials were 0.95 (SFO), 0.95 (SFO) and 0.97 (SFO) for trials -01, -02 and -03, respectively.
Calculation of initial concentration (C0) DT50/DT90 values
Sugar Beet Leaves
In trial -01 none of the models achieved the critical values < 15 for χ^2-error. In trial -02 the calculation of degradation rates for zoxamide in sugar beet leaves achieved critical values < 15 for χ^2-error for the FOMC and DFOP model. In trial -03 the calculation of degradation rates for zoxamide in sugar beet leaves achieved critical values < 15 for χ^2-error for the HS model. The determination coefficient of r2 > 0.85 was achieved for all trials for all models. In trial -01 the FOMC and DFOP models showed the highest r2 with 0.94. However, the DFOP model was not significant at p(k)<0.05. In trial -02 the FOMC and DFOP models also achieved the highest r2 with 0.99. Here, too, the DFOP model was not significant at p(k)<0.05. Therefore, for both trials (-01 and -02) the SFO model was used for calculation of degradation rates of zoxamide in sugar beet leaves. In trial -03 the HS model reached the highest r^2 with 0.98 and one of the two constants was significant at p(k)<0.05. The results of the calculation showed DT50 values between 1.81 and 3.55 days and DT90 values between 6.02 and 7.79 days, respectively for the degradation of zoxamide in sugar beet leaves. The calculated χ^2-errors for the three trials were 20.35 (SFO), 19.55 (SFO) and 10.34 (HS) for trials -01, -02 and -03, respectively. The calculated r^2 for the three trials were 0.92 (SFO), 0.93 (SFO) and 0.98 (HS) for trials -01, -02 and -03, respectively.

Table A 2-3: Results and parameters of the kinetic degradation of zoxamide in/on sugar beet leaves (Trial S16-05375-01)

Model	SFO	FOMC	DFOP	HS
DT50 [days]	1.94	1.43	1.37	2.05
DT90 [days]	6.45	8.91	9.86	6.56
CHI2-err [%]	20.35	18.02	19.01	24.22
r2	0.92	0.94	0.94	0.92
parameter	k = 0.35709	α = 1.4887	k1 = 0.80186	k1 = 0.002558
	M(0) = 6.95022	β = 2.4099	k2 = 0.11990	k2 = 0.357086
		M(0) = 7.5557	g = 0.67453	tb = 0.112641
			M(0) = 7.58528	M(0) = 6.678141
lower Cl	k = 0.16253	α = -0.8153	k1 = -0.29060	k1 = -3.931317
	M(0) = 5.33129	β = -3.5004	k2 = -0.17975	k2 = 0.102682
		M(0) = 5.5901	g = -0.04745	tb = -0.461000
			M(0) = 5.73028	M(0) = 2.656454
upper Cl	k = 0.552	α = 3.793	k1 = 1.894	k1 = 3.936
	M(0) = 8.569	β = 8.320	k2 = 0.420	k2 = 0.611
		M(0) = 9.521	g = 1.397	tb = 0.686
			M(0) = 9.440	M(0) = 10.700
t-test	p(k): 0.007794	-	p(k1): 0.12292	p(k1): 0.4995
			p(k2): 0.24506	p(k2): 0.0353

SFO = Single first order kinetic;

FOMC = First order multi compartment kinetic;
DFOP = Double first order kinetic;
HS = Hockey stick kinetic
M(0) = initial concentration
CI = confidence interval
t = time after time of initial concentration
k = rate constant (ln(2)/DT50)
α = 3.4735*N^-0.8629
β = DT50/(2^(1/ α)-1)
N = DT90/DT50-3.32
DT50 = estimated from the data
DT90 = estimated from the data
k1 = rate constant for the fast degradation phase (ln(2)/DT50f)
k2 = rate constant for the slow degradation phase (ln(2)/DT50s)
tb = break point time estimated from the data rate
g = 1-F
F = estimated residual fraction at the time when the fast degradation phase changes to the slow degradation phase

Table A 2-4: Results and parameters of the kinetic degradation of zoxamide in/on sugar beet leaves (Trial S16-05375-02)

Model	SFO	FOMC	DFOP	HS
DT50 [days]	1.81	0.86	0.93	1.82
DT90 [days]	6.02	10.99	10.17	6.02
CHI2-err [%]	19.55	7.27	8.62	23.27
r2	0.93	0.99	0.99	0.93
parameter	k = 0.3825	α = 0.77533	k1 = 1.40435	k1 = 0.004787
	M(0) = 7.2441	β = 0.59437	k2 = 0.12918	k2 = 0.382458
		M(0) = 8.84162	g = 0.62789	tb = 0.003585
			M(0) = 8.52512	M(0) = 7.234198
lower Cl	k = 0.1650	α = 0.43121	k1 = -0.18605	k1 = -4.077415
	M(0) = 5.6238	β = -0.04523	k2 = -0.02383	k2 = 0.058166
		M(0) = 7.54307	g = 0.033830	tb = -3.011862
			M(0) = 7.04669	M(0) = -3.057741
upper Cl	k = 0.600	α = 1.119	k1 = 2.995	k1 = 4.087
	M(0) = 8.864	β = 1.234	k2 = 0.282	k2 = 0.707
		M(0) = 10.140	g = 0.917	tb = 3.019
			M(0) = 10.004	M(0) = 17.526
t-test	p(k): 0.009152	-	p(k1): 0.090971	p(k1): 0.4992
			p(k2): 0.098278	p(k2): 0.0519

SFO = Single first order kinetic;
FOMC = First order multi compartment kinetic;
DFOP = Double first order kinetic;
HS = Hockey stick kinetic
M(0) = initial concentration
CI = confidence interval
t = time after time of initial concentration
k = rate constant (ln(2)/DT50)
α = 3.4735*N^-0.8629
β = DT50/(2^(1/ α)-1)

N = DT90/DT50-3.32
DT50 = estimated from the data
DT90 = estimated from the data
k1 = rate constant for the fast degradation phase (ln(2)/DT50f)
k2 = rate constant for the slow degradation phase (ln(2)/DT50s)
tb = break point time estimated from the data rate
g = 1-F
F = estimated residual fraction at the time when the fast degradation phase changes to the slow degradation phase

Table A 2-5: Results and parameters of the kinetic degradation of zoxamide in/on sugar beet leaves (Trial S16-05375-03)

Model	SFO	FOMC	DFOP	HS
DT50 [days]	3.09	3.65	3.09	3.55
DT90 [days]	10.25	12.14	10.25	7.79
CHI2-err [%]	21.02	23.75	25.02	10.34
r2	0.89	0.89	0.89	0.98
parameter	k = 0.22460	α = 1.365e+03	k1 = 0.224604	k1 = 1.704e-08
	M(0) = 6.92205	β = 7.187e+03	k2 = 0.085447	k2 = 3.798e-01
		M(0) = 6.588	g = 1.000000	tb = 1.725
			M(0) = 6.922004	M(0) = 6.070
lower Cl	k = 0.12070	α = -1.448e+04	k1 = -0.052762	k1 = -2.884e-01
	M(0) = 5.41794	β = -7.627e+04	k2 = -0.007701	k2 = 2.306e-01
		M(0) = 5.051	g = -0.218086	tb = 6.145e-01
			M(0) = 4.102791	M(0) = 4.901
upper Cl	k = 0.329	α = 17211.434	k1 = 0.502	k1 = 0.288
	M(0) = 8.426	β = 90641.070	k2 = 0.179	k2 = 0.529
		M(0) = 8.125	g = 2.218	tb = 2.835
			M(0) = 9.741	M(0) = 7.239
t-test	p(k): 0.00410	-	p(k1): 0.10534	p(k1): 0.50000
			p(k2): 0.08502	p(k2): 0.00775

SFO = Single first order kinetic;
FOMC = First order multi compartment kinetic;
DFOP = Double first order kinetic;
HS = Hockey stick kinetic
M(0) = initial concentration
CI = confidence interval
t = time after time of initial concentration
k = rate constant (ln(2)/DT50)
α = 3.4735*N^-0.8629
β = DT50/(2^(1/ α)-1)
N = DT90/DT50-3.32
DT50 = estimated from the data
DT90 = estimated from the data
k1 = rate constant for the fast degradation phase (ln(2)/DT50f)
k2 = rate constant for the slow degradation phase (ln(2)/DT50s)
tb = break point time estimated from the data rate
g = 1-F
F = estimated residual fraction at the time when the fast degradation phase changes to the slow degradation phase

Wheat Green Mass

In trials -01, -02 and -03 the calculation of degradation rates for zoxamide in wheat green mass achieved critical values < 15 for χ^2-error for all models, with one exception. In trial -01 the χ^2-error was > 15 for the HS model. All models showed a determination coefficient of r2 > 0.85. As the t-test of the SFO model for all trials was significant (p(k)<0.05) the SFO model was used to calculate the degradation rates of the trials. The results of the calculation showed DT50 values between 2.74 and 4.31 days and DT90 values between 9.10 and 14.32 days, respectively for the degradation of zoxamide in wheat green mass. The calculated χ^2-errors for the three trials were 13.65 (SFO), 10.22 (SFO) and 8.04 (SFO) for trials -01, -02 and -03, respectively. The calculated r^2 for the three trials were 0.95 (SFO), 0.95 (SFO) and 0.97 (SFO) for trials -01, -02 and -03, respectively.

Table A 2-6: Results and parameters of the kinetic degradation of zoxamide in/on wheat green mass (Trial S16-05375-01)

Model	SFO	FOMC	DFOP	HS
DT50 [days]	2.74	2.07	1.97	2.74
DT90 [days]	9.10	12.59	12.64	9.10
CHI2-err [%]	13.65	11.42	11.85	16.24
r2	0.95	0.97	0.97	0.95
parameter	k = 0.25314	α = 1.5420	k1 = 0.68299	k1 = 0.25314
	M(0) = 10.04582	β = 3.6464	k2 = 0.11975	k2 = 0.13547
		M(0) = 10.8462	g = 0.54619	tb = 19.77503
			M(0) = 10.92615	M(0) = 10.04581
lower Cl	k = 0.16501	α = -0.2238	k1 = -0.13113	k1 = 0.12963
	M(0) = 8.62295	β = -2.7295	k2 = -0.04693	k2 = 0.02685
		M(0) = 8.6787	g = -0.03999	tb = -8.86985
			M(0) = 8.94225	M(0) = 7.91918
upper Cl	k = 0.341	α = 3.308	k1 = 1.497	k1 = 0.377
	M(0) = 11.469	β = 10.022	k2 = 0.286	k2 = 0.244
		M(0) = 13.014	g = 1.132	tb = 48.420
			M(0) = 12.910	M(0) = 12.172
t-test	p(k): 0.00123	-	p(k1): 0.09933	p(k1): 0.01385
			p(k2): 0.12691	p(k2): 0.04607

SFO = Single first order kinetic;
FOMC = First order multi compartment kinetic;
DFOP = Double first order kinetic;
HS = Hockey stick kinetic
M(0) = initial concentration
CI = confidence interval
t = time after time of initial concentration
k = rate constant (ln(2)/DT50)
α = 3.4735*N^-0.8629
β = DT50/(2^(1/ α)-1)
N = DT90/DT50-3.32
DT50 = estimated from the data
DT90 = estimated from the data
k1 = rate constant for the fast degradation phase (ln(2)/DT50f)
k2 = rate constant for the slow degradation phase (ln(2)/DT50s)
tb = break point time estimated from the data rate
g = 1-F

F = estimated residual fraction at the time when the fast degradation phase changes to the slow degradation phase

Table A 2-7: Results and parameters of the kinetic degradation of zoxamide in/on wheat green mass (Trial S16-05375-02)

Model	SFO	FOMC	DFOP	HS
DT50 [days]	4.31	4.29	4.31	4.31
DT90 [days]	14.32	14.28	14.32	14.32
CHI2-err [%]	10.22	11.05	12.16	12.16
r2	0.95	0.95	0.95	0.95
parameter	k = 0.16082	α = 5.809e+02	k1 = 0.16082	k1 = 0.16082
	M(0) = 9.73916	β = 3.595e+03	k2 = 0.09269	k2 = 0.09655
		M(0) = 9.747	g = 1.00000	tb = 15.87861
			M(0) = 9.73915	M(0) = 9.73915
lower Cl	k = 0.11922	α = -5.805e+03	k1 = 0.08106	k1 = 0.11136
	M(0) = 8.60284	β = -3.600e+04	k2 = -0.43351	k2 = 0.03006
		M(0) = 8.634	g = 0.51005	tb = -5.71376
			M(0) = 8.15279	M(0) = 8.21811
upper Cl	k = 0.202	α = 6966.81	k1 = 0.241	k1 = 0.210
	M(0) = 10.875	β = 43187.14	k2 = 0.619	k2 = 0.163
		M(0) = 10.86	g = 1.490	tb = 37.471
			M(0) = 11.326	M(0) = 11.260
t-test	p(k): 0.000318	-	p(k1): 0.014455	p(k1): 0.003912
			p(k2): 0.376352	p(k2): 0.032665

SFO = Single first order kinetic;
FOMC = First order multi compartment kinetic;
DFOP = Double first order kinetic;
HS = Hockey stick kinetic
M(0) = initial concentration
CI = confidence interval
t = time after time of initial concentration
k = rate constant (ln(2)/DT50)
α = 3.4735*N^-0.8629
β = DT50/(2^(1/ α)-1)
N = DT90/DT50-3.32
DT50 = estimated from the data
DT90 = estimated from the data
k1 = rate constant for the fast degradation phase (ln(2)/DT50f)
k2 = rate constant for the slow degradation phase (ln(2)/DT50s)
tb = break point time estimated from the data rate
g = 1-F
F = estimated residual fraction at the time when the fast degradation phase changes to the slow degradation phase

Table A 2-8: Results and parameters of the kinetic degradation of zoxamide in/on wheat green mass (Trial S16-05375-03)

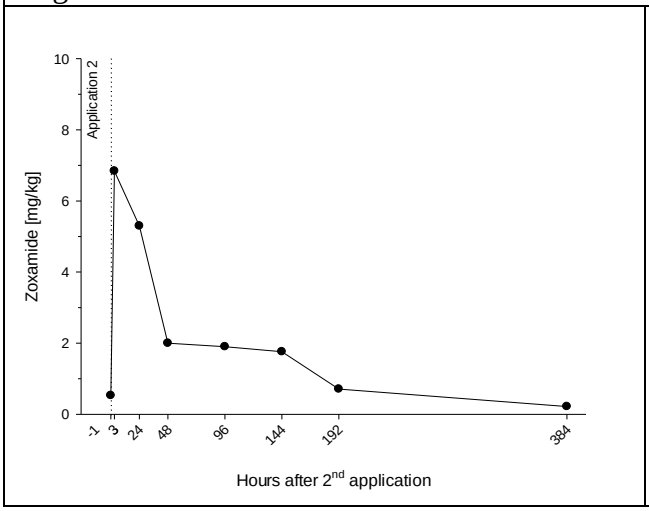
Model	SFO	FOMC	DFOP	HS
DT50 [days]	3.65	3.57	3.65	3.80
DT90 [days]	12.12	12.46	12.12	10.82
CHI2-err [%]	8.04	8.67	9.57	9.30
r2	0.97	0.97	0.97	0.98

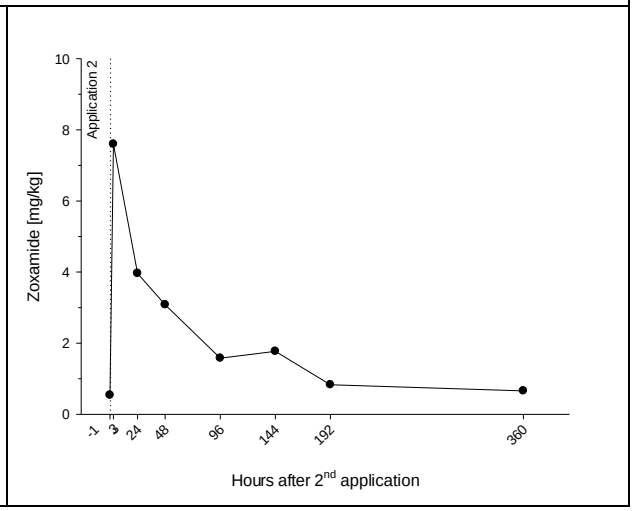
parameter	k = 0.18999	α = 17.0640	k1 = 1.431e-02	k1 = 0.182387
	M(0) = 9.33027	β = 86.2323	k2 = 1.900e-01	k2 = 0.250785
		M(0) = 9.3832	g = 1.088e-07	tb = 6.000000
			M(0) = 9.330	M(0) = 9.253163
lower Cl	k = 0.15018	α = -251.5509	k1 = -	k1 = 0.122714
	M(0) = 8.52435	β = -1327.5442	k2 = -7.374e-01	k2 = -0.008406
		M(0) = 8.0851	g = -1.914	tb = -3.942312
			M(0) = 4.747	M(0) = 8.143282
upper Cl	k = 0.23	α = 285.68	k1 = 15.759	k1 = 0.242
	M(0) = 10.14	β = 1500.01	k2 = 1.117	k2 = 0.510
		M(0) = 10.68	g = 1.914	tb = 15.942
			M(0) = 13.913	M(0) = 10.363
t-test	p(k): 0.000118	-	p(k1): 0.4993	p(k1): 0.004657
			p(k2): 0.3575	p(k2): 0.077089

SFO = Single first order kinetic;
FOMC = First order multi compartment kinetic;
DFOP = Double first order kinetic;
HS = Hockey stick kinetic
M(0) = initial concentration
CI = confidence interval
t = time after time of initial concentration
k = rate constant (ln(2)/DT50)
α = 3.4735*N^-0.8629
β = DT50/(2^(1/ α)-1)
N = DT90/DT50-3.32
DT50 = estimated from the data
DT90 = estimated from the data
k1 = rate constant for the fast degradation phase (ln(2)/DT50f)
k2 = rate constant for the slow degradation phase (ln(2)/DT50s)
tb = break point time estimated from the data rate
g = 1-F
F = estimated residual fraction at the time when the fast degradation phase changes to the slow degradation phase

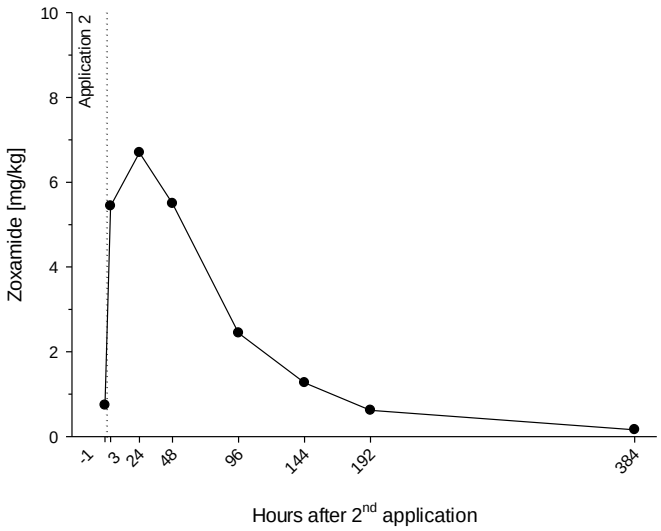
The following figures show residues of zoxamide.

Sugar beet leaves



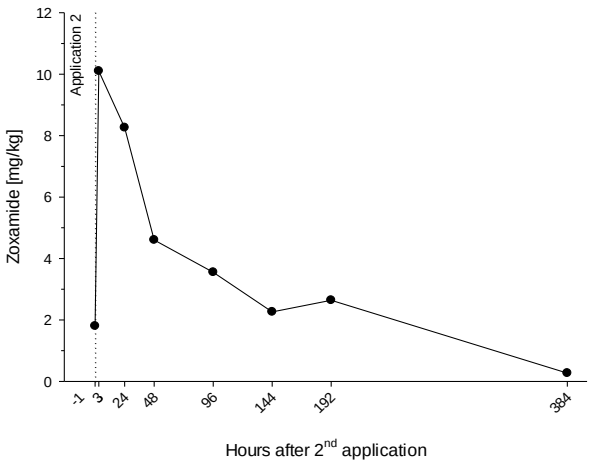


Residues of zoxamide in sugar beet leaves over time (trial 01)	Residues of zoxamide in sugar beet leaves over time (trial 02)

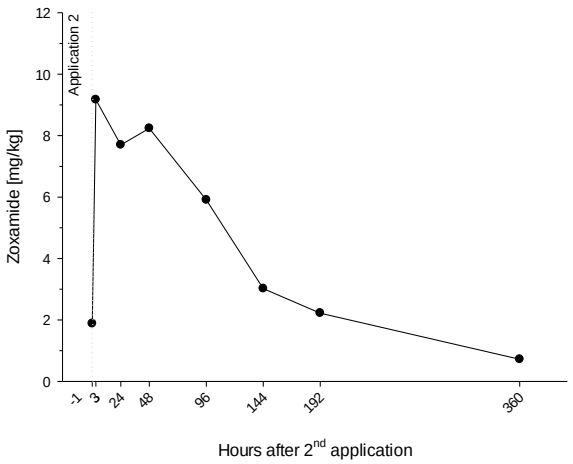


Residues of zoxamide in sugar beet leaves over time (trial 03)

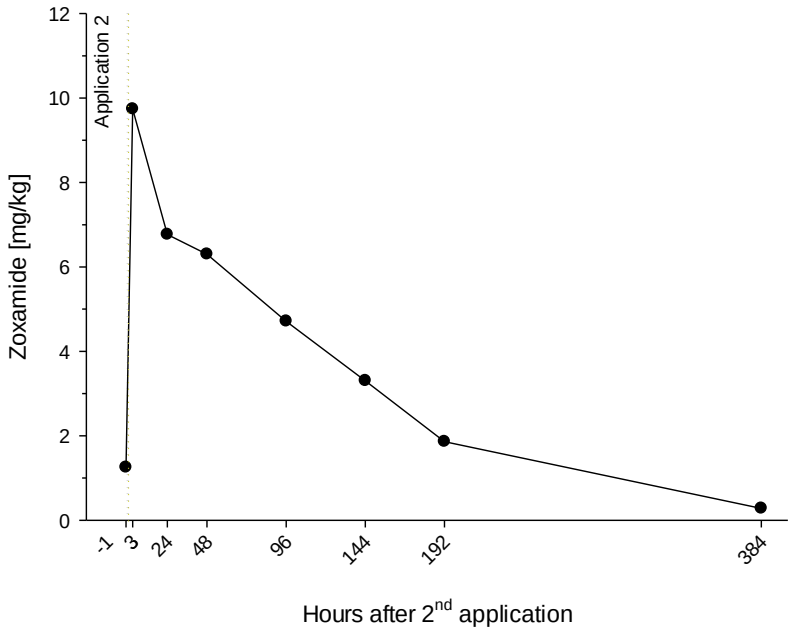
Wheat



Residues of zoxamide in wheat green mass over time (trial 01)



Residues of zoxamide in wheat green mass over time (trial 02)



Residues of zoxamide in wheat green mass over time (trial 03)

Conclusion

The residue decline of zoxamide on sugar beet leaves (as surrogate dicotyledonae plant) and wheat green mass (as surrogate monocotyledonae plant) has been studied in the field under representative growing conditions for Northern Europe (Germany). The degradation kinetics of the active ingredient were investigated after two applications of Zoxium 240 SC (240 g/L zoxamide, SC) at application rates for zoxamide of 180 g a.s./ha with an interval of 7 days (BBCH 14-24 for sugar beet and wheat).
The study was conducted in accordance to current guidelines.
For the degradation of zoxamide in sugar beet leaves, the single first order (SFO) degradation model was used for two trials (trials -01 and -02) – as recommended in Appendix H of the Birds and Mammals Guidance Document (2009) - and the hockey stick (HS) model was used for trial -03. DT50 values were calculated between 1.81 and 3.55 days and DT90 values were calculated between 6.02 and 7.79 days.
For the degradation of zoxamide in wheat green mass, the single first order (SFO) degradation model was used in all of the three trials – as recommended in Appendix H of the Birds and Mammals Guidance Document (2009). DT50 values were calculated between 2.74 and 4.31 days and DT90 values were calculated between 9.10 and 14.32 days.

(Appeltauer A. 2020)

Study 8 - Residue degradation of zoxamide in mono- and dicotyledonae plants under northern European growing conditions

Comments of zRMS:

The study is accepted (acc. SANCO/3029/99 rev. 4).
The data presented in this report demonstrate that the used method permits the determination of residues of Zoxamide in sugar beet leaves (representing the feed item group "non-grass herbs") and in wheat plants (representing the feed item group "grass and cereaIs") (without roots) with accuracy, precision and repeatability. The method is based on QuEChERS multi-residue method, validated by RICHTER (2014) according to SANCO/825/00 rev. 8.1 for the determination of Zoxamide in various crop commodities. This method was validated under the laboratory conditions of CIP for the determination of residues of Zoxamide in sugar beet leaves and wheat plants according to guideline SANCO/3029/99 rev. 4. For this purpose, recovery experiments were performed by fortifying control (untreated) specimens. The method employed extraction with acetonitrile/water, phase separation by addition of buffer salt mixture; Dispersive SPE clean up, followed by filtration through a 0.45 µm single-use syringe filter and dilution with acidified acetonitrile/water; final determination with HPLC - MS/MS detection. The LOQ is 0.01 mg/kg, the LOD is defined as 30% of the LOQ (i.e. 0.003 mg/kg).
Precision was already tested in former study (17E10095-01-RAVE). In the current study, the overall RSD for the quantification ion mass transition ranges from 2.9 % to 3.6 % for Zoxamide in sugar beet leaves and wheat green mass. These values are within the guideline requirements for relative standard deviations (≤ 20%). The calibration graphs (linearity) for Zoxamide are linear within the range of 0.1 µg/L to 250 µg/L with correlation coefficients of ≥ 0.9998 (matrix-matched standard solutions). Regarding specificity typical retention time with no significant interferences (< 30%); analysis of control specimens used for recoveries yielded no residues of Zoxamide above 30% of the LOQ, indicating that no interferences were present. Zoxamide parent compound was detected with two ion mass transitions (SRM 336 —> 187 for quantification, SRM 338 —> 189 for confirmation). Recoveries were analysed at the LOQ of 0.01 mg/kg and at a higher level of 50 mg/kg (sugar beet leaves) or100 mg/kg (wheat plant) to cover the estimated residues found in field samples.
The following recoveries were obtained with HPLC-MS/MS for Zoxamide:

Matrix	Fortification Level [mg/kg]	Recoveries Single Values [%]	Mean [%]	RSD [%]	No. of Analyses	Overall recovery Mean [%]	Overall recovery RSD [%]
Zoxamide SRM 336 → 187 (quantification)							
sugar beet leaves	0.01	102 / 102	102	n.a.	2	104	2.9
	50	108 / 102	105	n.a.	2		
Zoxamide SRM 338 → 189 (confirmation)							
sugar beet leaves	0.01	106 / 110	108	n.a.	2	106	3.5
	50	107 / 101	104	n.a.	2		
Zoxamide SRM 336 → 187 (quantification)							
wheat green mass	0.01	104 / 110	107	n.a.	2	105	3.6
	100	105 / 101	103	n.a.	2		
Zoxamide SRM 338 → 189 (confirmation)							
wheat green mass	0.01	103 / 111	107	n.a.	2	105	4.8
	100	105 / 99	102	n.a.	2		

RSD = Relative Standard Deviation, SRM: Single Reaction Monitoring; n.a. = not applicalble

From field trials in the sugar beet leaves specimens, residues of Zoxamide were at maximum 7.09 mg/kg and declined to 1.68 mg/kg within 21 days after the last application of Zoxium 240 SC. In the wheat green mass specimens, residues of Zoxamide were at maximum 7.43 mg/kg and declined to 0.85 mg/kg within 21 days after last application of Zoxium 240 SC. Residues of Zoxamide in sugar beet leaves and wheat green mass were below the limit of detection (< 0.003 mg/kg) in all control (untreated) specimens. The residues are summarised in the following tables:

Trial S19-01450-01:

Sample No. L19-01450--	Laboratory sample No.	Timing	Treat-ment	Matrix	Residue Zoxamide (mg/kg)
01-C-sbl-S01-A	1450-01	5 ± 5 DBA1	C	sugar beet leaves	<LOD
01-C-w-S01-A	1450-02	5 ± 5 DBA1	C	wheat green mass	<LOD
01-T-sbl-S02-A	1450-03	3 ± 1 HAA2	T	sugar beet leaves	7.09
01-T-sbl-S03-A	1450-04	24 ± 1 HAA2	T	sugar beet leaves	7.07
01-T-sbl-S04-A	1450-05	48 ± 1 HAA2	T	sugar beet leaves	6.16
01-T-sbl-S05-A	1450-06	4 ± 1 DAA2	T	sugar beet leaves	5.68
01-T-sbl-S06-A	1450-07	6 ± 1 DAA2	T	sugar beet leaves	4.83
01-T-sbl-S07-A	1450-08	8 ± 1 DAA2	T	sugar beet leaves	4.23
01-T-sbl-S08-A	1450-09	14 ± 1 DAA2	T	sugar beet leaves	3.51
01-T-sbl-S09-A	1450-10	21 ± 1 DAA2	T	sugar beet leaves	1.68
01-T-w-S02-A	1450-11	3 ± 1 HAA2	T	wheat green mass	6.95
01-T-w-S03-A	1450-12	24 ± 1 HAA2	T	wheat green mass	7.43
01-T-w-S04-A	1450-13	48 ± 1 HAA2	T	wheat green mass	5.96
01-T-w-S05-A	1450-14	4 ± 1 DAA2	T	wheat green mass	4.69
01-T-w-S06-A	1450-15	6 ± 1 DAA2	T	wheat green mass	3.55
01-T-w-S07-A	1450-16	8 ± 1 DAA2	T	wheat green mass	2.51
01-T-w-S08-A	1450-17	14 ± 1 DAA2	T	wheat green mass	1.82
01-T-w-S09-A	1450-18	21 ± 1 DAA2	T	wheat green mass	0.85

C: contro (untreated) sample, T:< LOD: below the limit of detection
Page 12 of 36 treated sample, HBA/DBA: hours/days before application, HAA/DAA (0.003mg/kg); LOQ: limit of quantification (0.01mg/kg) hours/days after application.

The extraction procedure used in this study was according to the QuEChERS method (validated for Zoxamide in study No P 3114 G (RICHTER, S., PTRL Europe 2014)). This method was checked for its extraction efficiency in study AS362 (Hein, W., RLP AgroScience
GmbH, Germany, 2014) in pea immature whole plant. Therefore, the extraction efficiency was considered to be proven and no additional assessment of extraction efficiency was performed in the current study.
The method was laboratory validated according to guideline SANCO/3029/99 rev. 4. The specimen extracts were analysed using liquid chromatography with mass selective detection (HPLC-MS/MS).
The procedural recovery data demonstrate that the method permits the determination of residues of Zoxamide in sugar beet leaves and wheat green mass matrices with satisfactory accuracy according to guideline SANCO/3029/99 rev.4 during the study.
Residues of Zoxamide in all control (untreated) sugar beet leaves and wheat green mass specimens were below the limit of detection (< 0.003 mg/kg)
In the sugar beet leaves specimens residues of Zoxamide were at maximum of 7.09 mg/kg and declined to 1.68 mg/kg within 21 days after the last application of Zoxium 240 SC. In the wheat green mass specimens, residues of Zoxamide were at maximum 7.43 mg/kg and declined to 0.85 mg/kg within 21 days after last application of Zoxium 240 SC.

Reference:	KCP 9.2.5/03
Report	Appeltauer, A., 2020: Determination of residues of zoxamide on/in typical feed items of herbivorous birds and mammals after two applications of Zoxium 240 SC on sugar beet and wheat in the Netherlands in 2019 Gowan Crop Protection Ltd., UK Eurofins GmbH, Germany, Report No. S19-01450, GLP, Not published
Guideline(s):	SANCO/4145/2000 EFSA Guidance on Risk Assessment for Birds and Mammals (2009) SANCO/3029/99 rev. 4 SANCO/825/00 rev. 8.1 EFSA Technical Report (2019): Outcome of the pesticides peer review meeting on general recurring issues in physical and chemical properties and analytical methods. FOCUS (2014)
Deviations:	No daily precipitation GLP data available. Reason for this deviation was a mistake during study conduct. Amount of rain in rain gauge was only recorded at every visit (not daily). Only non-GLP rain data are available for the report.
GLP:	Yes
Acceptability:	Yes

The objective of this study was to determine the residue decline of zoxamide on/in feed items of herbivorous birds and mammals under representative growing conditions in The Netherlands in the field: In sugar beet leaves (as surrogate dicotyledonae, representative for the feed item group "non-grass herbs") and in wheat green mass above soil (as surrogate monocotyledonae, representative for the feed item group "grass and cereals"). The residues and degradation kinetics of the active ingredient were investigated after two applications of Zoxium 240 SC (240 g/L zoxamide, SC) at application rates for zoxamide of 180 g a.i./ha with an interval of 7 days (BBCH 18-21 for sugar beet and wheat). The early application timing of zoxamide to both plant groups is representative for the more sensitive phase for wild birds and mammals.
The study consisted of one field trial, S19-01450-01 and one residue analysis trial, S19-01450-L1. The field part was carried out on a field located near PK Elst, Gelderland, The Netherlands. The field site of the trial covered an area of 675 m² sugar beet and 675 m² summer wheat.
Sugar beet plants were planted on 06 April 2019; wheat was sown on 03 May 2019. Samples were taken before first application and up to 21 days after the second application. One sampling before the 1st application served as control.
For the two specimen types, the sampling schedule was as follows: the first sampling before the 1st application (control), 3, 24, 48 hours after the 2nd application, and 4, 5, 7, 14 and finally 21 days after the 2nd application. The samples were weighed in the field and frozen immediately on dry ice. Retained samples were taken for all matrices. All residues samples were stored in the freezer within 1 hour after collection in the field. The samples were stored and shipped frozen. The maximum storage time of samples was 28 days for sugar beet leaves and wheat green mass. The stability of the analyte during storage of the final sample extracts is sufficiently proven. The maximum freezer storage time of samples was 28 days for sugar beet leaves and wheat green mass. Residue analysis took place within 5 days after extraction.
The residues of the active ingredients on/in sugar beet leaves and wheat green mass were analysed with fully validated analytical methods according to SANCO/3029/99 rev. 4. The method for the determination of zoxamide in sugar beet leaves and wheat green mass has been previously validated according to guideline SANCO/3029/99 rev. 4 in a former study (Witte, A.; CIP Phase ID 17E10095-01-RAVE, analytical part of EAS study S16-05375) at an LOQ of 0.01 mg/kg for the matrices under investigation. It takes into account additional requirements of SANCO/825/00 rev. 8.1 and EFSA Technical Report (2019): Outcome of the

pesticides peer review meeting on general recurring issues in physical and chemical properties and analytical methods. Specimens were extracted (in analogy to the QuEChERS multi residue method) with acetonitrile/water, phase separation was done by addition of buffer salt mixture. The final analysis was conducted with highly specific HPLC with MS/MS detection. Recoveries in the fortified samples were within the acceptable range of 70 - 110 %, therefore the stability of the analyte during storage of the final sample extracts is sufficiently proven.
The degradation kinetics of the active ingredient was analysed according to the recommendations of the EFSA Guidance document on Risk Assessment for Birds and Mammals (2009) and the Guidance document on Estimating Persistence and Degradation Kinetics from Environmental Studies on Pesticides in EU Registration (FOCUS 2014). The calculation of the DT50 values and DT90 values as well as the fitting of the kinetic degradation models was done using the computer software KinGU II (version 2.2012). Four different kinetic degradation models were taken into account: single first-order, first order multi-compartment (GUSTAFSON & HOLDEN, 1990), hockey stick (bi-phasic) and double first-order (bi-exponential) kinetics. The operating system was Microsoft Windows 10 Professional.
Materials and methods
Materials

1. Test material	ZOXIUM 240 SC
Lot/batch:	SB 2401
Active substance content:	240 g/ zoxamide (nominal), 232 g/L zoxamide (analysed)
Expiry date:	February 2020

Methods
Experimental conditions
The study comprised one sugar beet and wheat field, trial S19-01450-01. The trial was treated with two applications of Zoxium 240 SC at a nominal rate of 180 g zoxamide/ha. The field site was located near PK Elst, Gelderland, in The Netherlands. The agricultural practices and sugar beet / wheat varieties were in accordance with the local farming practice.
The trial was designed to produce a single sample for each food type at each sampling date (i.e. to provide an assessment of the average residue level as well as to ensure that sufficient material was collected for the actual residue analysis).
During the study, weather data obtained from weather station in the vicinity of the field site including precipitation and air temperature was taken. During applications and samplings, the climatic conditions (GLP data) were measured at the field site with a portable thermo-hygrometer, a soil thermometer and a portable anemometer.
No other formulations containing zoxamide were applied during the trial period onto the plot.
Sampling
Samples of different food items for birds and mammals were collected for residue analysis. Two categories of potential bird and mammalian food items were considered:
1. Sugar beet leaves
2. Wheat

For each trial 9 samplings per category were carried out. The first sampling took place before the 1st application and was used as control sample.
For the two specimen types, the sampling schedule was as follows: the first sampling before the 1st application (control), 3, 24, 48 hours after the 2nd application, and 4, 5, 7, 14 and finally 21 days after the 2nd application. The samples were weighed in the field and frozen immediately on dry ice. Retained samples were taken for all matrices. All residues samples were stored in the freezer within 1 hour after collection in the field. The samples were stored and shipped frozen. The maximum storage time of samples was 28 days for sugar beet leaves and wheat green mass. The stability of the analyte during storage of the final sample extracts is sufficiently proven. The maximum freezer storage time of samples was 28 days for sugar beet leaves and wheat green mass. Residue analysis took place within 5 days after extraction.
The samples were taken randomly on at least 12 locations per sample. There were at least 500 g plant material taken at each sampling occasion. Samplings were done by hand or with a knife. Samples were taken with a minimum distance of 0.5 m to the border of the plot. The samples of all locations of one field were put together to one pooled sample per sampling occasion.

The samples were weighed in the field and frozen immediately on dry ice. Retained samples were taken for all matrices. All residues samples were stored in the freezer within 1 hour after collection in the field. The samples were stored and shipped frozen. The maximum storage time of samples was 28 days for sugar beet leaves and wheat green mass.
The samples were weighed in the field and frozen immediately on dry ice. Retained samples were taken for all matrices. All residues samples were stored in the freezer within 1 hour after collection in the field. The samples were stored and shipped frozen. The maximum storage time of samples was 28 days for sugar beet leaves and wheat green mass.
Description of the analytical procedure
The data presented in this report demonstrate that the used method permits the determination of residues of zoxamide in in sugar beet leaves (representing the feed item group "non-grass herbs") and in wheat plants without roots (representing the feed item group "grass and cereals") with accuracy, precision and repeatability. The method is based on QuEChERS multi-residue method, validated by RICHTER (2014) according to SANCO/825/00 rev. 8.1 for the determination of zoxamide in various crop commodities. This method was validated under the laboratory conditions of CIP for the determination of residues of zoxamide in sugar beet leaves and wheat green mass according to guideline SANCO/3029/99 rev. 4. For this purpose, recovery experiments were performed by fortifying control (untreated) specimens.
10 g (± 0.1 g) of sugar beet leaves and wheat green mass specimens were weighed into 50 mL single-use centrifuge tubes. Recovery samples were fortified at this step. 10 mL acetonitrile were added and the samples were homogenised for at least 2 min using a vortex mixer. Thereafter, QuEChERS EN15662 salt-mixture (1 g sodium citrate, 0.5 g sodium hydrogencitrate sequihydrate, 4 g magnesium sulphate, 1 g sodium chloride) was added, thoroughly shaken and mixed again on a vortex mixer for at least 1 min. The samples were centrifuged at 4000 min-1 for at least 5 minutes. An aliquot of 1 mL of the supernatant was transferred into a tube prepared with 25 mg PSA (primary-secondary amino phase) and 150 mg anhydrous magnesium sulphate and mixed on a Vortex mixer for 1 min. The extract was filtered through a single–use syringe filter (0.45 µm) into an autosampler vial (1.8 mL). 0.5 mL of this solution were transferred into a second vial, 5 µL of acetonitrile + 5 % formic acid were added, the vial capped and thoroughly shaken. 50 µL of this sample extract were then diluted with 950 µL acetonitrile/water (20:80, v/v) plus 0.1 % formic acid. If necessary, these final extracts were diluted further with final extract of untreated samples to achieve final concentrations falling within the calibrated concentration range of the detection system.
For detailed information on the analytical method validation, please refer to Part B, Section 5.
Calculation of initial concentration (C0) and DT50/DT90 values
The degradation time of zoxamide was calculated, including information about the kinetics of the decay according to the recommendations of the guidance document on estimating persistence and degradation kinetics from environmental studies on pesticides in EU registration (FOCUS 2014).
The calculation of the DT50 values and DT90 values as well as the fitting of the kinetic degradation models was done using the computer software KinGU II (version 2.2012). The fitting of the analysed data was calculated for four kinetic degradation models – single first order kinetic (simple first order), first order multi compartment kinetic (GUSTAFSON & HOLDEN, 1990), hockey stick kinetic (bi-phasic) and double first order kinetic (bi-exponential). The operating system was Microsoft Windows 7 Professional. The significance of the used models was determined, considering the significance of the parameters k, k1 and k2, which was part of the results obtained from the calculation with KinGU II.
For both commodities, the analysed residues after the last application (2nd application), i.e. starting from three hours after the last application to 21 days after the last application were chosen to establish degradation kinetics. For the two commodities timings were calculated separately from the end of application until the samples were put on dry ice (i.e. time degradation of residues stops). Times were rounded to full days (two digits).
Results and discussions
Weather conditions
During the trial period the daily average temperatures were between 13.7 °C and 28.2 °C. The rainfall recorded at the field site was 38 mm during the period from first sampling before application to last sampling.
Zoxamide residues

In the control samples of sugar beet leaves and wheat green mass, taken one day before the 1st application, concentrations analysed were below the LOD (0.003 mg/kg).

Zoxamide in/on sugar beet leaves

Zoxamide concentrations in sugar beet leaves were highest at the first sampling after the second application (3HAA2) with 7.09 mg/kg. Subsequently residues decreased to 1.68 mg/kg at the last sampling (21DAA2). A summary of the residue levels found in sugar beet leaf samples is shown in the following table.

Table A 2-9: Zoxamide residues in/on sugar beet leaves

Timing	Trial
	S19-01450-01
	[mg/kg]
1DBA1	<LOD
3HAA2	7.09
24HAA2	7.07
48HAA2	6.16
4DAA2	5.68
5DAA2	4.83
7DAA2	4.23
14DAA2	3.51
21DAA2	1.68

DBA: days before application, DAA: days after application,
HAA: hours after application, HBA: hours before application,
LOD: level of detection (0.003 mg/kg)

Zoxamide in/on wheat green mass

Zoxamide concentrations in wheat samples were at 6.95 mg/kg at the first sampling after the second application (3HAA2). On the following sampling (24HAA2) the highest residue was observed with 7.43 mg/kg. Subsequently residues decreased to 0.85 mg/kg at the last sampling (21DAA2). A summary of the residue levels found in wheat green mass samples is shown in the following table.

Table A 2-10: Zoxamide residues in/on wheat green mass

Timing	Trial
	S19-01450-01
	[mg/kg]
1DBA1	<LOD
3HAA2	6.95
24HAA2	7.43
48HAA2	5.96
4DAA2	4.69
5DAA2	3.55
7DAA2	2.51
14DAA2	1.82
21DAA2	0.85

DBA: days before application, DAA: days after application,
HAA: hours after application, HBA: hours before application,
LOD: level of detection (0.003 mg/kg)

Calculation of initial concentration (C0) DT50/DT90 values

Sugar Beet Leaves

For sugar beet samples all of the models achieved the critical values < 15 for χ^2-error and for the determination coefficient of r2 > 0.85. The FOMC, DFOP and HS models showed either negative confidence intervals or were not statistically significant. For the SFO model the calculated χ^2-error was 5.01 and the calculated r^2 was 0.97. The results of the calculation showed a DT50 value of 10.81 days and a DT90 value of 35.90 days, respectively.

Table A 2-11: Results and parameters of the kinetic degradation of zoxamide in/on sugar beet leaves (Trial S19-01450-01)

Model	SFO	FOMC	DFOP	HS
DT50 [days]	10.81	10.33	10.37	10.96
DT90 [days]	35.90	43.62	38.68	35.98
CHI2-err [%]	5.01	5.20	5.46	5.77
r2	0.97	0.97	0.97	0.97
parameter	k = 0.064146	α = 3.5906	k1 = 0.33656	k1 = 0.0002994
	M(0) = 7.165004	β = 48.5250	k2 = 0.05661	k2 = 0.006432
		M(0) = 7.2753	g = 0.10653	tb = 0.1845
			M(0) = 7.34484	M(0) = 7.090
lower Cl	k = 0.051628	α = -10.6976	k1 = -0.84082	k1 = -1.108
	M(0) = 6.732990	β = -171.3987	k2 = 0.02303	k2 = 0.04233
		M(0) = 6.6238	g = -0.32957	tb = -11.90
			M(0) = 6.68011	M(0) = 3.912
upper Cl	k = 0.077	α = 17.879	k1 =1.514	k1 = 1.109
	M(0) = 7.597	β = 268.449	k2 = 0.090	K = 0.086
		M(0) = 7.927	g = 0.543	tb = 12.267
			M(0) = 8.010	M(0) = 10.268
t-test	p(k): 2.82e-05	-	p(k1): 0.3026	p(k1): 0.49980
			p(k2): 0.0149	p(k2): 0.00229

SFO = Single first order kinetic;
FOMC = First order multi compartment kinetic;
DFOP = Double first order kinetic;
HS = Hockey stick kinetic
M(0) = initial concentration
CI = confidence interval
t = time after time of initial concentration
k = rate constant (ln(2)/DT50)
α = 3.4735*N^-0.8629
β = DT50/(2^(1/ α)-1)
N = DT90/DT50-3.32
DT50 = estimated from the data
DT90 = estimated from the data
k1 = rate constant for the fast degradation phase (ln(2)/DT50f)
k2 = rate constant for the slow degradation phase (ln(2)/DT50s)
tb = break point time estimated from the data rate
g = 1-F
F = estimated residual fraction at the time when the fast degradation phase changes to the slow degradation phase

Wheat Green Mass

For wheat samples all of the models achieved the critical values < 15 for χ^2-error and for the determination coefficient of r2 > 0.85. The FOMC, DFOP and HS models showed either negative confidence intervals or where not statistically significant. For the SFO model the calculated χ^2-error was 5.44 and the calculated r^2 was 0.96. The results of the calculation showed a DT50 value of 5.44 days and a DT90 value of 18.06 days, respectively.

Table A 2-12: Results and parameters of the kinetic degradation of zoxamide in/on wheat green mass (Trial S19-01450-01)

Model	SFO	FOMC	DFOP	HS
DT50 [days]	5.44	5.09	4.99	5.83

DT90 [days]	18.06	21.32	28.75	17.05
CHI2-err [%]	9.00	9.06	9.48	8.83
r2	0.96	0.96	0.96	0.97
parameter	k = 0.12751	α = 3.7007	k1 = 1.599e-01	k1 = 1.149e-05
	M(0) = 7.56718	β = 24.7017	k2 = 2.868e-07	k2 = 1.435e-01
		M(0) = 7.7249	g = 9.091e-01	tb = 1.000
			M(0) = 7.736	M(0) = 7.027
lower Cl	k = 0.09371	α = -6.2107	k1 = 1.076e-02	k1 = -2.326e-01
	M(0) = 6.85027	β = -51.9133	k2 = -3.560e-01	k2 = 9.284e-02
		M(0) = 6.8304	g = 1.679e-01	tb = 1.998e-01
			M(0) = 6.733	M(0) = 5.780
upper Cl	k = 0.161	α = 13.612	k1 = 0.309	k1 = 0.233
	M(0) = 8.284	β = 101.317	k2 = 0.356	k2 = 0.194
		M(0) = 8.619	g = 1.650	tb = 1.800
			M(0) = 8.739	M(0) = 8.274
t-test	p(k): 0.000157	-	p(k1): 0.0517	p(k1): 0.499964
			p(k2): 0.5000	p(k2): 0.002574

SFO = Single first order kinetic;
FOMC = First order multi compartment kinetic;
DFOP = Double first order kinetic;
HS = Hockey stick kinetic
M(0) = initial concentration
CI = confidence interval
t = time after time of initial concentration
k = rate constant (ln(2)/DT50)
α = 3.4735*N^-0.8629
β = DT50/(2^(1/ α)-1)
N = DT90/DT50-3.32
DT50 = estimated from the data
DT90 = estimated from the data
k1 = rate constant for the fast degradation phase (ln(2)/DT50f)
k2 = rate constant for the slow degradation phase (ln(2)/DT50s)
tb = break point time estimated from the data rate
g = 1-F
F = estimated residual fraction at the time when the fast degradation phase changes to the slow degradation phase

The following figures show residues of zoxamide.

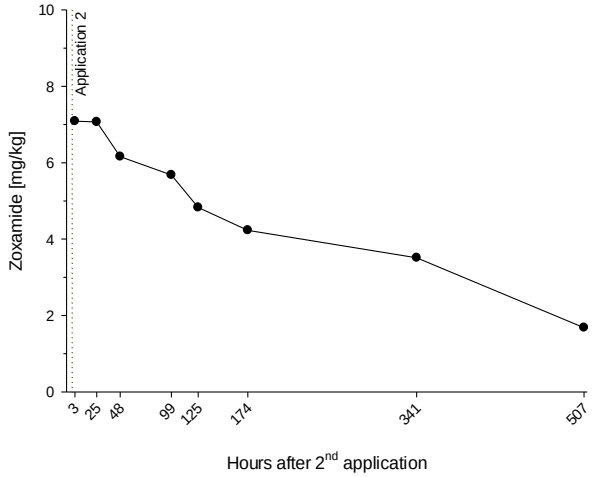


Figure A 9: Residues of zoxamide in sugar beet leaves over time (trial 01)

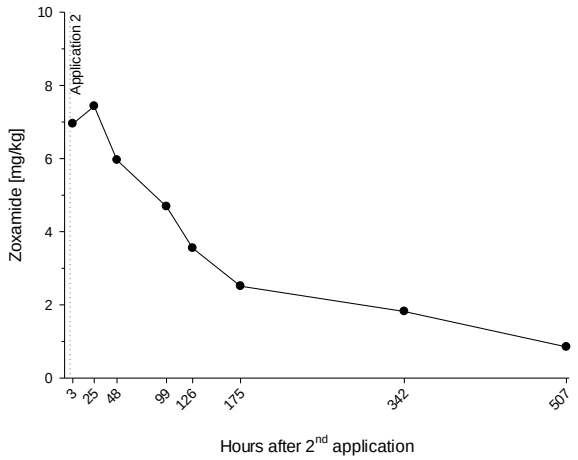


Figure A 10: Residues of zoxamide in wheat green mass over time (trial 01)

Conclusion

The residue decline of zoxamide on sugar beet leaves (as surrogate dicotyledonae plant) and wheat green mass (as surrogate monocotyledonae plant) has been studied in the field under representative growing conditions for Northern Europe (Netherlands). The degradation kinetics of the active ingredient were investigated after two applications of Zoxium 240 SC (240 g/L zoxamide, SC) at application rates for zoxamide of 180 g a.s./ha with an interval of 7 days (BBCH 18-21 for sugar beet and wheat).

The study was conducted in accordance to current guidelines.

For the degradation of zoxamide in sugar beet leaves, the single first order (SFO) degradation model was used – as recommended in Appendix H of the EFSA Birds and Mammals Guidance Document (2009). The DT50 value was calculated at 10.81 days and the DT90 was calculated at 35.90 days.

For the degradation of zoxamide in wheat green mass, the single first order (SFO) degradation model was used – as recommended in Appendix H of the EFSA Birds and Mammals Guidance Document (2009). The DT50 value was calculated at 5.44 days and the DT90 was calculated at 18.06 days.

(Appeltauer A. 2020)

Study 9 - Residue degradation of zoxamide in mono- and dicotyledonae plants under southern European growing conditions

Comments of zRMS:	The study is accepted (acc. SANCO/3029/99 rev. 4).

The data presented in this report demonstrate that the used method permits the determination of residues of Zoxamide in In sugar beet leaves (representing the feed item group "non-grass herbs") and in wheat plants without roots (representing the feed item group "grass and cereals") with accuracy, precision and repeatability. The method is based on QuEChERS multi-residue method, validated by RICHTER (2014) according to SANCO/825/00 rev. 8.1 for the determination of Zoxamide in various crop commodities. This method was validated under the laboratory conditions of CIP for the determination of residues of Zoxamide in sugar beet leaves and wheat plants according to guideline SANCO/3029/99 rev. 4. For this purpose, recovery experiments were performed by fortifying control (untreated) specimens.

The analytical method is characterised as follows:
Extraction with acetonitrile/water, phase separation by addition of buffer salt mixture; Clean up: Dispersive SPE clean up, followed by filtration through a 0.45 µm single-use syringe filter and dilution with acidified acetonitrile/water; Final analysis: HPLC with MS/MS detection; LOQ is 0.01 mg/kg; LOD is defined as 30% of the LOQ (i.e. 0.003 mg/kg); The RSD for the quantification ion mass transition per fortification level ranges from 1.4 % to 4.8 % for Zoxamide in sugar beet leaves and wheat plant. These values are within the guideline requirements for relative standard deviations (≤20%). The calibration graphs for Zoxamide are linear within the range of 0.1 µg/L to 250 µg/L with correlation coefficients of r ≥ 0.9998 (matrix-matched standard solutions); Typical retention time with no significant interferences; Zoxamide parent compound detected with two ion mass transitions (SRM 336 —> 187 for quantification, SRM 338 —> 189 for confirmation). Analysis of control specimens used for recoveries with HPLC-MS/MS yielded no residues of Zoxamide above 30% of the LOQ, indicating that no interferences were present. Recoveries were analysed at the LOQ of 0.01 mg/kg and at a higher level of 50 mg/kg (sugar beet leaves) or 100 mg/kg (wheat plant) to cover the estimated residues found in field samples. The method validation part of this study was performed together with the analytical part of study S16-05375 (CIP phase ID 17E10095-01-RAVE).

The following recoveries were obtained with HPLC-MS/MS for Zoxamide:

Matrix	Fortification Level [mg/kg]	Recoveries Single Values [%]	Mean [%]	RSD [%]	No. of Analyses	Overall recovery Mean [%]	Overall recovery RSD [%]
Zoxamide SRM 336 → 187 (quantification)							
sugar beet leaves	0.01	96 / 95 / 102 / 102 / 95 / 106 / 98	99	4.3	7	104	5.4
	50	110 / 108 / 109 / 109 / 108 / 105 / 108	108	1.5	7		
Zoxamide SRM 338 → 189 (confirmation)							
sugar beet leaves	0.01	106 / 105 / 102 / 107 / 104 / 98 / 104	104	2.9	7	105	3.0
	50	109 / 109 / 108 / 109 / 107 / 102 / 105	107	2.5	7		
Zoxamide SRM 336 → 187 (quantification)							
wheat plant	0.01	96 / 100 / 104 / 92 / 98 / 102 / 92	98	4.8	7	103	6.1
	100	106 / 108 / 109 / 110 / 109 / 106 / 108	108	1.4	7		
Zoxamide SRM 338 → 189 (confirmation)							
wheat plant	0.01	88 / 94 / 99 / 94 / 93 / 94 / 84	92	5.3	7	100	8.7
	100	110 / 107 / 109 / 108 / 109 / 105 / 105	108	1.8	7		

RSD = Relative Standard Deviation, SRM: Single Reaction Monitoring

All mean recoveries obtained by HPLC-MS/MS for Zoxamide at all fortification levels comply with the standard acceptance criteria of SANCO/3029/99, which demand that the mean recovery at each fortification level should be in the range of 70 — 110%.

In the sugar beet leaves specimens residues of Zoxamide were at maximum in the range of 7 - 11 g/kg and declined to values between 0.08 and 0.8 mg/kg within 16 days after the last application of Zoxium 240 SC. In the wheat plant specimens, residues of Zoxamide were at maximum in the range of 5.8 – 8 mg/kg and declined to values between 0.02 and 2 mg/kg within 16 days after last application. Residues of Zoxamide in untreated sugar beet leaves and wheat plants were below the limit of detection (< 0.003 mg/kg) in all untreated specimens.
The residues are summarised in the following tables:

Trial S16-05376-01:

Sample No. L16-05376-	Laboratory sample No.	Timing	Treat-ment	Matrix	Residue Zoxamide (mg/kg)
01-C-sbl-S01-A	5376-01-01	5 ± 5 DBA1	C	sugar beet leaves	n.d.
01-C-w-S01-A	5376-01-02	5 ± 5 DBA1	C	wheat plant	n.d.
01-T-sbl-S02-A	5376-01-03	2 ± 2 HBA2	T	sugar beet leaves	0.47
01-T-sbl-S03-A	5376-01-04	3 ± 1 HAA2	T	sugar beet leaves	9.48
01-T-sbl-S04-A	5376-01-05	24 ± 1 HAA2	T	sugar beet leaves	9.11
01-T-sbl-S05-A	5376-01-06	48 ± 1 HAA2	T	sugar beet leaves	9.08
01-T-sbl-S06-A	5376-01-07	4 ± 1 DAA2	T	sugar beet leaves	1.28
01-T-sbl-S07-A	5376-01-08	6 ± 1 DAA2	T	sugar beet leaves	1.12
01-T-sbl-S08-A	5376-01-09	8 ± 1 DAA2	T	sugar beet leaves	0.78
01-T-sbl-S09-A	5376-01-10	16 ± 1 DAA2	T	sugar beet leaves	0.08
01-T-w-S02-A	5376-01-11	2 ± 2 HBA2	T	wheat plant	0.52
01-T-w-S03-A	5376-01-12	3 ± 1 HAA2	T	wheat plant	7.97
01-T-w-S04-A	5376-01-13	24 ± 1 HAA2	T	wheat plant	5.56
01-T-w-S05-A	5376-01-14	48 ± 1 HAA2	T	wheat plant	5.27
01-T-w-S06-A	5376-01-15	4 ± 1 DAA2	T	wheat plant	1.60
01-T-w-S07-A	5376-01-16	6 ± 1 DAA2	T	wheat plant	1.15
01-T-w-S08-A	5376-01-17	8 ± 1 DAA2	T	wheat plant	0.39
01-T-w-S09-A	5376-01-18	16 ± 1 DAA2	T	wheat plant	0.02

C: control sample, T: treated sample, HBA/DBA: hours/days before application, HAA/DAA: hours/days after application, < LOD: below the limit of detection (0.003mg/kg); LOQ: limit of quantification (0.01mg/kg)

Trial S16-05376-02:

Sample No. L16-05376-	Laboratory sample No.	Timing	Treat-ment	Matrix	Residue Zoxamide (mg/kg)
02-C-sbl-S01-A	5376-02-01	5 ± 5 DBA1	C	sugar beet leaves	n.d.
02-C-w-S01-A	5376-02-02	5 ± 5 DBA1	C	wheat plant	n.d.
02-T-sbl-S02-A	5376-02-03	2 ± 2 HBA2	T	sugar beet leaves	1.85
02-T-sbl-S03-A	5376-02-04	3 ± 1 HAA2	T	sugar beet leaves	10.2
02-T-sbl-S04-A	5376-02-05	24 ± 1 HAA2	T	sugar beet leaves	11.1
02-T-sbl-S05-A	5376-02-06	48 ± 1 HAA2	T	sugar beet leaves	10.2
02-T-sbl-S06-A	5376-02-07	4 ± 1 DAA2	T	sugar beet leaves	7.53
02-T-sbl-S07-A	5376-02-08	6 ± 1 DAA2	T	sugar beet leaves	4.31
02-T-sbl-S08-A	5376-02-09	8 ± 1 DAA2	T	sugar beet leaves	3.14
02-T-sbl-S09-A	5376-02-10	16 ± 1 DAA2	T	sugar beet leaves	0.69
02-T-w-S02-A	5376-02-11	2 ± 2 HBA2	T	wheat plant	1.42
02-T-w-S03-A	5376-02-12	3 ± 1 HAA2	T	wheat plant	7.66
02-T-w-S04-A	5376-02-13	24 ± 1 HAA2	T	wheat plant	5.81
02-T-w-S05-A	5376-02-14	48 ± 1 HAA2	T	wheat plant	5.44
02-T-w-S06-A	5376-02-15	4 ± 1 DAA2	T	wheat plant	5.59
02-T-w-S07-A	5376-02-16	6 ± 1 DAA2	T	wheat plant	2.42
02-T-w-S08-A	5376-02-17	8 ± 1 DAA2	T	wheat plant	4.12
02-T-w-S09-A	5376-02-18	16 ± 1 DAA2	T	wheat plant	0.51

C: control sample, T: treated sample, HBA/DBA: hours/days before application, HAA/DAA: hours/days after application, < LOD: below the limit of detection (0.003mg/kg); LOQ: limit of quantification (0.01mg/kg)

Trial S16-05376-03:

Sample No. L16-05376-	Laboratory sample No.	Timing	Treat-ment	Matrix	Residue Zoxamide (mg/kg)
03-C-sbl-S01-A	5376-03-01	5 ± 5 DBA1	C	sugar beet leaves	n.d.
03-C-w-S01-A	5376-03-02	5 ± 5 DBA1	C	wheat plant	n.d.
03-T-sbl-S02-A	5376-03-03	2 ± 2 HBA2	T	sugar beet leaves	0.77
03-T-sbl-S03-A	5376-03-04	3 ± 1 HAA2	T	sugar beet leaves	6.53
03-T-sbl-S04-A	5376-03-05	24 ± 1 HAA2	T	sugar beet leaves	7.55
03-T-sbl-S05-A	5376-03-06	48 ± 1 HAA2	T	sugar beet leaves	4.83
03-T-sbl-S06-A	5376-03-07	4 ± 1 DAA2	T	sugar beet leaves	1.97
03-T-sbl-S07-A	5376-03-08	6 ± 1 DAA2	T	sugar beet leaves	1.57
03-T-sbl-S08-A	5376-03-09	8 ± 1 DAA2	T	sugar beet leaves	1.49
03-T-sbl-S09-A	5376-03-10	16 ± 1 DAA2	T	sugar beet leaves	0.76
03-T-w-S02-A	5376-03-11	2 ± 2 HBA2	T	wheat plant	1.76
03-T-w-S03-A	5376-03-12	3 ± 1 HAA2	T	wheat plant	5.83
03-T-w-S04-A	5376-03-13	24 ± 1 HAA2	T	wheat plant	5.37
03-T-w-S05-A	5376-03-14	48 ± 1 HAA2	T	wheat plant	5.23
03-T-w-S06-A	5376-03-15	4 ± 1 DAA2	T	wheat plant	3.00
03-T-w-S07-A	5376-03-16	6 ± 1 DAA2	T	wheat plant	2.74
03-T-w-S08-A	5376-03-17	8 ± 1 DAA2	T	wheat plant	2.74
03-T-w-S09-A	5376-03-18	16 ± 1 DAA2	T	wheat plant	2.02

C: control sample, T: treated sample, HBA/DBA: hours/days before application, HAA/DAA: hours/days after application, < LOD: below the limit of detection (0.003mg/kg); LOQ: limit of quantification (0.01mg/kg)

The extraction procedure used in this study was according to the QuEChERS method (validated for Zoxamide in study No P 3114 G (RICHTER, S., PTRL Europe 2014)). This method was checked for its extraction efficiency in study AS362 (Hein, W., RLP AgroScience GmbH, Germany, 2014) in pea immature whole plant. Therefore, the extraction efficiency was considered to be proven and no additional assessment of extraction efficiency was performed in the current study.
The recovery data demonstrate that the method permits the determination of residues of Zoxamide in sugar beet leaves and wheat plant matrices with satisfactory accuracy, precision and repeatability according to guideline SANCO/3029/99 rev.4.

Reference:	KCP 9.2.5/04
Report	Appeltauer, A., 2020: Determination of residues of zoxamide on/in typical feed items of herbivorous birds and mammals after two applications of Zoxium 240 SC on sugar beet and wheat in Southern Europe 2017 Gowan Crop Protection Ltd., UK Eurofins GmbH, Germany, Report No. S16-05376, GLP, Not published
Guideline(s):	SANCO/4145/2000 EFSA Guidance on Risk Assessment for Birds and Mammals (2009) SANCO/3029/99 rev. 4 (2000) FOCUS (2014)
Deviations:	No daily precipitation GLP data available. Reason for this deviation was a mistake during study conduct. Amount of rain in rain gauge was only recorded at every visit (not daily). Only non-GLP rain data are available for the report.
GLP:	Yes
Acceptability:	Yes

The objective of this study was to determine the residue decline of zoxamide on/in feed items of herbivorous birds and mammals under representative growing conditions in Southern Europe in the field: In sugar beet green mass (as surrogate dicotyledonae plant representative for the feed item group “non-grass herbs”) and in wheat green mass above soil (as surrogate monocotyledonae plant representative for the feed item group

"grass and cereals"). The residues and degradation kinetics of the active ingredient were investigated after two applications of Zoxium 240 SC (240 g/L zoxamide, SC) at application rates for zoxamide of 180 g a.s./ha with an interval of 7 days (BBCH 15-29 for sugar beet and wheat). The early application timing of zoxamide to both plant groups is representative for the more sensitive phase of wild birds and mammals in nature.

The study consisted of three field trials, S16-05376-01 to -03 and one residue analytical phase, S16-05376-L1. The field parts were carried out in three fields located near Latnitsa (trial -01), Boshulya (trial -02) in Bulgaria and near Alpera (trial -03), in Spain. The field sites covered an area of approx. 255 m² sugar beet and 255 m² wheat (trial -01), approx. 270 m² sugar beet and 270 m² wheat (trial -02) and approx.300 m² sugar beet and 300 m² wheat (trial -03).

Sugar beet plants were planted on 20 Mar 2017 (sugar beet) in trial -01, on 10 April 2017 in trial -02 and on 20 April 2017 in trial -03. Wheat was sown on 10 April 2017 in trial -01, on 10 April 2017 in trial -02 and on 20 April 2017 in trial -03. Samples were taken before each application and up to 16 days after the last application. One sampling before the 1st application served as control.

For the two specimen types, the sampling schedule was as follows: In trial -01, first sampling two days before the 1st application (control), 2 hours before the 2nd application, 2, 24, 48 hours after the 2nd application, and 5, 7, 8 and finally 16 days after the 2nd application. In trial -02, first sampling before the 1st application (control), 1 hour before the 2nd application, 2, 24, 48 hours after the 2nd application, and 4, 7, 8 and finally 16 days after the 2nd application. In trial -03, first sampling one day before the 1st application (control), directly before the 2nd application, 2, 24, 48 hours after the 2nd application, and 4, 6, 8 and finally 15 days after the 2nd application.

The samples were weighed in the field and frozen immediately on dry ice. Retained samples were taken for all matrices. All residues samples were stored in the freezer within 5 hours after collection in the field. The samples were stored and shipped frozen. At the testing sites (including shipment) the samples were stored deep frozen at ≤ -18°C for a maximum of 101 days (sugar beet green mass) and 102 days (wheat green mass) until extraction and residue analysis. Residue analysis took place within 72 hours after extraction.

The method for the determination of zoxamide in sugar beet leaves and wheat green mass was validated according to SANCO Guideline 3029/99 rev. 4 in the analytical phase during the course of this study (EAS study number S16-05375 and CIP Phase ID 17E10095-02-RAVE). Specimens were extracted (in analogy to the QuEChERS multi residue method) with acetonitrile/water, phase separation was done by addition of buffer salt mixture. The final analysis was conducted with highly specific HPLC with MS/MS detection. Recoveries in the fortified samples were within the acceptable range of 70 - 110 %, therefore the stability of the analyte during storage of the final sample extracts is sufficiently proven.

The degradation kinetics of the active ingredient was analysed according to the recommendations of the EFSA Guidance document on Risk Assessment for Birds and Mammals (2009) and the Guidance document on Estimating Persistence and Degradation Kinetics from Environmental Studies on Pesticides in EU Registration (FOCUS 2014). The calculation of the DT50 values and DT90 values as well as the fitting of the kinetic degradation models was done using the computer software KinGU II (version 2.2012). Four different kinetic degradation models were taken into account: single first-order, first order multi-compartment (GUSTAFSON & HOLDEN, 1990), hockey stick (bi-phasic) and double first-order (bi-exponential) kinetics. The operating system was Microsoft Windows 7 Professional.

Materials and methods

Materials

1. Test material	ZOXIUM 240 SC
Lot/batch:	SB 2401
Active substance content:	240 g/ zoxamide (nominal), 232 g/L zoxamide (analysed)
Expiry date:	April 2018

Methods

Experimental conditions

The study comprised three sugar beet and wheat fields, one per trial. All trials were treated with two applications of Zoxium 240 SC at a nominal rate of 180 g zoxamide/ha. Plot specifications (minimum distances and plot size) are given in Table 10 (Appendix A). The field sites were located near Letnitsa (trial -01) and Boshulya (trial -02) in Bulgaria and near Alpera (trial -03) in Spain. The agricultural practices and sugar beet varieties were in accordance with the local farming practices.

Each trial was designed to produce a single sample for each food type at each sampling date (i.e. to provide an assessment of the average residue level as well as to ensure sufficient material was collected for the actual residue analysis). To minimise edge effects from neighbouring fields, sampling was not carried out at the outer 50 cm of the field.
During the study weather data obtained from portable data loggers on the field sites and from weather stations in the vicinity of the field sites including precipitation and air temperature were taken. During application and samplings, the climatic conditions (GLP data) were measured at the field site with a portable thermo-hygrometer, a soil thermometer and a portable anemometer. Additional data for the long-term average were taken from official weather stations (non-GLP data).
No other formulations containing zoxamide were applied during the trial period onto the plot.
Sampling
Samples of different food items for birds and mammals were collected for residue analysis. Two categories of potential bird and mammalian food items were considered:
1. Sugar beet leaves / green mass
2. Wheat leaves / green mass

For each trial 9 samplings per category were carried out. The first sampling took place before the 1st application and was used as control sample.
For the two specimen types, the sampling schedule was as follows: In trial -01, first sampling two days before the 1st application (control), 2 hours before the 2nd application, 2, 24, 48 hours after the 2nd application, and 5, 7, 8 and finally 16 days after the 2nd application. In trial -02, first sampling before the 1st application (control), 1 hour before the 2nd application, 2, 24, 48 hours after the 2nd application, and 4, 7, 8 and finally 16 days after the 2nd application. In trial -03, first sampling one day before the 1st application (control), directly before the 2nd application, 2, 24, 48 hours after the 2nd application, and 4, 6, 8 and finally 15 days after the 2nd application.
The samples were collected randomly on 12 locations per trial. There was at least 50 g plant material taken per field at each sampling occasion. Samplings were done by hand or with scissors. Samples were taken with a minimum distance of 0.5 m to the border of the plot. The samples of all locations of one field were put together to one pooled sample.
The samples were weighed in the field and frozen immediately on dry ice. Retained samples were taken for all matrices. All residues samples were stored in the freezer within 5 hours after collection in the field. The samples were stored and shipped frozen. At the testing sites (including shipment) the samples were stored deep frozen at ≤ -18°C for a maximum of 101 days (sugar beet green mass) and 102 days (wheat green mass) until extraction and residue analysis.
Description of the analytical procedure
The data presented in this report demonstrate that the used method permits the determination of residues of zoxamide in sugar beet leaves (representing the feed item group “non-grass herbs”) and in wheat plants without roots (representing the feed item group “grass and cereals”) with accuracy, precision and repeatability. The method is based on QuEChERS multi-residue method, validated by RICHTER (2014) according to SANCO/825/00 rev. 8.1 for the determination of zoxamide in various crop commodities. This method was validated under the laboratory conditions of CIP for the determination of residues of zoxamide in sugar beet leaves and wheat plants according to guideline SANCO/3029/99 rev. 4. For this purpose, recovery experiments were performed by fortifying control (untreated) specimens.
10 g (± 0.1 g) of sugar beet leaves and wheat plant specimens were weighed into 50 mL singleuse centrifuge tubes. Recovery samples were fortified at this step. 10 mL acetonitrile were added and the samples were homogenised for at least 2 min using a vortex mixer. Thereafter, QuEChERS EN15662 salt-mixture (1 g sodium citrate, 0.5 g sodium hydrogencitrate sequihydrate, 4 g magnesium sulphate, 1 g sodium chloride) was added, thoroughly shaken and mixed again on a vortex mixer for at least 1 min. Residue analysis took place within 72 hours after extraction. The samples were centrifuged at 4000 min-1 for at least 5 minutes. An aliquot of 1 mL of the supernatant was transferred into a tube prepared with 25 mg PSA (primary-secondary amino phase) and 150 mg anhydrous magnesia sulphate and mixed on a Vortex mixer for 1 min. The extract was filtered through a single–use syringe filter (0.45 µm) into an autosampler vial (1.8 mL). 0.5 mL of this solution were transferred into a second vial, 5 µL of acetonitrile + 5 % formic acid were added, the vial capped and thoroughly shaken. 50 µL of this sample extract were then diluted with 950 µL acetonitrile/water (20:80, v/v) plus 0.1 % formic acid. If necessary, these final extracts were diluted further with

final extract of untreated samples to achieve final concentrations falling within the calibrated concentration range of the detection system.
For detailed information on the analytical method validation, please refer to Part B, Section 5.
Calculation of initial concentration (C0) and DT50/DT90 values
The degradation time of zoxamide was calculated, including information about the kinetics of the decay according to the recommendations of the guidance document on estimating persistence and degradation kinetics from environmental studies on pesticides in EU registration (FOCUS 2014).
The calculation of the degradation of zoxamide residues in sugar beet leaves and wheat green mass was done based on the analysed residue data. The degradation kinetics of the active ingredient was analysed according to the recommendations of the EFSA Guidance document on Risk Assessment for Birds and Mammals (2009) and the Guidance document on Estimating Persistence and Degradation Kinetics from Environmental Studies on Pesticides in EU Registration (FOCUS 2014). The calculation of the DT50 values and DT90 values as well as the fitting of the kinetic degradation models was done using the computer software KinGU II (version 2.2012). Four different kinetic degradation models were taken into account: single first-order, first order multi-compartment (GUSTAFSON & HOLDEN, 1990), hockey stick (bi-phasic) and double first-order (bi-exponential) kinetics. The operating system was Microsoft Windows 7 Professional.
For both commodities, the analysed residues after the last application (2nd application), i.e. starting from two or three hours after the last application to 15 to 16 days after the last application were chosen to establish degradation kinetics for the single trials. For the two commodities timings were calculated separately from the end of application until the samples were put on dry ice (i.e. time degradation of residues stops). Times were rounded to full hours.
Results and discussions
Weather conditions
The climatic conditions during trial -01 compared to the long-term average (1961-1990) revealed higher average temperatures for May and June 2017. During the trial period the rainfall recorded at the field site was 121 mm.
The climatic conditions during trial -02 compared to the long-term average (1961-1990) revealed slightly lower average temperatures for May 2017 and higher average temperatures for June 2017. During the trial period the rainfall recorded at the field site was 25 mm.
The climatic conditions during trial -03 compared to the long-term average (2000-2017) revealed higher average temperatures for May and June 2017. During the trial period the rainfall recorded at the field site was 25 mm.
Zoxamide residues
In the control samples, taken directly before the 1st application, concentrations analysed were below the LOD (0.003 mg/kg) in all trials.
Zoxamide in/on sugar beet leaves
Zoxamide concentrations in sugar beet leaves of the three trials shortly before the 2nd application (0-2 HBA2) were 0.47 mg/kg (trial -01), 1.85 mg/kg (trial -02), and 0.77 mg/kg (trial 03). The highest concentrations were analysed two hours after the 2nd application in trial -01 and 24 hours after the 2nd application in trial -02 and -03 (trial -01: 9.48 mg/kg; trial -02: 11.1 mg/kg, trial -03: 7.55 mg/kg). At the last sampling 15/16DAA2 concentrations for the field trials were 0.08 mg/kg (trial -01), 0.69 mg/kg (trial -02) and 0.76 mg/kg (trial -03). In trial -01, analysed zoxamide concentrations increased to a peak of 9.48 mg/kg at 2HAA2 and decreased subsequently until the last sampling (16DAA2) when 0.08 mg/kg were measured. In trial -02 the highest concentrations of all trials were analysed for zoxamide. Highest concentrations were measured 24 hours after the 2nd application with 11.1 mg/kg. Concentrations decreased in the following samplings to 0.69 mg/kg at 16DAA2. In trial -03, analysed zoxamide concentrations increased to a peak of 7.55 mg/kg at 24HAA2 and decreased subsequently until the last sampling (15DAA2) when 0.76 mg/kg were measured A summary of the residue levels found in sugar beet leaves samples is shown in the following table.
Table A 2-13: Zoxamide residues in/on sugar beet leaves (individual values of all field sites)

Timing	Trial		
(trial -01/-02/-03)	-01	-02	-03

	[mg/kg]	[mg/kg]	[mg/kg]
2/0/1DBA1	<LOD	<LOD	<LOD
2/1/0HBA2	0.47	1.85	0.77
2/3/3HAA2	9.48	10.2	6.53
24/24/24HAA2	9.11	11.1	7.55
48/49/48HAA2	9.08	10.2	4.83
5/4/4DAA2	1.28	7.53	1.97
7/7/6DAA2	1.12	4.31	1.57
8/8/8DAA2	0.78	3.14	1.49
16/16/15DAA2	0.08	0.69	0.76

DBA: days before application, DAA: days after application,
HAA: hours after application, HBA: hours before application,
LOD: level of detection (0.003 mg/kg)

Zoxamide in/on wheat green mass

In the control samples, taken directly before the 1st application, concentrations analysed were below the LOD (0.003 mg/kg) in all trials. zoxamide concentrations in wheat green mass shortly before the 2nd application (0-2 HBA2) were 0.52 mg/kg (trial -01), 1.42 mg/kg (trial -02) and 1.76 mg/kg (trial -03). The highest concentrations were analysed two hours after the 2nd application in trial -01, -02 and -03 (trial -01: 7.97 mg/kg, trial -02: 7.66 mg/kg, trial -03: 5.83 mg/kg). At the last sampling 15/16DAA2 concentrations for the field trials were 0.02 mg/kg (trial -01), 0.51 mg/kg (trial -02) and 2.02 mg/kg (trial -03). In trial -01, analysed zoxamide concentrations in wheat green mass, increased to 7.97 mg/kg two hours after the 2nd application (2HAA2). In subsequent samplings concentrations decreased to 0.02 mg/kg at 16DAA2. In trial -02 concentrations of zoxamide in wheat green mass increased to 7.66 mg/kg two hours after the 2nd application (2HAA2). At the following two samplings (24 and 48HAA2) concentrations decreased to 5.44 mg/kg followed by a slight increase to 5.59 mg/kg at 4DAA2. In sampling 7DAA2 concentrations decreased to 2.42 mg/kg again followed by an increase to 4.12 mg/kg. At the last sampling 16DAA2 concentrations decreased to 0.51 mg/kg. In trial -03 the highest concentration was analysed at 2HAA2 with 5.83 mg/kg. Afterwards concentrations decreased to 2.02 mg/kg at 15DAA2. A summary of the residue levels found in cereal green mass samples is shown in the following table.

Table A 2-14: Zoxamide residues in/on wheat green mass (individual values of all field sites)

Timing (trial -01/-02/-03)	Trial		
	-01	-02	-03
	[mg/kg]	[mg/kg]	[mg/kg]
2/0/1DBA1	<LOD	<LOD	<LOD
2/1/0HBA2	0.52	1.42	1.76
2/3/3HAA2	7.97	7.66	5.83
24/24/24HAA2	5.56	5.81	5.37
48/49/48HAA2	5.27	5.44	5.23
5/4/4DAA2	1.60	5.59	3.00
7/7/6DAA2	1.15	2.42	2.74
8/8/8DAA2	0.39	4.12	2.74
16/16/15DAA2	0.02	0.51	2.02

DBA: days before application, DAA: days after application,
HAA: hours after application, HBA: hours before application,
LOD: level of detection (0.003 mg/kg)

Calculation of initial concentration (C0) DT50/DT90 values

Sugar Beet Leaves

In trial -02 the calculation of degradation rates for zoxamide in sugar beet leaves achieved critical values < 15 for χ^2-error for all models. In trials -01 and -03 none of the models achieved the critical values < 15 for χ^2-error. The determination coefficient of r2 > 0.85 was achieved for all trials and for all models. In trial -01 the visual fit of the DFOP model was better than for the SFO model. However, the confidence intervals of the DFOP model showed negative values and p(k2) was not statistically significant. Therefore, the DFOP model was not appropriate for the residue data. In trial -02 the HS model showed lowest χ^2-error and the

highest r2 with 0.99. But the HS model was not appropriate because the confidence intervals revealed negative values and p(k1) was not statistically significant (> 0.05). In trial -03 all three biphasic models (FOMC, DFOP and HS) were not appropriate because of negative values within the confidence intervals. Therefore, for all three trials the SFO model was used for calculation of degradation rates of zoxamide in sugar beet leaves. The results of the calculation showed DT50 values between 2.60 and 5.02 days and DT90 values between 8.64 and 16.67 days, respectively for the degradation of zoxamide in sugar beet leaves. The calculated χ^2-errors for the three trials were 23.80, 10.74 and 18.22 for trials -01, -02 and -03, respectively. The calculated r^2 for the three trials were 0.90, 0.94 and 0.90 (SFO) for trials -01, -02 and -03, respectively.

Table A 2-15: Results and parameters of the kinetic degradation of zoxamide in/on sugar beet leaves (Trial S16-05376-01)

Model	SFO	FOMC	DFOP	HS
DT50 [days]	2.60	1.70	2.60	2.60
DT90 [days]	8.64	5.64	8.64	8.64
CHI2-err [%]	23.80	33.21	28.33	28.33
r2	0.90	0.85	0.90	0.90
parameter	k = 0.26655	α = 1133.445	k1 = 0.26655	k1 = 0.26655
	M(0) = 11.11148	β = 2771.880	k2 = 0.07474	k2 = 0.15911
		M(0) = 12.227	g = 1.00000	tb = 16.04790
			M(0) = 11.11147	M(0) = 11.11147
lower Cl	k = 0.14017	α = -26834.081	k1 = 0.08845	k1 = 0.10511
	M(0) = 8.51574	β = -65653.126	k2 = -1.57811	k2 = 0.04712
		M(0) = 8.301	g = -0.72896	tb = -18.42802
			M(0) = 5.96783	M(0) = 7.98092
upper Cl	k = 0.393	α = 29100.97	k1 = 0.445	k1 = 0.428
	M(0) = 13.707	β = 71196.89	k2 = 1.728	k2 = 0.271
		M(0) = 16.15	g = 2.729	tb = 50.524
			M(0) = 16.255	M(0) = 14.242
t-test	p(k): 0.004525	-	p(k1): 0.0304	p(k1): 0.02400
			p(k2): 0.4675	p(k2): 0.03436

SFO = Single first order kinetic;
FOMC = First order multi compartment kinetic;
DFOP = Double first order kinetic;
HS = Hockey stick kinetic
M(0) = initial concentration
CI = confidence interval
t = time after time of initial concentration
k = rate constant (ln(2)/DT50)
α = 3.4735*N^-0.8629
β = DT50/(2^(1/ α)-1)
N = DT90/DT50-3.32
DT50 = estimated from the data
DT90 = estimated from the data
k1 = rate constant for the fast degradation phase (ln(2)/DT50f)
k2 = rate constant for the slow degradation phase (ln(2)/DT50s)
tb = break point time estimated from the data rate
g = 1-F
F = estimated residual fraction at the time when the fast degradation phase changes to the slow degradation phase

Table A 2-16: Results and parameters of the kinetic degradation of zoxamide in/on sugar beet leaves (Trial S16-05376-02)

Model	SFO	FOMC	DFOP	HS
DT50 [days]	5.02	5.49	5.02	5.65
DT90 [days]	16.67	18.25	16.67	14.38
CHI2-err [%]	10.74	11.98	12.79	4.32
r2	0.94	0.95	0.94	0.99
parameter	k = 0.13813	α = 1966	k1 = 0.13813	k1 = 6.915e-09
	M(0) = 11.92396	β = 15580	k2 = 0.06732	k2 = 0.1844
		M(0) = 11.64	g = 1.00000	tb = 1.893
			M(0) = 11.92394	M(0) = 10.65
lower Cl	k = 0.09551	α = -1008	k1 = -0.23822	k1 = -0.4135
	M(0) = 10.35633	β = -7987	k2 = -0.88271	k2 = 0.1522
		M(0) = 10.15	g = -2.07191	tb = -1.144
			M(0) = 9.48538	M(0) = 8.135
upper Cl	k = 0.181	α = 14008.41	k1 = 0.514	k1 = 0.413
	M(0) = 13.492	β = 111023.55	k2 = 1.017	k2 = 0.217
		M(0) = 13.12	g = 4.072	tb = 4.930
			M(0) = 14.363	M(0) = 13.165
t-test	p(k): 0.000714	-	p(k1): 0.26195	p(k1): 0.500
			p(k2): 0.44917	p(k2): 0.000758

SFO = Single first order kinetic;
FOMC = First order multi compartment kinetic;
DFOP = Double first order kinetic;
HS = Hockey stick kinetic
M(0) = initial concentration
CI = confidence interval
t = time after time of initial concentration
k = rate constant (ln(2)/DT50)
α = 3.4735*N^-0.8629
β = DT50/(2^(1/ α)-1)
N = DT90/DT50-3.32
DT50 = estimated from the data
DT90 = estimated from the data
k1 = rate constant for the fast degradation phase (ln(2)/DT50f)
k2 = rate constant for the slow degradation phase (ln(2)/DT50s)
tb = break point time estimated from the data rate
g = 1-F
F = estimated residual fraction at the time when the fast degradation phase changes to the slow degradation phase

Table A 2-17: Results and parameters of the kinetic degradation of zoxamide in/on sugar beet leaves (Trial S16-05376-03)

Model	SFO	FOMC	DFOP	HS
DT50 [days]	2.89	2.87	2.74	2.72
DT90 [days]	9.60	9.55	11.19	14.91
CHI2-err [%]	18.22	19.68	21.24	20.45
r2	0.90	0.90	0.90	0.91
parameter	k = 0.23973	α = 282.0	k1 = 0.2755	k1 = 0.25437
	M(0) = 7.66682	β = 1165	k2 = 7.951e-08	k2 = 0.08713
		M(0) = 7.684	g = 0.9432	tb = 6.00000

			M(0) = 7.763	M(0) = 7.76221
lower Cl	k = 0.13219	α = -6331	k1 = -0.06204	k1 = 0.11551
	M(0) = 6.04061	β = -26250	k2 = -1.775	k2 = -0.35909
		M(0) = 6.173	g = -0.2244	tb = -7.43117
			M(0) = 5.608	M(0) = 5.71589
upper Cl	k = 0.347	α = 6895.135	k1 = 0.613	k1 = 0.393
	M(0) = 9.293	β = 28582.483	k2 = 1.775	k2 = 0.533
		M(0) = 9.196	g = 2.111	tb = 19.431
			M(0) = 9.918	M(0) = 9.809
t-test	p(k): 0.003614	-	p(k1): 0.10398	p(k1): 0.01851
			p(k2): 0.500	p(k2): 0.36372

SFO = Single first order kinetic;
FOMC = First order multi compartment kinetic;
DFOP = Double first order kinetic;
HS = Hockey stick kinetic
M(0) = initial concentration
CI = confidence interval
t = time after time of initial concentration
k = rate constant (ln(2)/DT50)
α = 3.4735*N^-0.8629
β = DT50/(2^(1/ α)-1)
N = DT90/DT50-3.32
DT50 = estimated from the data
DT90 = estimated from the data
k1 = rate constant for the fast degradation phase (ln(2)/DT50f)
k2 = rate constant for the slow degradation phase (ln(2)/DT50s)
tb = break point time estimated from the data rate
g = 1-F
F = estimated residual fraction at the time when the fast degradation phase changes to the slow degradation phase

Wheat Green Mass

In trials -01 to -03 the calculation of degradation rates for zoxamide in wheat green mass showed critical values < 15 for χ^2-error for the SFO model. For all models the determination coefficient of r2 was higher than 0.85. As the t-test of the SFO model for all trials was statistically significant (p(k)<0.05) the SFO model was used to calculate the degradation rates of all trials. The results of the calculation showed DT50 values between 2.35 and 6.94 days and DT90 values between 7.80 and 23.04 days, respectively for the degradation of zoxamide in wheat green mass. The calculated χ^2-errors for the three trials were 9.83, 13.91 and 10.44 for trials -01, -02 and -03, respectively. The calculated r^2 for the three trials were 0.98, 0.87 and 0.88 for trials -01, -02 and -03, respectively.

Table A 2-18: Results and parameters of the kinetic degradation of zoxamide in/on wheat green mass (Trial S16-05376-01)

Model	SFO	FOMC	DFOP	HS
DT50 [days]	2.35	2.15	2.35	2.61
DT90 [days]	7.80	7.15	7.80	7.19
CHI2-err [%]	9.83	11.31	11.70	9.98
r2	0.98	0.98	0.98	0.99
parameter	k = 0.29527	α = 2926.7619	k1 = 0.29527	k1 = 0.23865
	M(0) = 8.13430	β = 9079.6642	k2 = 0.08440	k2 = 0.35174
		M(0) = 8.3194	g = 1.00000	tb = 2.00000
			M(0) = 8.13432	M(0) = 7.85397
lower Cl	k = 0.23309	α = -2435.3737	k1 = 0.10916	k1 = 0.11678
	M(0) = 7.37933	β = -7562.7069	k2 = 0.06279	k2 = 0.01787

		M(0) = 7.4606	g = 0.52738	tb = -10.41645
			M(0) = 7.01281	M(0) = 6.88518
upper Cl	k = 0.357	α = 8288.898	k1 = 0.481	k1 = 0.361
	M(0) = 8.889	β = 25722.035	k2 = 0.106	k2 = 0.686
		M(0) = 9.178	g = 1.473	tb = 14.416
			M(0) = 9.256	M(0) = 8.823
t-test	p(k): 0.000121	-	p(k1): 0.026449	p(k1): 0.015594
			p(k2): 0.002317	p(k2): 0.065443

SFO = Single first order kinetic;
FOMC = First order multi compartment kinetic;
DFOP = Double first order kinetic;
HS = Hockey stick kinetic
M(0) = initial concentration
CI = confidence interval
t = time after time of initial concentration
k = rate constant (ln(2)/DT50)
α = 3.4735*N^-0.8629
β = DT50/(2^(1/ α)-1)
N = DT90/DT50-3.32
DT50 = estimated from the data
DT90 = estimated from the data
k1 = rate constant for the fast degradation phase (ln(2)/DT50f)
k2 = rate constant for the slow degradation phase (ln(2)/DT50s)
tb = break point time estimated from the data rate
g = 1-F
F = estimated residual fraction at the time when the fast degradation phase changes to the slow degradation phase

Table A 2-19: Results and parameters of the kinetic degradation of zoxamide in/on wheat green mass (Trial S16-05376-02)

Model	SFO	FOMC	DFOP	HS
DT50 [days]	6.19	5.39	6.19	6.68
DT90 [days]	20.57	17.93	20.57	17.98
CHI2-err [%]	13.91	15.59	16.56	15.84
r2	0.87	0.87	0.87	0.88
parameter	k = 0.11194	α = 620.7	k1 = 0.11195	k1 = 0.08008
	M(0) = 7.31253	β = 4823	k2 = 0.11193	k2 = 0.14244
		M(0) = 7.564	g = 0.70471	tb = 4.14381
			M(0) = 7.31256	M(0) = 7.01999
lower Cl	k = 0.06184	α = -12880	k1 = -0.02011	k1 = -0.06658
	M(0) = 6.01954	β = -100100	k2 = -0.23785	k2 = -0.07974
		M(0) = 6.276	g = -1.79763	tb = -11.61031
			M(0) = 5.69192	M(0) = 5.07482
upper Cl	k = 0.162	α = 14120	k1 = 0.244	k1 = 0.227
	M(0) = 8.606	β = 109800	k2 = 0.462	k2 = 0.365
		M(0) = 8.852	g = 3.207	tb = 19.898
			M(0) = 8.933	M(0) = 8.965
t-test	p(k): 0.00358	-	p(k1): 0.09761	p(k1): 0.18150
			p(k2): 0.28750	p(k2): 0.14893

SFO = Single first order kinetic;
FOMC = First order multi compartment kinetic;
DFOP = Double first order kinetic;
HS = Hockey stick kinetic

M(0) = initial concentration
CI = confidence interval
t = time after time of initial concentration
k = rate constant (ln(2)/DT50)
α = 3.4735*N^-0.8629
β = DT50/(2^(1/ α)-1)
N = DT90/DT50-3.32
DT50 = estimated from the data
DT90 = estimated from the data
k1 = rate constant for the fast degradation phase (ln(2)/DT50f)
k2 = rate constant for the slow degradation phase (ln(2)/DT50s)
tb = break point time estimated from the data rate
g = 1-F
F = estimated residual fraction at the time when the fast degradation phase changes to the slow degradation phase

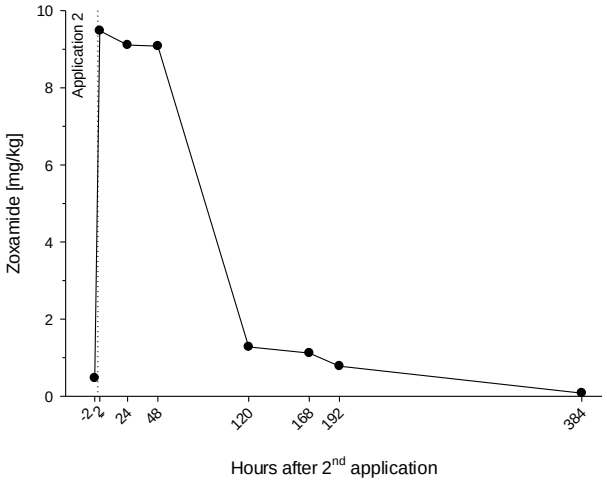
Table A 2-20: Results and parameters of the kinetic degradation of zoxamide in/on wheat green mass (Trial S16-05376-03)

Model	SFO	FOMC	DFOP	HS
DT50 [days]	6.94	5.73	5.32	6.99
DT90 [days]	23.04	62.53	n.a.	23.09
CHI2-err [%]	10.44	8.76	8.76	12.43
r2	0.88	0.93	0.94	0.88
parameter	k = 0.09993	α = 0.8608	k1 = 0.2349	k1 = 0.02609
	M(0) = 5.79592	β = 4.6269	k2 = 1.179e-07	k2 = 0.09993
		M(0) = 6.1990	g = 0.7008	tb = 0.07029
			M(0) = 6.205	M(0) = 5.76592
lower Cl	k = 0.05761	α = -0.3385	k1 = -0.1460	k1 = -0.06720
	M(0) = 4.98950	β = -5.7636	k2 = -0.2139	k2 = 0.04430
		M(0) = 5.1972	g = -0.3177	tb = -2.44704
			M(0) = 5.255	M(0) = 4.30653
upper Cl	k = 0.142	α = 2.060	k1 = 0.616	k1 = 0.119
	M(0) = 6.602	β = 15.018	k2 = 0.214	k2 = 0.156
		M(0) = 7.201	g = 1.719	tb = 2.588
			M(0) = 7.155	M(0) = 7.225
t-test	p(k): 0.00285	-	p(k1): 0.156697	p(k1): 0.31088
			p(k2): 0.500	p(k2): 0.01945

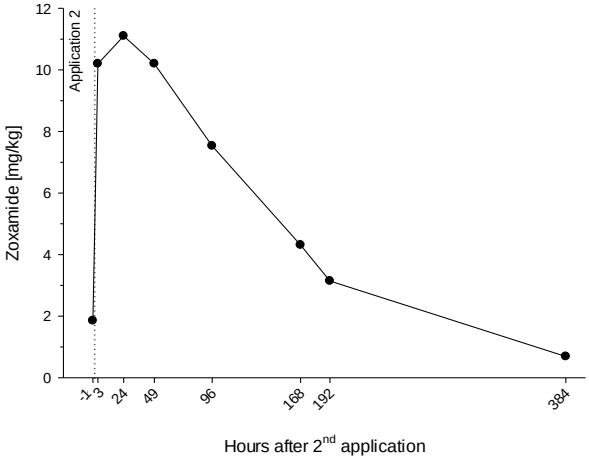
SFO = Single first order kinetic;
FOMC = First order multi compartment kinetic;
DFOP = Double first order kinetic;
HS = Hockey stick kinetic
M(0) = initial concentration
CI = confidence interval
t = time after time of initial concentration
k = rate constant (ln(2)/DT50)
α = 3.4735*N^-0.8629
β = DT50/(2^(1/ α)-1)
N = DT90/DT50-3.32
DT50 = estimated from the data
DT90 = estimated from the data
k1 = rate constant for the fast degradation phase (ln(2)/DT50f)
k2 = rate constant for the slow degradation phase (ln(2)/DT50s)
tb = break point time estimated from the data rate

g = 1-F
F = estimated residual fraction at the time when the fast degradation phase changes to the slow degradation phase
The following figures show residues of zoxamide.

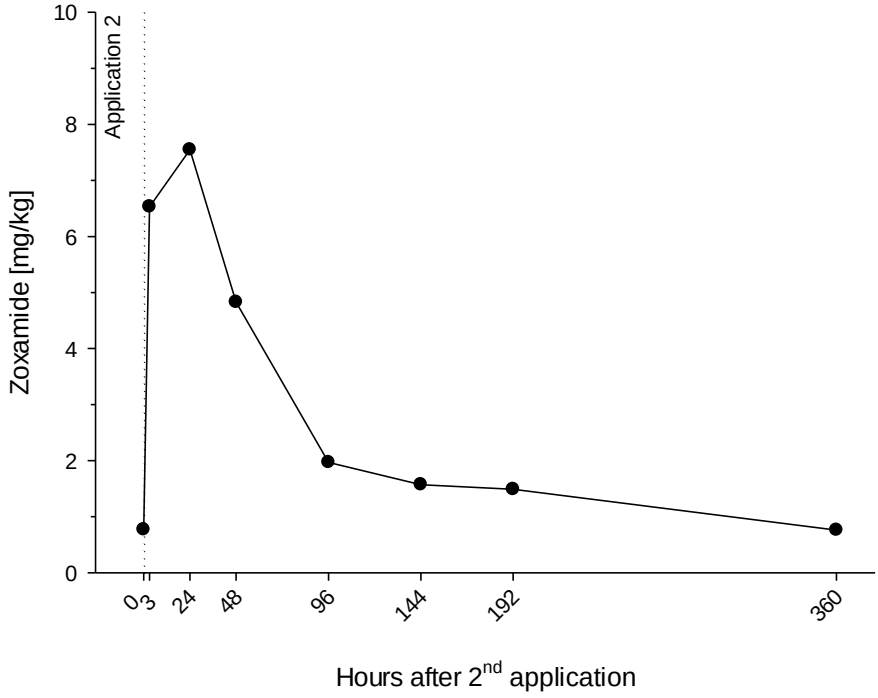
Sugar beet leaves



Residues of zoxamide in sugar beet leaves over time (trial 01)

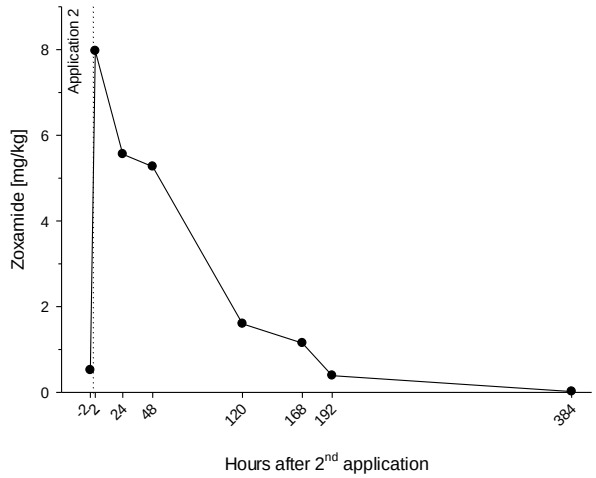


Residues of zoxamide in sugar beet leaves over time (trial 02)

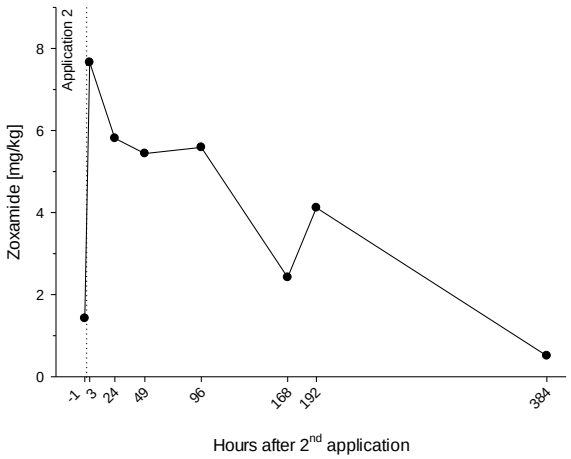


Residues of zoxamide in sugar beet leaves over time (trial 03)

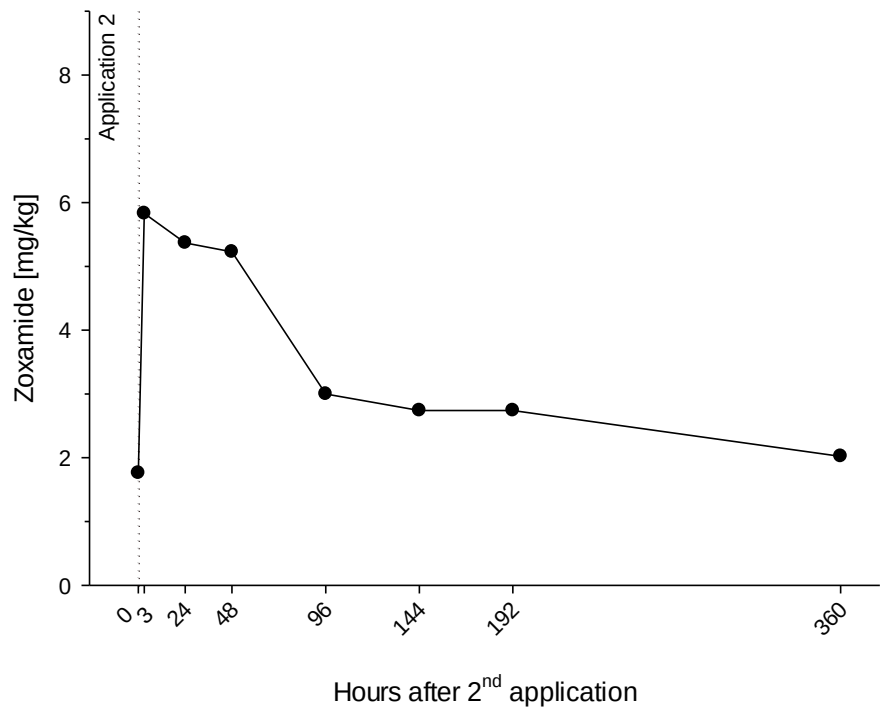
Wheat



Residues of zoxamide in wheat green mass over time (trial 01)



Residues of zoxamide in wheat green mass over time (trial 02)



Residues of zoxamide in wheat green mass over time (trial 03)

Conclusion

The residue decline of zoxamide on sugar beet leaves (as surrogate dicotyledonae plant) and wheat green mass (as surrogate monocotyledonae plant) has been studied in the field under representative growing conditions for Southern Europe. The residues and degradation kinetics of the active ingredient were investigated after two applications of Zoxium 240 SC (240 g/L zoxamide, SC) at application rates for zoxamide of 180 g a.s./ha with an interval of 7 days (BBCH 15-29 for sugar beet and wheat).

The resulting SFO DT50 values for all three trials on sugar beet performed under southern European growing conditions were calculated between 2.60 and 5.02 days and DT90 values were calculated between

8.64 and 16.67 days. For wheat green mass the single first order (SFO) DT50 values were calculated between 2.35 and 6.94 days and DT90 values were calculated between 7.80 and 23.04 days.
The study was conducted in accordance to current guidelines.

(Appeltauer A. 2020)

Study 10 - Residue degradation of zoxamide in mono- and dicotyledonae plants under southern European growing conditions

Comments of zRMS:

The study is acceptable.

The data presented in this report demonstrate that the used method permits the determination of residues of Zoxamide in In sugar beet leaves (representing the feed item group "non-grass herbs") and in wheat plants without roots (representing the feed item group "grass and cereals") (without roots) with accuracy, precision and repeatability. The method is based on QuEChERS multi-residue method, validated by RICHTER (2014) according to SANCO/825/00 rev. 8.1 for the determination of Zoxamide in various crop commodities. This method was validated under the laboratory conditions of CIP for the determination of residues of Zoxamide in sugar beet leaves and wheat green masss according to guideline SANCO/3029/99 rev. 4. For this purpose, recovery experiments were performed by fortifying control (untreated) specimens. The analytical method is characterised as follows:
Extraction with acetonitrile/water, phase separation by addition of buffer salt mixture; Clean-up: Dispersive SPE clean-up, followed by filtration through a 0.45 µm single-use syringe filter, and dilution with acidified acetonitrile/water; Final analysis: HPLC with MS/MS detection with LOQ is 0.01 mg/kg; LOD is defined as 30% of the LOQ (i.e. 0.003 mg/kg); Precision was already tested in former study (17E10095-01-RAVE). The calibration graphs for Zoxamide are linear within the range of 0.1 µg/L to 250 µg/L with determination coefficients of r2 ≥ 0.9999 (matrix-matched standard solutions). Specificity: Typical retention time with no significant interferences; Zoxamide parent compound detected with two ion mass transitions (SRM 336 —> 187 for quantification, SRM 338 —> 189 for confirmation). Analysis of control samples used for recoveries with HPLC-MS/MS yielded no residues of Zoxamide above 30% of the LOQ, indicating that no interferences were present.
Procedural recoveries were analysed at the LOQ of 0.01 mg/kg and at a higher level of 50 mg/kg (sugar beet leaves) or 100 mg/kg (wheat green mass) to cover the estimated residues found in field samples.

The following recoveries were obtained with HPLC-MS/MS for Zoxamide:

Matrix	Fortification level [mg/kg]	Recoveries Single values [%]	No of analyses
Zoxamide SRM 336 → 187 (quantification)			
Sugar beet leaves	0.01	104	1
	50	93	1
Zoxamide SRM 338 → 189 (confirmation)			
Sugar beet leaves	0.01	96	1
	50	95	1
Zoxamide SRM 336 → 187 (quantification)			
Wheat green mass	0.01	110	1
	100	92	1
Zoxamide SRM 338 → 189 (confirmation)			
Wheat green mass	0.01	104	1
	100	92	1

SRM: Single Reaction Monitoring

All recoveries obtained by HPLC-MS/MS for Zoxamide at all fortification levels comply with the standard acceptance criteria of SANCO/3029/99 rev.4, which demands that the mean recovery at each fortification level should be in the range of 70 — 110%.

In the sugar beet leaves samples, residues of Zoxamide were at maximum of 11.4 mg/kg and declined to 2.82 mg/kg within 16 days after the last application of Zoxium 240 SC. In the wheat green mass samples, residues of Zoxamide were at maximum 9.58 mg/kg and declined to 0.941 mg/kg within 16 days after the last application. Residues of Zoxamide in sugar beet leaves and wheat green mass were below the limit of detection (< 0.003 mg/kg) in all control (untreated) samples.

The residues are summarised in the following table:

Sample No. L19-23773-01-	Laboratory sample No.	Timing	Treatment	Matrix	Residue Zoxamide [mg/kg]
C-sbl-S01-A	23773-01	0 - 1 DBA1	C	Sugar beet leaves	n.d.
T-sbl-S02-A	23773-02	1HBA2	T	Sugar beet leaves	2.04
T-sbl-S03-A	23773-03	3 ± 1 HAA2	T	Sugar beet leaves	11.4
T-sbl-S04-A	23773-04	24 ± 1 HAA2	T	Sugar beet leaves	8.24
T-sbl-S05-A	23773-05	48 ± 1 HAA2	T	Sugar beet leaves	6.93
T-sbl-S06-A	23773-06	4 ± 1 DAA2	T	Sugar beet leaves	7.27
T-sbl-S07-A	23773-07	6 ± 1 DAA2	T	Sugar beet leaves	5.65
T-sbl-S08-A	23773-08	8 ± 1 DAA2	T	Sugar beet leaves	3.27
T-sbl-S09-A	23773-09	16 ± 1 DAA2	T	Sugar beet leaves	2.82
C-w-S01-A	23773-10	0 - 1 DBA1	C	Wheat green mass	n.d.
T-w-S02-A	23773-11	1HBA2	T	Wheat green mass	1.92
T-w-S03-A	23773-12	3 ± 1 HAA2	T	Wheat green mass	9.58
T-w-S04-A	23773-13	24 ± 1 HAA2	T	Wheat green mass	7.97
T-w-S05-A	23773-14	48 ± 1 HAA2	T	Wheat green mass	6.98
T-w-S06-A	23773-15	4 ± 1 DAA2	T	Wheat green mass	4.64
T-w-S07-A	23773-16	6 ± 1 DAA2	T	Wheat green mass	4.06
T-w-S08-A	23773-17	8 ± 1 DAA2	T	Wheat green mass	2.30
T-w-S09-A	23773-18	16 ± 1 DAA2	T	Wheat green mass	0.941

C: control (untreated) sample, T: treated sample, HBA/DBA: hours/days before application, HAAIDAA: hours/days after application; n.d: not detectable (below the limit of detection (0.003 mg/kg)); LOQ: limit of quantification (0.01 mg/kg)

The extraction procedure used in this study was according to the QuEChERS method (validated for Zoxamide in study No P 3114 G (RICHTER, S., PTRL Europe 2014)). This method was checked for its extraction efficiency in study AS362 (Hein, W., RLP AgroScience GmbH, Germany, 2014) in pea immature whole plant. Therefore, the extraction efficiency was considered to be proven and no additional assessment of extraction efficiency was performed in the current study.

Reference:	KCP 9.2.5/05
Report	Appeltauer, A., 2020: Determination of Residues of zoxamide on/in Typical Feed Items of Herbivorous Birds and Mammals after Two Applications of the test item on Sugar Beet and Wheat in Italy in 2020 Gowan Crop Protection Ltd., UK Eurofins GmbH, Germany, Report No. S19-23773, GLP, Not published
Guideline(s):	SANCO/4145/2000 EFSA Guidance on Risk Assessment for Birds and Mammals (2009) SANCO/3029/99 rev. 4 SANCO/825/00 rev. 8.1

	EFSA Technical Report (2019): Outcome of the pesticides peer review meeting on general recurring issues in physical and chemical properties and analytical methods. FOCUS (2014)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

The objective of this study was to determine the residue decline of zoxamide on/in feed items of herbivorous birds and mammals under representative growing conditions in Italy in the field: In sugar beet leaves (as surrogate dicotyledonae, representative for the feed item group "non-grass herbs") and in wheat green mass above soil (as surrogate monocotyledonae, representative for the feed item group "grass and cereals"). The residues and degradation kinetics of the active ingredient were investigated after two applications of Zoxium 240 SC (240 g/L zoxamide, SC) at application rates for zoxamide of 180 g a.i./ha with an interval of 7 days (BBCH 17-21 for sugar beet and wheat).
The study consisted of one field trial, S19-23773-01 and one residue analysis trial, S19-23773-L1. The field part was carried out on a field located near Mezzolara, Bologna, Italy. The field site of the trial covered an area of 540 m² sugar beet and 540 m² summer wheat. Sugar beet plants were planted on 11 Mar 2020; wheat was sown on 24 Feb 2020.
For the two specimen types, the sampling schedule was as follows: the first sampling before the 1st application (control), 1 hour before 2nd application, 3, 24, 48 hours after the 2nd application, and 4, 7, 9 and finally 15 days after the 2nd application. One sampling before the 1st application served as control.
All residues samples were stored in the freezer within 3 hours after collection in the field. The samples were stored and shipped frozen. The maximum storage time of samples was 30 days for sugar beet leaves and 31 days for wheat green mass (control samples: 37 days for sugar beet leaves and 38 days for wheat green mass) until extraction and residue analysis. Residue analysis took place within 1 day after extraction. Retained samples were taken for all matrices.
The residues of the active ingredients on/in sugar beet leaves and wheat green mass were analysed with fully validated analytical methods according to SANCO/3029/99 rev. 4. The method has previously been validated in a study of Witte A. (CIP Phase ID 17E10095-01-RAVE, analytical part of EAS study S16-05375) at an LOQ of 0.01 mg/kg for the matrices under investigation. It takes into account additional requirements of SANCO/825/00 rev. 8.1 and EFSA Technical Report (2019): Outcome of the pesticides peer review meeting on general recurring issues in physical and chemical properties and analytical methods. Specimens were extracted (in analogy to the QuEChERS multi residue method) with acetonitrile/water, phase separation was done by addition of buffer salt mixture. The final analysis was conducted with highly specific HPLC with MS/MS detection. Recoveries in the fortified samples were within the acceptable range of 70 - 110 %, therefore the stability of the analyte during storage of the final sample extracts is sufficiently proven.
The degradation kinetics of the active ingredient was analysed according to the recommendations of the EFSA Guidance document on Risk Assessment for Birds and Mammals (2009) and the Guidance document on Estimating Persistence and Degradation Kinetics from Environmental Studies on Pesticides in EU Registration (FOCUS 2014). The calculation of the DT50 values and DT90 values as well as the fitting of the kinetic degradation models was done using the computer software KinGU II (version 2.2012). Four different kinetic degradation models were taken into account: single first-order, first order multi-compartment (GUSTAFSON & HOLDEN, 1990), hockey stick (bi-phasic) and double first-order (bi-exponential) kinetics. The operating system was Microsoft Windows 10 Professional.
Materials and methods
Materials

1. Test material	ZOXIUM 240 SC
Lot/batch:	18011201-72-52
Active substance content:	21.8% w/w (240 g/ zoxamide nominal), 21.49% zoxamide (analysed)
Expiry date:	January 2022

Methods
Experimental conditions
The study comprised one sugar beet and wheat field, trial S19-23773-01. The trial was treated with two applications of Zoxium 240 SC at a nominal rate of 180 g zoxamide/ha. Plot specifications (minimum distances to the edge of the field and plot size) are given in Table 6 (Appendix A). The field site was located near Mezzolara, Bologna, in Italy. The agricultural practices and sugar beet / wheat varieties were in accordance with the local farming practice.
The trial was designed to produce a single sample for each food type at each sampling date (i.e. to provide an assessment of the average residue level as well as to ensure that sufficient material was collected for the actual residue analysis). To minimise edge effects from neighbouring fields, sampling was not carried out at the outer 50 cm of the plot.

During the study, weather data obtained from weather equipment placed on the field site including precipitation and air temperature was taken (GLP data). During applications and samplings, the climatic conditions (GLP data) were measured at the field site with a portable thermo-hygrometer, a soil thermometer and a portable anemometer.
No other formulations containing zoxamide were applied during the trial period onto the plot.
Sampling
Samples of different food items for birds and mammals were collected for residue analysis. Two categories of potential bird and mammalian food items were considered:
1. Sugar beet leaves
2. Wheat
For each trial 9 samplings per category were carried out. The first sampling took place before the 1st application and was used as control sample.
For the two specimen types, the sampling schedule was as follows: the first sampling before the 1st application (control), 1 hour before 2nd application, 3, 24, 48 hours after the 2nd application, and 4, 7, 9 and finally 15 days after the 2nd application. One sampling before the 1st application served as control.
The specimens were sampled randomly on at least 12 locations per sample. There were at least 500 g plant material taken at each sampling occasion. Samplings were done by hand or with a knife. Samples were taken with a minimum distance of 0.5 m to the border of the plot. The samples of all locations of one field were put together to one pooled sample per sampling occasion.
All residues samples were weighed and stored in the freezer within 3 hours after collection in the field. The samples were stored and shipped frozen until extraction and residue analysis. Retained samples were taken for all matrices.
Description of the analytical procedure
The data presented in this report demonstrate that the used method permits the determination of residues of zoxamide in in sugar beet leaves and in wheat plants with accuracy, precision and repeatability. The method is based on QuEChERS multi-residue method, validated by RICHTER (2014) according to SANCO/825/00 rev. 8.1 for the determination of zoxamide in various crop commodities. This method was validated under the laboratory conditions of CIP for the determination of residues of zoxamide in sugar beet leaves and wheat green mass according to guideline SANCO/3029/99 rev. 4. For this purpose, recovery experiments were performed by fortifying control (untreated) specimens.
10 g (± 0.1 g) of sugar beet leaves and wheat plant specimens were weighed into 50 mL singleuse centrifuge tubes. Recovery samples were fortified at this step. 10 mL acetonitrile were added and the samples were homogenised for at least 2 min using a vortex mixer. Thereafter, QuEChERS EN15662 salt-mixture (1 g sodium citrate, 0.5 g sodium hydrogencitrate sequihydrate, 4 g magnesium sulphate, 1 g sodium chloride) was added, thoroughly shaken and mixed again on a vortex mixer for at least 1 min. The samples were centrifuged at 4000 min-1 for at least 5 minutes. An aliquot of 1 mL of the supernatant was transferred into a tube prepared with 25 mg PSA (primary-secondary amino phase) and 150 mg anhydrous magnesium sulphate and mixed on a Vortex mixer for 1 min. The extract was filtered through a single–use syringe filter (0.45 µm) into an autosampler vial (1.8 mL). 0.5 mL of this solution were transferred into a second vial, 5 µL of acetonitrile + 5 % formic acid were added, the vial capped and thoroughly shaken. 50 µL of this sample extract were then diluted with 950 µL acetonitrile/water (20:80, v/v) plus 0.1 % formic acid. If

necessary, these final extracts were diluted further with final extract of unfortified control samples to achieve final concentrations falling within the calibrated concentration range of the detection system.
For detailed information on the analytical method validation, please refer to Part B, Section 5.
Calculation of initial concentration (C0) DT50/DT90 values
The calculation of the degradation of zoxamide residues in sugar beet leaves and wheat green mass was done based on the analysed residue data.
The degradation kinetics of the active ingredient was analysed according to the recommendations of the EFSA Guidance document on Risk Assessment for Birds and Mammals (2009) and the Guidance document on Estimating Persistence and Degradation Kinetics from Environmental Studies on Pesticides in EU Registration (FOCUS 2014).
The calculation of the DT50 values and DT90 values as well as the fitting of the kinetic degradation models was done using the computer software KinGU II (version 2.2012). Four different kinetic degradation models were taken into account: single first-order, first order multi-compartment (GUSTAFSON & HOLDEN, 1990), hockey stick (bi-phasic) and double first-order (bi-exponential) kinetics. The operating system was Microsoft Windows 10 Professional.
For both commodities, the analysed residues after the last application (2nd application), i.e. starting from three hours after the last application to 15 days after the last application were chosen to establish degradation kinetics. For the two commodities timings were calculated separately from the end of application until the samples were put on dry ice (i.e. time degradation of residues stops). Times were rounded to days (2 digits). For wheat samples the exact timing of 2.53 hours was used to calculate the of 0.11 days. For the tables in the report the rounded value is given with 3 hours.
Results and discussions
Weather conditions
During the trial period the daily average temperatures were between 19.0 °C and 27.8 °C. The rainfall recorded at the field site was 7 mm during the period from first sampling before application to last sampling.
Zoxamide residues
In the control samples, taken directly before the 1st application, concentrations analysed were below the LOD (0.003 mg/kg) in all trials.
Zoxamide in/on sugar beet leaves
Zoxamide concentrations in sugar beet leaves were highest at the first sampling after the 2nd application (3HAA2) with 11.4 mg/kg. Subsequently residues decreased to 2.82 mg/kg at the last sampling (15DAA2), with a second smaller peak (7.27 mg/kg) at sampling S6 (4DAA2). A summary of the residue levels found in sugar beet leaf samples is shown in the following table.
Table A 2-21: Zoxamide residues in/on sugar beet leaves

Timing	Trial
	S19-23773-01
	[mg/kg]
0DBA1	<LOD
1HBA2	2.04
3HAA2	11.4
24HAA2	8.24
48HAA2	6.93
4DAA2	7.27
7DAA2	5.65
9DAA2	3.27
15DAA2	2.82

DBA: days before application, DAA: days after application,
HAA: hours after application, HBA: hours before application,
LOD: level of detection (0.003 mg/kg)
Zoxamide in/on wheat green mass
Zoxamide concentrations in wheat samples were at 9.58 mg/kg at the first sampling after the 2nd application (3HAA2). Subsequently residues decreased to 0.941 mg/kg at the last sampling (15DAA2). A summary of the residue levels found in wheat green mass samples is shown in the following table.

Table A 2-22: Zoxamide residues in/on wheat green mass

Timing	Trial
	S19-23773-01
	[mg/kg]
0DBA1	<LOD
1HBA2	1.92
3HAA2	9.58
24HAA2	7.97
48HAA2	6.98
4DAA2	4.64
7DAA2	4.06
9DAA2	2.30
15DAA2	0.941

DBA: days before application, DAA: days after application,
HAA: hours after application, HBA: hours before application,
LOD: level of detection (0.003 mg/kg)

Sugar Beet Leaves

For sugar beet samples all of the models achieved the critical values < 15 for χ^2-error and for the determination coefficient of r2 > 0.85. The FOMC and DFOP models showed either negative confidence intervals or were not statistically significant. The HS and the SFO model showed no negative confidence intervals and were statistically significant. For the SFO model the calculated χ^2-error was 11.57 and the calculated r^2 was 0.88. The results of the calculation for the SFO model showed a DT50 value of 6.86 days and a DT90 value of 22.78 days, respectively.

Table A 2-23: Results and parameters of the kinetic degradation of zoxamide in/on sugar beet leaves (Trial S19-23773-01)

Model	SFO	FOMC	DFOP	HS
DT50 [days]	6.86	4.37	3.60	4.93
DT90 [days]	22.78	111.40	23.73	25.14
CHI2-err [%]	11.57	10.80	8.76	8.76
r2	0.88	0.91	0.95	0.95
parameter	k = 0.10110	α = 0.5511	k1 = 4.28828	k1 = 0.368839
	M(0) = 10.10576	β = 1.7346	k2 = 0.07994	k2 = 0.079620
		M(0) = 11.4773	g = 0.33345	tb = 1.040435
			M(0) = 13.26376	M(0) = 11.915719
lower Cl	k = 0.05897	α = -0.3318	k1 = -25.26500	k1 = 0.085717
	M(0) = 8.63988	β = -4.4168	k2 = 0.04159	k2 = 0.043485
		M(0) = 8.4290	g = -0.43621	tb = 0.006781
			M(0) = -3.00006	M(0) = 9.859875
upper Cl	k = 0.143	α = 1.434	k1 = 33.842	k1 = 0.652
	M(0) = 11.572	β = 7.886	k2 = 0.118	k2 = 0.116
		M(0) = 14.526	g = 1.103	tb = 2.074
			M(0) = 29.528	M(0) = 13.972
t-test	p(k): 0.00266	-	p(k1): 0.3973	p(k1): 0.041849
			p(k2): 0.0132	p(k2): 0.011438

SFO = Single first order kinetic;
FOMC = First order multi compartment kinetic;
DFOP = Double first order kinetic;
HS = Hockey stick kinetic
M(0) = initial concentration

CI = confidence interval
t = time after time of initial concentration
k = rate constant (ln(2)/DT50)
α = 3.4735*N^-0.8629
β = DT50/(2^(1/ α)-1)
N = DT90/DT50-3.32
DT50 = estimated from the data
DT90 = estimated from the data
k1 = rate constant for the fast degradation phase (ln(2)/DT50f)
k2 = rate constant for the slow degradation phase (ln(2)/DT50s)
tb = break point time estimated from the data rate
g = 1-F
F = estimated residual fraction at the time when the fast degradation phase changes to the slow degradation phase

Wheat Green Mass

For wheat samples all of the models achieved the critical values < 15 for χ^2-error and for the determination coefficient of r2 > 0.85. The FOMC and DFOP models showed either negative confidence intervals or were not statistically significant. The HS and the SFO model showed no negative confidence intervals and were statistically significant. For the SFO model the calculated χ^2-error was 5.77 and the calculated r^2 was 0.98. The results of the calculation for the SFO model showed a DT50 value of 4.62 days and a DT90 value of 15.35 days, respectively.

Table A 2-24: Results and parameters of the kinetic degradation of zoxamide in/on wheat green mass (Trial S19-23773-01)

Model	SFO	FOMC	DFOP	HS
DT50 [days]	4.62	4.38	4.25	4.62
DT90 [days]	15.35	16.71	16.04	15.39
CHI2-err [%]	5.77	6.05	6.22	6.86
r2	0.98	0.98	0.99	0.98
parameter	k = 0.15005	α = 6.0561	k1 = 0.98740	k1 = 0.15005
	M(0) = 9.46630	β = 36.1182	k2 = 0.13622	k2 = 0.09333
		M(0) = 9.6010	g = 0.11052	tb = 15.26760
			M(0) = 9.81765	M(0) = 9.46630
lower Cl	k = 0.12470	α = -22.6811	k1 = -3.05244	k1 = 0.11760
	M(0) = 8.82664	β = -154.1444	k2 = 0.07967	k2 = 0.04410
		M(0) = 8.6810	g = -0.22503	tb = 3.21631
			M(0) = 8.76221	M(0) = 8.61498
upper Cl	k = 0.175	α = 34.79	k1 = 5.027	k1 = 0.182
	M(0) = 10.106	β = 226.38	k2 = 0.193	k2 = 0.143
		M(0) = 10.52	g = 0.446	tb = 27.319
			M(0) = 10.873	M(0) = 10.318
t-test	p(k): 4.17e-05	-	p(k1): 0.33233	p(k1): 0.001418
			p(k2): 0.00900	p(k2): 0.016949

SFO = Single first order kinetic;
FOMC = First order multi compartment kinetic;
DFOP = Double first order kinetic;
HS = Hockey stick kinetic
M(0) = initial concentration
CI = confidence interval
t = time after time of initial concentration
k = rate constant (ln(2)/DT50)

α = 3.4735*N^-0.8629
β = DT50/(2^(1/ α)-1)
N = DT90/DT50-3.32
DT50 = estimated from the data
DT90 = estimated from the data
k1 = rate constant for the fast degradation phase (ln(2)/DT50f)
k2 = rate constant for the slow degradation phase (ln(2)/DT50s)
tb = break point time estimated from the data rate
g = 1-F
F = estimated residual fraction at the time when the fast degradation phase changes to the slow degradation phase
The following figures show residues of zoxamide.

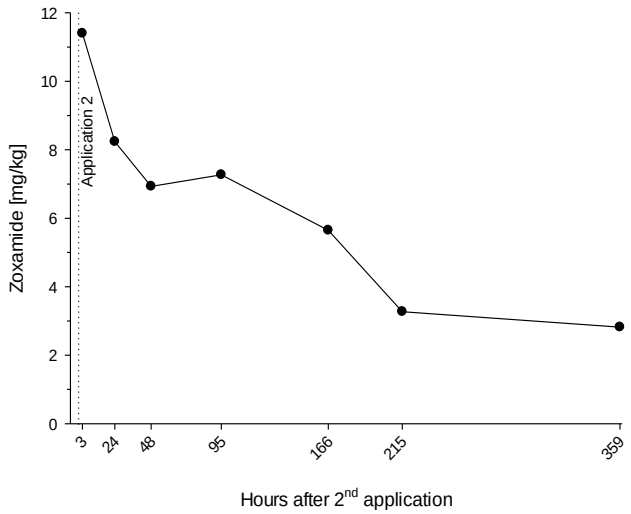


Figure A 11: Residues of zoxamide in sugar beet leaves over time (trial 01)

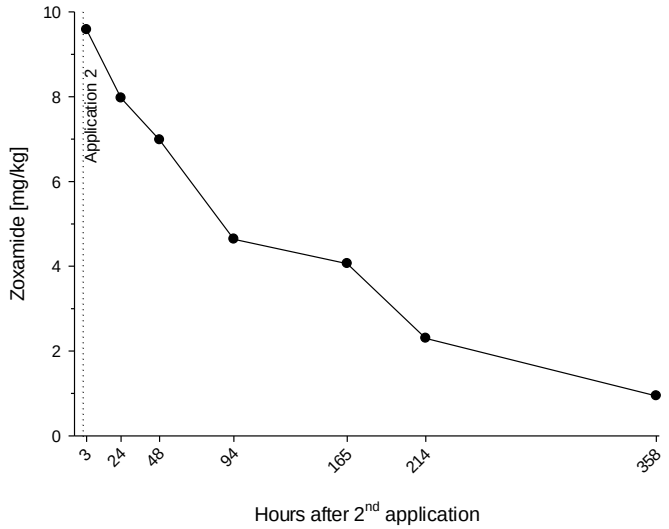


Figure A 12: Residues of zoxamide in wheat green mass over time (trial -01)
Conclusion
The residue decline of zoxamide on sugar beet leaves (as surrogate dicotyledonae plant) and wheat green mass (as surrogate monocotyledonae plant) has been studied in the field under representative growing conditions for Southern Europe (Italy) in the field. In sugar beet leaves (as surrogate dicotyledonae plant) and in wheat green mass (as surrogate monocotyledonae plant), the residues and degradation kinetics of the

active ingredient were investigated after two applications of Zoxium 240 SC (240 g/L zoxamide, SC) at application rates for zoxamide of 180 g a.i./ha with an interval of 7 days (BBCH 17-21 for sugar beet and wheat).
For the degradation of zoxamide in sugar beet leaves, the single first order (SFO) degradation was calculated at 6.86 days and the DT90 was calculated at 22.78 days. For the degradation of zoxamide in wheat green mass, the single first order (SFO) DT50 was calculated at 4.62 days and the DT90 was calculated at 15.35 days.

(Appeltauer A. 2020)

A 2.2 Cymoxanil

A 2.2.1 Stability of residues

A 2.2.1.1 Stability of residues during storage of samples

A 2.2.1.1.1 Storage stability of residues in plant products

A 2.2.1.1.1.1 Study 1

Report:	**Report: KCA 6.1/03 Lucini L. (2006);**
Title:	Freezer storage stability of cymoxanil residue in grape bunches
Document No.:	Sipcam SpA, Italy, unpublished report no. SIP 1380
Guidelines:	FAO guidelines on producing residue data from supervised trials, 1990; EC Directive 91/414; SANCO/3029/99 rev 4. Deviations: No major variations to the recommended test guideline EU Directive 91/414/EEC (EU Commission Working Document 1607/97 Appendix H: Storage Stability of Residue Samples)
GLP:	Yes

Material and Methods:

The storage stability data for Cymoxanil residues in/on grape bunches was studied by fortifying samples of whole grape bunches with Cymoxanil at 0.524 mg/kg and homogenised grape at 0.526 mg/kg, and storing in the freezer at approximately -20°C. Samples were analysed on 0-Day and after 7, 15 and 30 days (homogenised and whole fruit samples) and after 3, 6, 12, 18 and 24 months (whole fruit samples only) of frozen storage. The analytical method consisted in an extraction by blender with ethyl acetate as extraction solvent, followed by a clean-up by partitioning and then on a column with Silica Gel. Analysis for Cymoxanil was conducted by gas chromatography with a NPD detector. The LOQ of the method was 0.05 mg/kg.

Findings:

Procedural recoveries at each sampling time were within acceptable limits (in the range 70 to 110%). All normalised recoveries of Cymoxanil residues in homogenised and whole fruit were 98% or greater, showing that Cymoxanil residues were stable in a freezer for at least 1 month when homogenized and 2 years as whole fruit at approximately -20°C prior to analysis. The results are presented in the following table.

Table A 47: Storage stability of Cymoxanil in grapes

Crop commodity	Storage interval	% Recovery				
		Freeze stored fortifications	Fresh fortifications	Average recovery		Normalised recovery*
				Stored	Fresh	
Whole grape bunches	0	96.6, 99.6, 104	85.0	100	85.0	118
	7 days	88.9, 92.4, 80.7	85.2	87.2	85.2	102
	15 days	101.9, 100, 109	84.4	103.5	84.4	123
	30 days	98.4, 96.2, 101.9	92.2	98.8	92.2	107
	3 months	93.9, 97.7, 105	80.8	98.8	80.8	122
	6 months	90.4, 90.4, 88.9	82.1	89.8	82.1	110
	12 months	109, 95.2, 101.7	85.1	99.3	85.1	117
	18 months	107.8, 102.0, 103.0	81.2	104.3	81.2	128
	24 months	86.1, 90.3, 86.2	86.1	87.5	86.1	102
Homogenised	0	97, 102, 101.3	87.3	100	87.3	114
	7 days	82, 86, 85.4	86.2	84.5	86.2	98

grape bunches	15 days	95.4, 99.6, 98	88.9	97.7	88.9	110
	30 days	104, 103, 107	94.2	104	94.2	110

* Normalised recovery = average freezer storage value/fresh fortification value

Conclusion/endpoint:

Following storage at approximately -20°C, residues of Cymoxanil in grape were stable for 24 months.

A 2.2.1.1.1.2 Study 2

Report:	**Report: KCA 6.1/04 Lucini L. (2006)**
Title:	Freezer storage stability of cymoxanil in whole and processed tomatoes.
Document No.:	Sipcam SpA, Italy. unpublished report no. SIP 1381
Guidelines:	FAO guidelines on producing residue data from supervised trials, 1990; EC Directive 91/414; SANCO/3029/99 rev 4. Deviations: No major variations to the recommended test guideline EU Directive 91/414/EEC (EU Commission Working Document 1607/97 Appendix H: Storage Stability of Residue Samples)
GLP:	Yes

Material and Methods:

The storage stability data for Cymoxanil residues in/on tomato was studied by fortifying samples of whole and homogenised tomato berries with Cymoxanil at 0.506 mg/kg and storing in the freezer at approximately -20°C. Samples were analysed on 0-Day and after 7, 15 and 30 days (homogenised and whole fruit samples) and after 3, 6, 12, 18 and 24 months (whole fruit samples only) of frozen storage. The analytical method consisted in an extraction by blender with ethyl acetate as extraction solvent, followed by a clean-up by partitioning and then on a column with Silica Gel. Analysis for Cymoxanil was conducted by gas chromatography with a NPD detector. The LOQ of the method was 0.05 mg/kg.

Findings:

Procedural recoveries at each sampling time were within acceptable limits (in the range 70 to 110%). All normalised recoveries of Cymoxanil residues in homogenised and whole fruit were 76.7% or greater, Cymoxanil residues were stable in tomato homogenized berry for the whole period tested (76.7% of the initial after 30 days of storage) while residues in whole tomato were stable up to 18 months of storage (82.7% of the initial). At the end of the study (2 years), Cymoxanil residues were decreased down to the 63.9% of initial. The results are presented in the following table.

Table A 48: Storage stability of Cymoxanil in tomato

Crop commodity	**Storage interval**	**% Recovery**				
		Freeze stored fortifications	**Fresh fortifications**	**Average recovery**		**Normalised recovery***
				Stored[§]	**Fresh**	
Whole tomato	0	96.8, 105, 98.4	85.0	100	85.0	118
	7 days	98.2, 99.1, 93.7	85.2	97.0	85.2	114
	15 days	96.5, 100.9, 97.3	93.2	98.2	93.2	105
	30 days	89.6, 90.7, 91.1	98.3	90.5	98.3	92
	3 months	90.9, 70.1, 58.1,	76.8	73.0	76.8	95
	6 months	108.2, 87.6, 114.7	81.8	103.5	81.8	126
	12 months	88.3, 100.3, 90.1	81.1	92.9	81.1	114
	18 months	71.7, 86.4, 89.9, 87.2, 91.4	80.5	82.7	80.5	103
	24 months	78.0, 55.4, 58.2	87.0	63.9	87.0	73.4

Homogenised tomato	0	124.1, 129.4, 132.8	92.6	128.8	92.6	122
	7 days	70.0, 76.1, 71.2	88.1	72.5	88.1	82.3
	15 days	75.1, 73.4, 76.6	97.1	75.1	97.1	77.3
	30 days	72.2, 82.4, 75.5	91.8	76.7	91.8	83.5

*Normalised recovery = average freezer storage value/fresh fortification value
§Mean initial value 0.510 mg/kg for whole tomato, 0.652 for homogenised tomato.

Conclusion/endpoint:

Cymoxanil residues were stable in tomato homogenized berry for the whole period tested (76.7% of the initial after 30 days of storage) while residues in whole tomato were stable up to 18 months of storage.

A 2.2.1.1.2 Storage stability of residues in animal products

No new data submitted.

A 2.2.1.1.3 Storage stability of residues in sample extracts

The stability of residues of cymoxanil in sample extracts was checked as part of individual studies in case necessary (i.e. stored for > 24 hours in the refrigerator). Recovery experiments were performed concurrently with the analysed samples. The results do not indicate residue decrease within the necessary storage periods.

A 2.2.2 Nature of residues in plants, livestock and processed commodities

A 2.2.2.1 Nature of residue in plants

A 2.2.2.1.1 Nature of residue in primary crops

A 2.2.2.1.1.1 Study 1

Report:	**KCA 6.2.1/01, Melkebeke, van Noorloos (2003)**
Title:	Metabolism, distribution, and expression of Cymoxanil residues in tomato
Document No.:	NOTOX B.V., 's-Hertogenbosch, The Netherlands; unpublished report no 257783, 05.03.2003
Guidelines:	EEC Commission Directive 96/68/EC (1996);
GLP:	Yes

Material and Methods:

In a metabolism study 3 tomato plants were treated four times at a dose rate of 240 g a.i./ha. The first application was at BBCH 5. Spraying intervals were 15, 27 and 16 days. Harvest took place 13 days after the last application (BBCH 81). The total radioactive residue (TRR) in fruits was determined by combustion: 0.06% was left in crop at harvest, equivalent to 125 µg/kg fresh weight (ppb, Cymoxanil equivalents. After extraction with consecutively acetonitrile/water, hexane, ethylacetate, strong acid and strong alkali, the aqueous fraction was analysed on several TLC and HPLC systems and co-chromatographed with references.

Findings:

Extraction of tomato matrix revealed 73.3% of TRR (92 ppb) extractable residue. Fractionation of the acetonitrile:water extract revealed <1% of TRR (<1 ppb) in the organic fraction (dichloromethane, hexane or ethylacetate).as organosoluble compounds, and 73.0% of TRR (91 ppb) in the aqueous fraction as polar compounds. Acid and base hydrolysable conjugates accounted for 6.4% of TRR; 8 ppb). Unextractables were to 13.2% of TRR (16 ppb) (see following Figure).

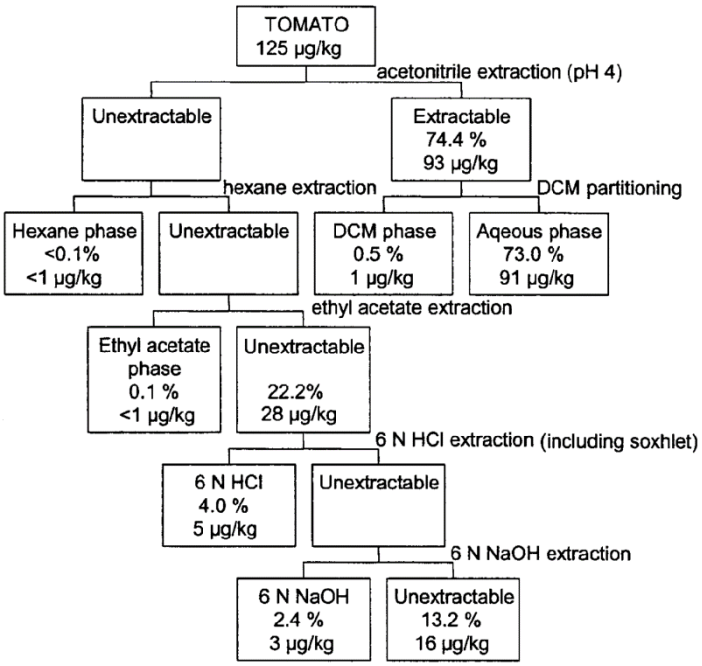


Figure A 13: Fractionation of radioactivity in tomato

The best fractionation was obtained with TLC2. Up to two radioactive zones were shown in the aqueous fraction representing 32.3% of TRR (40 ppb) and 40.7% of TRR (51 ppb) in TLC 2, but they were not identified. They were characterized as unidentified polar components.

Table A 49: Distribution of activity in aqueous fraction

TLC	Rf	% on TLC plate	% of TRR	Cymoxanil equivalents (µg/kg)
TLC 2	0.04	55.8	40.7	51
	0.69	44.2	32.3	40

Conclusion/endpoint:

No Cymoxanil was present in tomatoes.

No conjugated Cymoxanil or conjugated metabolites were shown in the aqueous fraction after hydrolysis with 3-glucosidase. Neither free glycine nor conjugated glycine was identified in the aqueous tomato extract.

In general, the major unidentified activity at harvest is present as polar metabolites or conjugates, or incorporated into plant constituents (bio-unavailable).

A 2.2.2.1.2 Nature of residue in rotational crops

No new data submitted.

A 2.2.2.1.3 Nature of residues in processed commodities

No new data submitted.

A 2.2.2.2 Nature of residues in livestock

No new data are submitted in the framework of this application.

A 2.2.3 Magnitude of residues in plants

A 2.2.3.1 Fruiting crops – grapes

A 2.2.3.1.1 Study 1

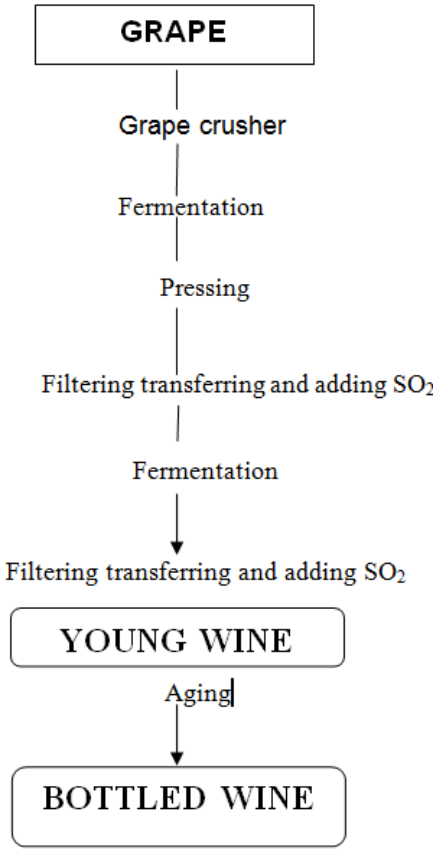
Report:	**KCA 6.3/01, Romanini M., 2011**
Title:	Determination of cymoxanil and zoxamide residues at harvest in raw and processed agricultural commodity grape (bunch, must young and bottled wine) following five applications of Harpon W (Cymoxanil 33% + Zoxamide 33% WG) - Four trials, Italy 2010 -
Document No:	Sipcam S.p.A. report no. CREG 2117
Guidelines:	EC working document, 1607/VI/97, rev. 2, Appendix B, 7029/VI/95 Rev.5 SANCO/3029/99, rev. 4, SANCO/825/00, rev. 7, ENV /JM/MONO(2007)17, ENV/MC/CHEM(98)17
GLP	Yes

Report:	**KCA 6.3/02, Romanini M., 2011**
Title:	Determination of cymoxanil and zoxamide residues at harvest in raw and processed agricultural commodity grape (bunch, must young and bottled wine) following five applications of Harpon W (Cymoxanil 33% + Zoxamide 33% WG) - Four trials, northern Europe 2010 -
Document No:	Sipcam S.p.A. report no. CREG 2120
Guidelines:	EC working document, 1607/VI/97, rev. 2, Appendix B, 7029/VI/95 Rev.5 SANCO/3029/99, rev. 4, SANCO/825/00, rev. 7, ENV /JM/MONO(2007)17, ENV/MC/CHEM(98)17
GLP	**Yes**

Material and Methods:

Four trials in Northern and 4 trials in Southern Europe are available in grapes, treated with 5 applications of 'Cymoxanil 33% + Zoxamide 33% WG' (Harpon WG, batch XE28160A11, Cymoxanil 32.7% , Zoxamide 33.5%) at 0.45 kg fp/ha each, 7 days interval. Plant samples were collected 28 days after the last application. Samples arising from trials I/CZ10/GR02 and F/CZ10/GR02 underwent processing steps in order to obtain must, young wine and bottled wine to be analyzed.

Figure 1 – Flowchart of the processed Grape



A sample of bunches was taken and analyzed straight after deep freezing. For Cymoxanil the sample was extracted by Ultra-Turrax (bunch samples) or by shaking (processed samples) with ethyl acetate, purified by liquid-liquid partition and analysed by gas chromatography with a Nitrogen Phosphorus Detector (GC/NPD). Zoxamide was determined using an internal analytical method that consisted of a solvent extraction by Ultraturrax (bunch samples) or by shaking (processed samples). The extract containing the active ingredient was cleaned up by liquid-liquid partition and by SPE chromatography, then analysed by gas chromatography equipped with an ECD. The Limit of Quantification (LOQ) was 0.05 mg/kg for Cymoxanil on bunches samples and 0.01 mg/kg on processed samples; for Zoxamide the LOQ was 0.01 mg/kg for all matrices. Limit of Detection (LOD) was 0.0290 mg/kg for bunch samples and 0.0058 mg/kg for processed samples (must, young wine and bottled wine) for Cymoxanil, and 0.005 mg/kg for Zoxamide, all matrices.

Table A 50: Summary of 'Cymoxanil 33% + Zoxamide 33% WG' Residue Trials on Grapes

Trial	Crop / Variety	Country / Region	Application rate			Crop growth stage	Portion analyzed	Residues (mg/kg)		PHI (days)	Method / Recovery
			kg as/hL	Water (L/ha)	kg as/ha			Cymoxa-nil	Zoxa-mide		
CREG2120 G/CZ10/GR 01	Grapevine/ Chardonnay	D-79356 Eichstetten, Baden-Würtenberg Germany	1st: 0.015 2nd: 0.015 3rd: 0.015 4th: 0.015 5th: 0.015	1st: 1000 2nd: 1000 3rd: 1000 4th: 1000 5th: 1000	1st: 0.1485 2nd: 0.1485 3rd: 0.1485 4th: 0.1485 5th: 0.1485	1st: BBCH 79 2nd: BBCH 79-81 3rd: BBCH 83 4th: BBCH 83-85 5th: BBCH 83-85	Grape bunch	<0.05	0.8598	28	Mean recovery/RSD Cymoxanil: 90.78%/6.1%, 106.61%/0.5%, 96.67%/3.8%, 103.72%/3.1%. Zoxamide: 85.49%/11.0%, 91.71%/0.8, 105.20%/2.9%, 79.39%/3.7%. For grape bunch, must, young wine, bottled wine.
CREG2120 F/CZ10/GR 02	Grapevine/ Auxerrois	F-67117 Furdenheim, Alsace France	1st: 0.015 2nd: 0.015 3rd: 0.015 4th: 0.015 5th: 0.015	1st: 1000 2nd: 1000 3rd: 1000 4th: 1000 5th: 1000	1st: 0.1485 2nd: 0.1485 3rd: 0.1485 4th: 0.1485 5th: 0.1485	1st: BBCH 77 2nd: BBCH 77-79 3rd: BBCH 79 4th: BBCH 79-81 5th: BBCH 81	Grape bunch Must Young wine Bottled wine	<0.05 <0.01 0.0151 <0.01	0.2980 0.0346 0.0228 <0.01	28	
CREG2120 F/CZ10/GR 03	Grapevine/ Pinot Blanc	F-67990 Osthoffen, Alsace France	1st: 0.015 2nd: 0.015 3rd: 0.015 4th: 0.015 5th: 0.015	1st: 1000 2nd: 1000 3rd: 1000 4th: 1000 5th: 1000	1st: 0.1485 2nd: 0.1485 3rd: 0.1485 4th: 0.1485 5th: 0.1485	1st: BBCH 77 2nd: BBCH 77-79 3rd: BBCH 79 4th: BBCH 79-81 5th: BBCH 81	Grape bunch	<0.05	0.4586	28	
CREG2120 F/CZ10/GR 04	Grapevine/ Grolleau	F-49540 Martigne-Briand, Pays de la Loire France	1st: 0.015 2nd: 0.015 3rd: 0.015 4th: 0.015 5th: 0.015	1st: 1000 2nd: 1000 3rd: 1000 4th: 1000 5th: 1000	1st: 0.1485 2nd: 0.1485 3rd: 0.1485 4th: 0.1485 5th: 0.1485	1st: BBCH 79 2nd: BBCH 79 3rd: BBCH 81 4th: BBCH 83 5th: BBCH 83	Grape bunch	< 0.05	0.2656	28	
CREG2117 I/CZ10/GR 01	Grape/Barbera	I-15058 Viguzzolo (AL) Italy	1st: 0.015 2nd: 0.015 3rd: 0.015 4th: 0.015 5th: 0.015	1st: 1000 2nd: 1000 3rd: 1000 4th: 1000 5th: 1000	1st: 0.1485 2nd: 0.1485 3rd: 0.1485 4th: 0.1485 5th: 0.1485	1st: BBCH 80 2nd: BBCH 81 3rd: BBCH 82 4th: BBCH 83 5th: BBCH 84	Grape bunch	<0.05	0.2594	28	Mean recovery/RSD Cymoxanil: 83.98%/15.9%, 100.49%/6.1%, 101.33%/10.1%, 97.76%/10.6%. Zoxamide: 77.11%/8.5%, 78.95%/13.3%, 84.40%/18.1%,
CREG2117 I/CZ10/GR 02	Grape/Barbera	I-26857 Salerano sul Lambro (LO) Italy	1st: 0.015 2nd: 0.015 3rd: 0.015 4th: 0.015 5th: 0.015	1st: 1000 2nd: 1000 3rd: 1000 4th: 1000 5th: 1000	1st: 0.1485 2nd: 0.1485 3rd: 0.1485 4th: 0.1485 5th: 0.1485	1st: BBCH 73 2nd: BBCH 75 3rd: BBCH 77 4th: BBCH 79 5th: BBCH 81	Grape bunch Must Young wine Bottled wine	<0.05 <0.01 <0.01 <0.01	0.2748 0.0166 0.0191 0.0186	28	

CREG2117 I/CZ10/GR 03	Grape /Montepulciano d'Abruzzo	I-64013 Corropoli (TE) Italy	1st: 0.015 2nd: 0.015 3rd: 0.015 4th: 0.015 5th: 0.015	1st: 1000 2nd: 1000 3rd: 1000 4th: 1000 5th: 1000	1st: 0.1485 2nd: 0.1485 3rd: 0.1485 4th: 0.1485 5th: 0.1485	1st: BBCH 79 2nd: BBCH 81 3rd: BBCH 83 4th: BBCH 85 5th: BBCH 85	Grape bunch	<0.05	0.0733	28	76.40%/5.7%. For grape bunch, must, young wine, bottled wine.
CREG2117 I/CZ10/GR 04	Grape /Montepulciano d'Abruzzo	I-64010 Morro D'Oro (TE) Italy	1st: 0.015 2nd: 0.015 3rd: 0.015 4th: 0.015 5th: 0.015	1st: 1000 2nd: 1000 3rd: 1000 4th: 1000 5th: 1000	1st: 0.1485 2nd: 0.1485 3rd: 0.1485 4th: 0.1485 5th: 0.1485	1st: BBCH 81 2nd: BBCH 81 3rd: BBCH 83 4th: BBCH 83 5th: BBCH 85	Grape bunch	< 0.05	0.0833	28	

Conclusions

For Cymoxanil residues were below the method limit of quantification (LOQ = 0.05 mg/kg for bunches and 0.01 mg/kg for processed samples) in all the specimens analysed (treated, untreated and processed), except for young wine residues were slightly above the LOQ.
The results above are below the respective MRL values set forth according to Reg. (EC) No. 396/2005 for grapes (0.2 mg/kg and 5 mg/kg for Cymoxanil and Zoxamide, respectively).

A 2.2.3.2 Tomato

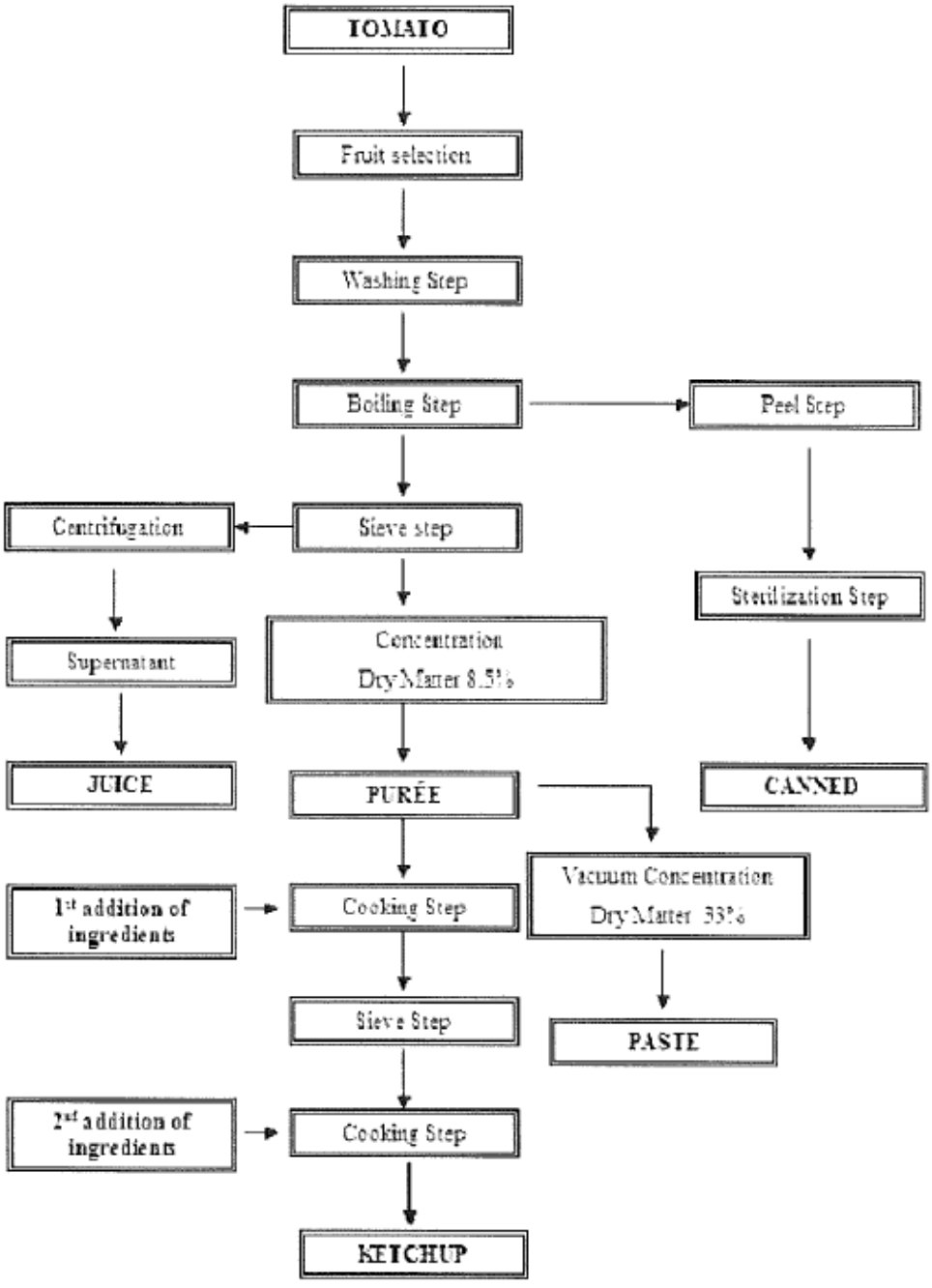
A 2.2.3.2.1 Study 1

Report:	**KCA 6.3/16, Romanini M., 2011**
Title:	Determination of cymoxanil and zoxamide residues at harvest in raw and processed agricultural commodity tomato (fruit, juice, puree and canned) following five applications of Harpon W(Cymoxanil 33% + Zoxamide 33% WG) - Four trials, Italy 2010 -
Document No:	Sipcam S.p.A. report no. CREG 2118
Guidelines:	EC working document, 1607/VI/97, rev. 2, Appendix B, 7029/VI/95 Rev.5 SANCO/3029/99, rev. 4, SANCO/825/00, rev. 7, ENV /JM/MONO(2007)17, ENV/MC/CHEM(98)17
GLP	Yes

Material and Methods:

Four trials in southern Europe are available on tomatoes treated with Cymoxanil 33% + Zoxamide 33% WG (Harpon WG, batch XE28160A11, Cymoxanil 32.7% , Zoxamide 33.5%) with 5 applications at 0.45 kg fp/ha, 7 days interval. Samples were collected 3 days after the last application. Samples arising from trial I/CZ10/TO02 underwent processing steps in order to obtain juice, puree and canned tomato to be analyzed.

Figure 1 – Flowchart of the processed tomato



For Cymoxanil the sample was extracted by Ultra-Turrax with ethyl acetate, purified by liquid-liquid partition and analysed by gas chromatography with a Nitrogen Phosphorus Detector (GC/NPD). Zoxamide was determined using an internal analytical method that consisted in a solvent extraction by Ultraturrax (bunch samples) or by shaking (processed samples). The extract containing the active ingredient was cleaned up by liquid-liquid partition and by SPE chromatography, then analysed by gas chromatography equipped with an ECD. The Limit of Quantification (LOQ) was 0.05 mg/kg for Cymoxanil on tomato samples; for Zoxamide the LOQ was 0.01 mg/kg for all matrices. Limit of Detection (LOD) was 0.0249 mg/kg for Cymoxanil, and 0.0051 for Zoxamide in all matrices.

Table A 51: Summary of 'Cymoxanil 33% + Zoxamide 33% WG' Residue Trials on Tomatoes (outdoors)

Trial	Crop / Variety	Country / Region	Application rate			Crop growth stage	Portion analyzed	Residues (mg/kg)		PHI (days)	Method / Recovery
			kg as/hL	Water (L/ha)	kg as/ha			Cymoxa-nil	Zoxa-mide		
CREG2118 I/CZ10/TO01	Tomato/ Heinz 3402	Salerano sul Lambro (LO) Italy	1st: 0.019 2nd: 0.019 3rd: 0.019 4th: 0.019 5th: 0.019	1st: 800 2nd: 800 3rd: 800 4th: 800 5th: 800	1st: 0.1485 2nd: 0.1485 3rd: 0.1485 4th: 0.1485 5th: 0.1485	1st: BBCH 716 2nd: BBCH 716 3rd: BBCH 717 4th: BBCH 719 5th: BBCH 805 (89)	fruit	<0.05	0.3073	3	Mean recovery/RSD Cymoxanil: 77.34%/6.1%, 88.23%/11.8%, 90.18%/11.3%, 86.99%/0.4%. Zoxamide: 99.01%/9.4%, 92.01%/8.4%, 87.77%/9.5%, 100.64%/10.6%. For fruit, juice, puree, canned.
CREG2118 I/CZ10/TO02	Tomato/ Leader	I-29029 Rivergaro (PC) Italy	1st: 0.019 2nd: 0.019 3rd: 0.019 4th: 0.019 5th: 0.019	1st: 800 2nd: 800 3rd: 800 4th: 800 5th: 800	1st: 0.1485 2nd: 0.1485 3rd: 0.1485 4th: 0.1485 5th: 0.1485	1st: BBCH 79 2nd: BBCH 81 3rd: BBCH 83 4th: BBCH 85 5th: BBCH 89	fruit juice puree canned	<0.05 <0.05 <0.05 <0.05	0.3031 <0.01 <0.01 <0.01	3	
CREG2118 I/CZ10/TO03	Tomato/ Doget	I-64013 Corropoli (TE) Italy	1st: 0.019 2nd: 0.019 3rd: 0.019 4th: 0.019 5th: 0.019	1st: 800 2nd: 800 3rd: 800 4th: 800 5th: 800	1st: 0.1485 2nd: 0.1485 3rd: 0.1485 4th: 0.1485 5th: 0.1485	1st: BBCH 82 2nd: BBCH 85 3rd: BBCH 87 4th: BBCH 88 5th: BBCH 89	fruit	<0.05	0.1613	3	
CREG2118 I/CZ10/TO04	Tomato/ Perfect peel	I-64010 Morro D'Oro (TE) Italy	1st: 0.019 2nd: 0.019 3rd: 0.019 4th: 0.019 5th: 0.019	1st: 800 2nd: 800 3rd: 800 4th: 800 5th: 800	1st: 0.1485 2nd: 0.1485 3rd: 0.1485 4th: 0.1485 5th: 0.1485	1st: BBCH 81 2nd: BBCH 82 3rd: BBCH 85 4th: BBCH 85 5th: BBCH 88	fruit	< 0.05	0.2746	3	

Conclusions

For Cymoxanil residues were below the limit of quantification (LOQ) and below the limit of detection (LOD) in all the specimens analysed (treated, untreated and processed).
For Zoxamide residues were below the method LOQ (and LOD) in all the untreated specimens while the treated tomato fruit samples had residues higher than the LOQ. Highest Zoxamide residue observed in tomato fruits was 0.3073 mg/kg. Residues in processed tomato matrices were below LOQ.
The results above are below the respective MRL values set forth according to Reg. (EC) No. 396/2005 for tomato fruits (0.2 mg/kg and 0.5 mg/kg for Cymoxanil and Zoxamide, respectively).

A 2.2.3.2.2 Study 1

Report:	**KCA 6.3/29, Lucini, 2008**
Title:	Determination of cymoxanil residues in raw agricultural commodity tomato (fruit) following application of SIP40936 (CYMOXANIL 33% + ZOXAMIDE 33% WG) SIPCAM S.p.A., Italy
Document No:	SIP 1551
Guidelines:	EC working document, 1607/VI/97, rev. 2, Appendix B, 7029/VI/95 Rev.5 SANCO/3029/99, rev. 4, SANCO/825/00, rev. 7, ENV /JM/MONO(2007)17, ENV/MC/CHEM(98)17
GLP	Yes

Active substance (common name):	**Cymoxanil**	**Commercial Product (name):**	
Crop/crop group:	**Tomato**	**Producer of commercial product:**	**Oxon**
Responsible body for reporting (name, address):	**Oxon** **Via Sempione 195- Pero (Milan)**	**Indoor/Glasshouse/Outdoor:**	**Outdoor**
Country:	**Italy**	**Other active substance in the formulation (common name and content):**	**Zoxamide 33%**
Content of active substance (g/kg or g/L):	**33%**	**Residues calculated as:**	**Cymoxanil (zoxamide was not tested)**
Formulation (*e.g.* WP):	**WG**		

1	2	3	4	5			6	7	8	9	10	11
Report No. Location (region)	Commodity / Variety	Date of 1. Sowing or Planting 2. Flowering 3. Harvest	Method of treatment	Application rate Per treatment			Dates of treatment(s) or no. of treatment(s) and last date	Growth stage at last treatment or date	Portion analyzed	Residues (mg/kg)	PHI (days)	Remarks:
	(a)	(b)	(c)	kg as/hL	Water (L/ha)	kg as/ha	(d)	(e)	(a)		(f)	(g)
SIP 1551 **I/CZ/07/TO01** 29010 Monticelli d'Ongina (Piacenza) - Italy	Open field Tomato/ 9885	1) 30/04/07 2) 10/06/07 3) 13/08/07	spray	0.0248 0.0248 0.0248 0.0248 0.0248	608.89 622.22 604.44 600.00 613.33	0.151 0.154 0.150 0.149 0.152	09/07/07 16/07/07 23/07/07 30/07/07 06/08/07	BBCH 703-704 BBCH 704-705 BBCH 801 BBCH 805 BBCH 808	Fruits	<0.05 <0.05	3 7	GC-NPD; LOQ=0.05 mg/kg LOD=0.02 mg/kg
SIP 1551 **CY-ZO1/TO/S-03** 11140 Conil de la Frontera (Cadiz) - Spain	Open field Tomato/ Jaguar	1) 13/06/07 2) 26/07/07 3) 06/08/07	spray	0.0149 0.0149 0.0149 0.0149 0.0149	938.46 1038.46 984.62 1030.77 1023.08	0.139 0.154 0.146 0.153 0.152	30/07/07 06/08/07 13/08/07 20/08/07 27/08/07	BBCH 63-64 BBCH 65-81 BBCH 81-82 BBCH 82 BBCH 83	Fruits	<0.05 (0.033) <0.05 <0.05 <0.05	0 1 3 7	GC-NPD; LOQ=0.05 mg/kg LOD=0.02 mg/kg

n.i.=not indicated

(a) According to EEC and Codex classifications (both) should be used
(b) Only if relevant
(c) High or low volume spaying, spreading, dusting *etc*., overall, broadcast, type of equipment used must be indicated
(d) Year must be indicated
(e) BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4),
f) Minimum number of days after last application (Label pre-harvest interval, PHI, underline)
(g) Remarks may include: Climatic conditions; Reference to analytical method; Information concerning the metabolites included, the method of storage, storage stability, analysis date

A 2.2.3.3 Potato

A 2.2.3.3.1 Study 1

Report:	**KCA 6.3/23, Tetuan B., 2011**
Title:	Determination of residues at harvest in potatoes following six broadcast applications of Harpon WG under field conditions-Northern Europe, season 2010
Document No:	Promovert report no.10 F PT GW P/A
Guidelines:	EC working document, 1607/VI/97, rev. 2, Appendix B, 7029/VI/95 - rev.5 SANCO/3029/99, rev. 4, SANCO/825/00, rev. 7, ENV /JM/MONO(2007)17
GLP	Yes

Report:	**KCA 6.3/24, Tetuan B., 2011**
Title:	Determination of residues at harvest in potatoes following six broadcast applications of Harpon WG under field conditions-Southern Europe, season 2010
Document No:	Promovert report no.10 F PT GW P/B
Guidelines:	EC working document, 1607/VI/97, rev. 2, Appendix B, 7029/VI/95 - rev.5 SANCO/3029/99, rev. 4, SANCO/825/00, rev. 7, ENV /JM/MONO(2007)17
GLP	Yes

Material and Methods:

Two trials in Northern and 2 trials in Southern Europe are available in potatoes treated with 'Cymoxanil 33% + Zoxamide 33% WG' (Harpon WG, batch XE28160A11, Cymoxanil 32.7% , Zoxamide 33.5%) at 0.45 kg fp/ha, with 6 (Northern Europe) and 5 (Southern Europe) applications, at 7 days interval, and with samples collected at 6-7 days after the last application.
After extraction with acetonitrile/2% potassium bicarbonate aqueous solution *(80/20*, v/v) mixture and clean-up by dispersive solid phase extraction (D-SPE), the determination of Cymoxanil and Zoxamide was performed by liquid chromatography with detection by tandem mass spectrometry (LC-MS/MS) with a LOQ = 0.01mg/kg for both substances (see Part B, Section 2).

Table A 52: Summary of 'Cymoxanil 33% + Zoxamide 33% WG' Residue Trials on Potatoes

Trial	Crop / Variety	Country / Region	Application rate			Crop growth stage	Portion analyzed	Residues (mg/kg)		PHI (days)	Method / Recovery
			kg as/hL	Water (L/ha)	kg as/ha			Cymoxa-nil	Zoxa-mide		
10F PT GW P01	Potato/Alegria	D-04668 Motterwitz (Saxony) Germany	1st: 0.037 2nd: 0.037 3rd: 0.037 4th: 0.037 5th: 0.037 6th: 0.037	1st: 400 2nd: 400 3rd: 400 4th:400 5th: 400 6th: 400	1st: 0.1485 2nd: 0.1485 3rd: 0.1485 4th: 0.1485 5th: 0.1485 6th: 0.1485	1st: BBCH 81 2nd: BBCH 81 3rd: BBCH 85 4th: BBCH 85 5th: BBCH 85 6th: BBCH 87	tubers	< 0.01	< 0.01	7	Mean recovery: 106% Cy-moxanil, 107% Zoxamide. RDS: 6% Cymoxanil, 5% Zoxamide. Fortification range: 0.01-0.1 mg/kg.
10F PT GW P02	Potato/Albatros	D-18258 Mecklemburg (West Pomerania) Germany	1st: 0.037 2nd: 0.037 3rd: 0.037 4th: 0.037 5th: 0.037 6th: 0.037	1st: 400 2nd: 400 3rd: 400 4th:400 5th: 400 6th: 400	1st: 0.1485 2nd: 0.1485 3rd: 0.1485 4th: 0.1485 5th: 0.1485 6th: 0.1485	1st: BBCH 81 2nd: BBCH 81 3rd: BBCH 81-85 4th: BBCH 85 5th: BBCH 85 6th: BBCH 85	tubers	< 0.01	< 0.01	6	
10F PT GW P03	Potato/Carlita	E-11130 Chiclana de la frontera (Cadiz) Spain	1st: 0.037 2nd: 0.037 3rd: 0.037 4th: 0.037 5th: 0.037	1st: 400 2nd: 400 3rd: 400 4th:400 5th: 400	1st: 0.1485 2nd: 0.1485 3rd: 0.1485 4th: 0.1485 5th: 0.1485	1st: BBCH 40 2nd: BBCH 43 3rd: BBCH 44 4th: BBCH 45 5th: BBCH 46	tubers	< 0.01	< 0.01	7	
10F PT GW P04	Potato/Spunta	E-11140 Conil de la frontera (Cadiz) Spain	1st: 0.037 2nd: 0.037 3rd: 0.037 4th: 0.037 5th: 0.037	1st: 400 2nd: 400 3rd: 400 4th:400 5th: 400	1st: 0.1485 2nd: 0.1485 3rd: 0.1485 4th: 0.1485 5th: 0.1485	1st: BBCH 40 2nd: BBCH 43 3rd: BBCH 44 4th: BBCH 45 5th: BBCH 46	tubers	< 0.01	< 0.01	7	

Conclusion

No residues were found on treated and non-treated plant samples (potato tubers) for both active substances (<0.01 mg/kg, i.e. LOQ of the analytical method).

The residues in the edible part of potatoes (tubers) of the supervised residue trials with 'Cymoxanil 33% + Zoxamide 33% WG' are below the EU harmonized MRL values.

From the cymoxanil DAR (N-EU)

Active substance (common name):	**Cymoxanil**	**Commercial Product (name):**	
Crop/crop group:	**Potato/Potatoes**	**Producer of commercial product:**	**Oxon**
Responsible body for reporting (name, address):	**Oxon** **Via Sempione 195- Pero (Milan)**	**Indoor/Glasshouse/Outdoor:**	**Outdoor**
Country:	**Italy**	**Other active substance in the formulation (common name and content):**	
Content of active substance (g/kg or g/L):	**500 g/kg**	**Residues calculated as:**	**Cymoxanil**
Formulation (*e.g.* WP):	**WP**		

1	2	3	4	5			6	7	8	9	10	11
Report No. Location (region)	Commodity / Variety	Date of 1. Sowing or Planting 2. Flowering 3. Harvest	Method of treatment	Application rate Per treatment			Dates of treatment(s) or no. of treatment(s) and last date	Growth stage at last treatment or date	Portion analyzed	Residues (mg/kg)	PHI (days)	Remarks:
	(a)	(b)	(c)	kg as/hL	Water (L/ha)	kg as/ha	(d)	(e)	(a)		(f)	(g)
20021200/E1-FPPO Innenheim (Alsace), France	Potato Spunta	1. 03/04/2002 2. n.i. 3. 27/08/2002	Spray portable boom spray 6 nozzles, 50 cm, 2 bar 100 ml/s	1st: 0.0266 2nd: 0.0266 3rd: 0.0267 4th: 0.0267	1st: 467 2nd: 458 3rd: 469 4th: 464	1st: 0.1244 2nd: 0.1220 3rd: 0.1250 4th: 0.1238	1st: 02/08/2002 2nd: 08/08/2002 3rd: 15/08/2002 4th: 21/08/2002	1st: 89-91 2nd: 91 3rd: 91-93 4th: 95	Tuber	< 0.05	6	HS (Harvest Study) Field code: F02N028R Analytical report: SIP 1353 Silty loam, no irrigation Aug: mean 18.6-22.0ºC GC-NPD; LOQ=0.05mg/kg
20021200/E1-FPPO Dollern (Lower Saxony), Germany	Potato Cilena	1. 05/04/2002 2. n.i. 3. 29-30/07-01-05/08/2002	Spray portable boom spray 6 nozzles, 50 cm, 2-3 bar 54-71 ml/s	1st: 0.0300 2nd: 0.0300 3rd: 0.0300 4th: 0.0300	1st: 427 2nd: 392 3rd: 405 4th: 413	1st: 0.1280 2nd: 0.1175 3rd: 0.1216 4th: 0.1240	1st: 08/07/2002 2nd: 15/07/2002 3rd: 23/07/2002 4th: 29/07/2002	1st: 69 2nd: 71 3rd: 79 4th: 91	Tuber	< 0.05 < 0.05 < 0.05 < 0.05	0 1 3 7	DCS (Decline Curve Study) Field code: G02N008R Analytical report: SIP 1353 Loamy sand, no irrigation July: mean 18.2ºC Aug: mean 16.7-25.7ºC GC-NPD; LOQ=0.05mg/kg
20021200/E1-FPPO Perouse (Baden-Württemberg), Germany	Potato Granola	1. 04/04/2002 2. n.i. 3. 19-20-22-26/08/2002	Spray portable boom spray 6 nozzles, 50 cm, 2.2 bar 124.2 ml/s	1st: 0.0300 2nd: 0.0300 3rd: 0.0300 4th: 0.0300	1st: 412 2nd: 398 3rd: 400 4th: 408	1st: 0.1235 2nd: 0.1195 3rd: 0.1200 4th: 0.1225	1st: 29/07/2002 2nd: 05/08/2002 3rd: 13/08/2002 4th: 19/08/2002	1st: 81 2nd: 91 3rd: 93 4th: 93	Tuber	< 0.05 < 0.05 < 0.05 < 0.05	0 1 3 7	DCS (Decline Curve Study) Field code: G02N009R Analytical report: SIP 1353 Sandy clayey loam, no irrigation July: mean 19.3ºC Aug: mean 2.4-24.0ºC GC-NPD; LOQ=0.05mg/kg

20021200/E1-FPPO Slochteren (Groningen), The Netherlands	Potato Elkana	1. 08/04/2002 2. n.i. 3. 02/09/2002	Spray portable boom spray 6 nozzles, 50 cm, 2 bar 58-71 ml/s	1st: 0.0300 2nd: 0.0300 3rd: 0.0300 4th: 0.0300	1st: 420 2nd: 418 3rd: 422 4th: 398	1st: 0.1260 2nd: 0.1253 3rd: 0.1267 4th: 0.1193	1st: 04/08/2002 2nd: 12/08/2002 3rd: 19/08/2002 4th: 26/08/2002	1st: 73 2nd: 75 3rd: 76-78 4th: 90	Tuber	< 0.05	7	HS (Harvest Study) Field code: NL02N001R Analytical report: SIP 1353 Humous sand, no irrigation Aug: mean 20.1ºC Sept: mean 16.1-22.5ºC GC-NPD; LOQ=0.05mg/kg

n.i.=not indicated

(a) According to EEC and Codex classifications (both) should be used

(b) Only if relevant

(c) High or low volume spaying, spreading, dusting *etc.*, overall, broadcast, type of equipment used must be indicated

(d) Year must be indicated

(e) BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4),

f) Minimum number of days after last application (Label pre-harvest interval, PHI, underline)

(g) Remarks may include: Climatic conditions; Reference to analytical method; Information concerning the metabolites included, the method of storage, storage stability, analysis date

From the cymoxanil DAR (S-EU)

Active substance (common name):	**Cymoxanil**	**Commercial Product (name):**	
Crop/crop group:	**Potato/Potatoes**	**Producer of commercial product:**	**Oxon**
Responsible body for reporting (name, address):	**Oxon** **Via Sempione 195- Pero (Milan)**	**Indoor/Glasshouse/Outdoor:**	**Outdoor**
Country:	**Italy**	**Other active substance in the formulation (common name and content):**	**mancozeb 700 g/kg**
Content of active substance (g/kg or g/L):	**60 g/kg**	**Residues calculated as:**	**Cymoxanil**
Formulation (*e.g.* WP):	**WP**		

1	2	3	4	5			6	7	8	9	10	11
Report No. Location (region)	Commodity / Variety	Date of 1. Sowing or Planting 2. Flowering 3. Harvest	Method of treatment	Application rate Per treatment			Dates of treatment(s) or no. of treatment(s) and last date	Growth stage at last treatment or date	Portion analyzed	Residues (mg/kg)	PHI (days)	Remarks:
	(a)	(b)	(c)	kg as/hL	Water (L/ha)	kg as/ha	(d)	(e)	(a)		(f)	(g)
COF/1, I-03 Salerano sul Lambro (Lodi), Lombardia Italy	Potato Kennebec	1. n.i. 2. n.i. 3. 08/08/1996	Spray	1st: 0.0198 2nd: 0.0196 3rd: 0.0196 4th: 0.0196 5th: 0.0196	1st: 500 2nd: 500 3rd: 500 4th: 500 5th: 500	1st: 0.099 2nd: 0.098 3rd: 0.098 4th: 0.098 5th: 0.099	1st: 03/07/1996 2nd: 10/07/1996 3rd: 18/07/1996 4th: 25/07/1996 5th: 01/08/1996	1st: flowering 2nd: flowering 3rd: post-flower 4th: leaf wilting 5th: yellow	Tuber	< 0.05	7	HS (Harvest Study) Field code: COF/1/3I/P Analytical report: R 6124 Flooding irrigation July: 12-33, mean 17-28ºC Aug: 13-33, mean 17-28ºC GC-TSD; LOQ=0.05mg/kg LOD=0.010 mg/kg
COF/1, I-04 S. Agostino (FE), Emilia Romagna Region Italy	Potato Agata	1. n.i. 2. n.i. 3. 04/07/1996	Spray	1st: 0.0204 2nd: 0.0190 3rd: 0.0226 4th: 0.0206 5th: 0.0206	1st: 500 2nd: 500 3rd: 500 4th: 500 5th: 500	1st: 0.102 2nd: 0.095 3rd: 0.113 4th: 0.103 5th: 0.103	1st: 30/05/1996 2nd: 06/06/1996 3rd: 13/06/1996 4th: 20/06/1996 5th: 27/06/1996	1st: post-flower 2nd: tuber grow 3rd: tuber grow 4th: tuber grow 5th: leaf wilting	Tuber	< 0.05	7	HS (Harvest Study) Field code: COF/1/4I/P Analytical report: R 6124 Rain irrigation May: 10.4-30.3ºC June: 13-36.2ºC July: 12.7-34.9ºC GC-TSD; LOQ=0.05mg/kg LOD=0.010 mg/kg

COF/2, I-03 Cavazzana di Lusia (Rovigo) Veneto region Italy	Potato Jaerla	1. 03/02/1997 2. n.i. 3. 20/06/1997	Spray	1st: 0.0199 2nd: 0.0200 3rd: 0.0200 4th: 0.0200 5th: 0.0201	1st: 542 2nd: 454 3rd: 515 4th: 521 5th: 508	1st: 0.108 2nd: 0.091 3rd: 0.103 4th: 0.104 5th: 0.102	1st: 16/05/1997 2nd: 23/05/1997 3rd: 30/05/1997 4th: 06/06/1997 5th: 13/06/1997	1st: vegetative 2nd: pre-flowering 3rd: flowering 4th: post-flower 5th: tuber grow	Tuber	< 0.05	7	HS (Harvest Study) Field code: COF/2/3I/P Analytical report: R 7180 Sandy soil, sprinkler irrigation May: 5-32, mean 11.7-24.5ºC June 9-31, mean 15.6-25.9ºC GC-NPD; LOQ=0.05mg/kg

n.i.=not indicated

(a) According to EEC and Codex classifications (both) should be used
(b) Only if relevant
(c) High or low volume spaying, spreading, dusting *etc.*, overall, broadcast, type of equipment used must be indicated
(d) Year must be indicated
(e) BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4),
f) Minimum number of days after last application (Label pre-harvest interval, PHI, underline)
(g) Remarks may include: Climatic conditions; Reference to analytical method; Information concerning the metabolites included, the method of storage, storage stability, analysis date

From the cymoxanil DAR (S-EU)

Active substance (common name): **Cymoxanil**
Crop/crop group: **Potato/Potatoes**
Responsible body for reporting (name, address): **Oxon**
Via Sempione 195- Pero (Milan)
Country: **Italy**
Content of active substance (g/kg or g/L): **60 g/kg**
Formulation (*e.g.* WP): **WP**

Commercial Product (name):
Producer of commercial product: **Oxon**
Indoor/Glasshouse/Outdoor: **Outdoor**
Other active substance in the formulation (common name and content): **mancozeb 700 g/kg**
Residues calculated as: **Cymoxanil**

1	2	3	4	5			6	7	8	9	10	11
Report No. Location (region)	Commodity / Variety	Date of 1. Sowing or Planting 2. Flowering 3. Harvest	Method of treatment	Application rate Per treatment			Dates of treatment(s) or no. of treatment(s) and last date	Growth stage at last treatment or date	Portion analyzed	Residues (mg/kg)	PHI (days)	Remarks:
	(a)	(b)	(c)	kg as/hL	Water (L/ha)	kg as/ha	(d)	(e)	(a)		(f)	(g)
COF/2, I-04 S. Giovanni Persiceto (Bologna), Emilia Romagna Region Italy	Potato Primura	1. n.i. 2. n.i. 3. 09/07/1997	Spray	1st: 0.0200 2nd: 0.0200 3rd: 0.0200 4th: 0.0200 5th: 0.0200	1st: 480 2nd: 525 3rd: 515 4th: 520 5th: 515	1st: 0.096 2nd: 0.105 3rd: 0.103 4th: 0.104 5th: 0.103	1st: 04/06/1997 2nd: 11/06/1997 3rd: 18/06/1997 4th: 25/06/1997 5th: 02/07/1997	1st: flowering 2nd: post-flower 3rd: post-flower 4th: tuber grow 5th: tuber grow	Tuber	< 0.05	7	HS (Harvest Study) Field code: COF/2/4I/P Analytical report: R 7180 Sandy loam, rain irrigation May: 3-32, mean 9.3-26.6ºC June: 10-34, mean 15-28.5ºC July: 9-35, mean 15.9-32.2ºC GC-NPD; LOQ=0.05mg/kg
CY2/PA, I-02 Salerano sul Lambro (Lodi), Lombardia region Italy	Potato Kennebec	1. 26/04/2001 2. 12/06/2001 3. 06-07-09-13/08/2001	Spray air-compress 4 nozzles, 50 cm, 4 bar, 9.9-30.3 ml/s	1st: 0.0241 2nd: 0.0240 3rd: 0.0239 4th: 0.0240 5th: 0.0240	1st: 490 2nd: 487 3rd: 506 4th: 483 5th: 488	1st: 0.118 2nd: 0.117 3rd: 0.121 4th: 0.116 5th: 0.117	1st: 09/07/2001 2nd: 16/07/2001 3rd: 23/07/2001 4th: 30/07/2001 5th: 06/08/2001	1st: 69 2nd: 71 3rd: 75 4th: 83 5th: 89	Tuber	< 0.05 < 0.05 < 0.05 < 0.05	0 (+3h) 1 3 7	DCS (Decline Curve Study) Field code: CY2/I/02PA Analytical report: SIP 1298 Sandy clay loam, flowing irrigation July: 12-36, mean 18-32ºC Aug: 14-36, mean 19-33ºC GC-NPD; LOQ=0.05 mg/kg

n.i.=not indicated

(a) According to EEC and Codex classifications (both) should be used
(b) Only if relevant
(c) High or low volume spaying, spreading, dusting *etc.*, overall, broadcast, type of equipment used must be indicated
(d) Year must be indicated
(e) BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4),
f) Minimum number of days after last application (Label pre-harvest interval, PHI, underline)
(g) Remarks may include: Climatic conditions; Reference to analytical method; Information concerning the metabolites included, the method of storage, storage stability, analysis date

From the cymoxanil DAR (S-EU)

Active substance (common name): **Cymoxanil**
Crop/crop group: **Potato/Potatoes**
Responsible body for reporting (name, address): **Oxon**
Via Sempione 195- Pero (Milan)
Country: **Italy**
Content of active substance (g/kg or g/L): **40 g/kg**
Formulation (*e.g.* WP): **WP**

Commercial Product (name):
Producer of commercial product: **Oxon**
Indoor/Glasshouse/Outdoor: **Outdoor**
Other active substance in the formulation (common name and content): **mancozeb 400 g/kg**
Residues calculated as: **Cymoxanil**

1	2	3	4	5			6	7	8	9	10	11
Report No. Location (region)	Commodity / Variety	Date of 1. Sowing or Planting 2. Flowering 3. Harvest	Method of treatment	Application rate Per treatment			Dates of treatment(s) or no. of treatment(s) and last date	Growth stage at last treatment or date	Portion analyzed	Residues	PHI (days)	Remarks:
				kg as/hL	Water (L/ha)	kg as/ha				(mg/kg)		
	(a)	(b)	(c)				(d)	(e)	(a)		(f)	(g)
CY2/PO, S-01 Chipiona (Cádiz), Spain	Potato Espunta	1. 17/12/2000 2. n.i. 3. 09-16/04/ 2001	Spray air-compress 4 nozzles, 50 cm, 3 bar, 111-115 ml/s	1st: 0.0120 2nd: 0.0120 3rd: 0.0120 4th: 0.0120 5th: 0.0120	1st: 1020 2nd: 1012 3rd: 960 4th: 980 5th: 1020	1st: 0.1224 2nd: 0.1214 3rd: 0.1152 4th: 0.1176 5th: 0.1224	1st: 05/03/2001 2nd: 12/03/2001 3rd: 19/03/2001 4th: 26/03/2001 5th: 02/04/2001	1st: 43 2nd: 44 3rd: 45 4th: 46 5th: 47	Tuber	< 0.05 < 0.05	7 14	Harvest study (HS) Field code: CY2/PO/S-01 Analytical report: SIP 1265 Sandy soil, irrigation: None March: 8.3-26.0ºC April: 9.0-29.5ºC GC-NPD; LOQ=0.05mg/kg
CY2/PO, S-02 Sanlúcar la Mayor (Sevilla), Spain	Potato Espunta	1. 07/02/2001 2. n.i. 3. 14-15-17-21-28/05/2001	Spray air-compress 4 nozzles, 50 cm, 3 bar, 112-116 ml/s	1st: 0.0120 2nd: 0.0120 3rd: 0.0120 4th: 0.0120 5th: 0.0120	1st: 994 2nd: 1006 3rd: 1024 4th: 1022 5th: 1040	1st: 0.1193 2nd: 0.1207 3rd: 0.1229 4th: 0.1227 5th: 0.1248	1st: 16/04/2001 2nd: 23/04/2001 3rd: 30/04/2001 4th: 07/05/2001 5th: 14/05/2001	1st: 43 2nd: 44 3rd: 45 4th: 46 5th: 48	Tuber	< 0.05 < 0.05 < 0.05 < 0.05 < 0.05	0 1 3 7 14	Decline Curve Study (DCS) Field code: CY2/PO/S-02 Analytical report: SIP 1265 Loamy sand, trickle irrigation April: 7.0-30.5ºC May: 7.0-37.0ºC GC-NPD; LOQ=0.05mg/kg

n.i.=not indicated

(a) According to EEC and Codex classifications (both) should be used
(b) Only if relevant
(c) High or low volume spaying, spreading, dusting *etc.*, overall, broadcast, type of equipment used must be indicated
(d) Year must be indicated
(e) BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4),
f) Minimum number of days after last application (Label pre-harvest interval, PHI, underline)
(g) Remarks may include: Climatic conditions; Reference to analytical method; Information concerning the metabolites included, the method of storage, storage stability, analysis date

From the cymoxanil DAR (S-EU)

Active substance (common name): **Cymoxanil**
Crop/crop group: **Potato/Potatoes**
Responsible body for reporting (name, address): **Oxon**
Via Sempione 195- Pero (Milan)
Country: **Italy**
Content of active substance (g/kg or g/L): **40 g/kg**
Formulation (*e.g.* WP): **WP**
Commercial Product (name):
Producer of commercial product: **Oxon**
Indoor/Glasshouse/Outdoor: **Outdoor**
Other active substance in the formulation (common name and content): **Cu-oxychloride 400 g/kg**
Residues calculated as: **Cymoxanil**

1	2	3	4	5			6	7	8	9	10	11
Report No. Location (region)	Commodity / Variety	Date of 1. Sowing or Planting 2. Flowering 3. Harvest	Method of treatment	Application rate Per treatment			Dates of treatment(s) or no. of treatment(s) and last date	Growth stage at last treatment or date	Portion analyzed	Residues (mg/kg)	PHI (days)	Remarks:
	(a)	(b)	(c)	kg as/hL	Water (L/ha)	kg as/ha	(d)	(e)	(a)		(f)	(g)
COF/1, I-03 Salerano sul Lambro (Lodi), Lombardia region Italy	Potato Kennebec	1. n.i. 2. n.i. 3. 08/08/1996	Spray	1st: 0.0200 2nd: 0.0200 3rd: 0.0202 4th: 0.0198 5th: 0.0204	1st: 500 2nd: 500 3rd: 500 4th: 500 5th: 500	1st: 0.100 2nd: 0.100 3rd: 0.101 4th: 0.099 5th: 0.102	1st: 03/07/1996 2nd: 10/07/1996 3rd: 18/07/1996 4th: 25/07/1996 5th: 01/08/1996	1st: flowering 2nd: flowering 3rd: post-flower 4th: leaf wilting 5th: yellow	Tuber	< 0.05	7	HS (Harvest Study) Field code: COF/1/3I/P Analytical report: R 6124 Flooding irrigation July: 12-33, mean 17-28ºC Aug: 13-33, mean 17-28ºC GC-TSD; LOQ=0.05mg/kg LOD=0.010 mg/kg
COF/1, I-04 S. Agostino (FE), Emilia Romagna Region Italy	Potato Agata	1. n.i. 2. n.i. 3. 04/07/1996	Spray	1st: 0.0230 2nd: 0.0190 3rd: 0.0204 4th: 0.0206 5th: 0.0208	1st: 500 2nd: 500 3rd: 500 4th: 500 5th: 500	1st: 0.115 2nd: 0.095 3rd: 0.102 4th: 0.103 5th: 0.104	1st: 30/05/1996 2nd: 06/06/1996 3rd: 13/06/1996 4th: 20/06/1996 5th: 27/06/1996	1st: post-flower 2nd: tuber grow 3rd: tuber grow 4th: tuber grow 5th: leaf wilting	Tuber	< 0.05	7	HS (Harvest Study) Field code: COF/1/4I/P Analytical report: R 6124 Rain irrigation May: 10.4-30.3ºC June: 13-36.2ºC July: 12.7-34.9ºC GC-TSD; LOQ=0.05mg/kg LOD=0.010 mg/kg

COF/2, I-03 Cavazzana di Lusia (Rovigo) Veneto region Italy	Potato Jaerla	1. 03/02/1997 2. n.i. 3. 20/06/1997	Spray	1st: 0.0200 2nd: 0.0199 3rd: 0.0202 4th: 0.0200 5th: 0.0199	1st: 550 2nd: 567 3rd: 521 4th: 525 5th: 542	1st: 0.110 2nd: 0.113 3rd: 0.105 4th: 0.105 5th: 0.108	1st: 16/05/1997 2nd: 23/05/1997 3rd: 30/05/1997 4th: 06/06/1997 5th: 13/06/1997	1st: vegetative 2nd: pre-flowering 3rd: flowering 4th: post-flower 5th: tuber grow	Tuber	< 0.05	7	HS (Harvest Study) Field code: COF/2/3I/P Analytical report: R 7180 Sandy soil, sprinkler irrigation May: 5-32, mean 11.7-24.5ºC June 9-31, mean 15.6-25.9ºC GC-NPD; LOQ=0.05mg/kg

n.i.=not indicated

(a) According to EEC and Codex classifications (both) should be used
(b) Only if relevant
(c) High or low volume spaying, spreading, dusting *etc.*, overall, broadcast, type of equipment used must be indicated
(d) Year must be indicated
(e) BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4),
f) Minimum number of days after last application (Label pre-harvest interval, PHI, underline)
(g) Remarks may include: Climatic conditions; Reference to analytical method; Information concerning the metabolites included, the method of storage, storage stability, analysis date

From the cymoxanil DAR (S-EU)

Active substance (common name): **Cymoxanil**
Crop/crop group: **Potato/Potatoes**
Responsible body for reporting (name, address): **Oxon**
Via Sempione 195- Pero (Milan)
Country: **Italy**
Content of active substance (g/kg or g/L): **40 g/kg**
Formulation (*e.g.* WP): **WP**

Commercial Product (name):
Producer of commercial product: **Oxon**
Indoor/Glasshouse/Outdoor: **Outdoor**
Other active substance in the formulation (common name and content): **Cu-oxychloride 400 g/kg**
Residues calculated as: **Cymoxanil**

1	2	3	4	5			6	7	8	9	10	11
Report No. Location (region)	Commodity / Variety	Date of 1. Sowing or Planting 2. Flowering 3. Harvest	Method of treatment	Application rate Per treatment			Dates of treatment(s) or no. of treatment(s) and last date	Growth stage at last treatment or date	Portion analyzed	Residues (mg/kg)	PHI (days)	Remarks:
	(a)	(b)	(c)	kg as/hL	Water (L/ha)	kg as/ha	(d)	(e)	(a)		(f)	(g)
S. Giovanni Persiceto (Bologna), Emilia Romagna Region Italy	Potato Primura	1. n.i. 2. n.i. 3. 09/07/1997	Spray	1st: 0.0200 2nd: 0.0200 3rd: 0.0200 4th: 0.0200 5th: 0.0200	1st: 510 2nd: 495 3rd: 500 4th: 480 5th: 495	1st: 0.102 2nd: 0.099 3rd: 0.100 4th: 0.096 5th: 0.099	1st: 04/06/1997 2nd: 11/06/1997 3rd: 18/06/1997 4th: 25/06/1997 5th: 02/07/1997	1st: flowering 2nd: post-flower 3rd: post-flower 4th: tuber grow 5th: tuber grow	Tuber	< 0.05	7	HS (Harvest Study) Field code: COF/2/4I/P Analytical report: R 7180 Sandy loam, rain irrigation May: 3-32, mean 9.3-26.6ºC June: 10-34, mean 15-28.5ºC July: 9-35, mean 15.9-32.2ºC GC-NPD; LOQ=0.05mg/kg

n.i.=not indicated

(a) According to EEC and Codex classifications (both) should be used
(b) Only if relevant
(c) High or low volume spaying, spreading, dusting *etc.*, overall, broadcast, type of equipment used must be indicated
(d) Year must be indicated
(e) BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4),
f) Minimum number of days after last application (Label pre-harvest interval, PHI, underline)
(g) Remarks may include: Climatic conditions; Reference to analytical method; Information concerning the metabolites included, the method of storage, storage stability, analysis date

From the cymoxanil DAR (S-EU)

Active substance (common name): **Cymoxanil**
Crop/crop group: **Potato/Potatoes**
Responsible body for reporting (name, address): **Oxon**
Via Sempione 195- Pero (Milan)
Country: **Italy**
Content of active substance (g/kg or g/L): **50 g/l**
Formulation (*e.g.* WP): **SC**

Commercial Product (name):
Producer of commercial product: **Oxon**
Indoor/Glasshouse/Outdoor: **Outdoor**
Other active substance in the formulation (common name and content): **chlorothalonil 375 g/l**
Residues calculated as: **Cymoxanil**

1	2	3	4	5			6	7	8	9	10	11
Report No. Location (region)	Commodity / Variety	Date of 1. Sowing or Planting 2. Flowering 3. Harvest	Method of treatment	Application rate Per treatment			Dates of treatment(s) or no. of treatment(s) and last date	Growth stage at last treatment or date	Portion analyzed	Residues (mg/kg)	PHI (days)	Remarks:
	(a)	(b)	(c)	kg as/hL	Water (L/ha)	kg as/ha	(d)	(e)	(a)		(f)	(g)
COF/1, I-03 Salerano sul Lambro (Lodi), Lombardia region Italy	Potato Kennebec	1. n.i. 2. n.i. 3. 08/08/1996	Spray	1st: 0.0204 2nd: 0.0206 3rd: 0.0206 4th: 0.0200 5th: 0.0194	1st: 500 2nd: 500 3rd: 500 4th: 500 5th: 500	1st: 0.102 2nd: 0.103 3rd: 0.103 4th: 0.100 5th: 0.097	1st: 03/07/1996 2nd: 10/07/1996 3rd: 18/07/1996 4th: 25/07/1996 5th: 01/08/1996	1st: flowering 2nd: flowering 3rd: post-flower 4th: leaf wilting 5th: yellow	Tuber	< 0.05	7	HS (Harvest Study) Field code: COF/1/3I/P Analytical report: R 6124 Flooding irrigation July: 12-33, mean 17-28ºC Aug: 13-33, mean 17-28ºC GC-TSD; LOQ=0.05mg/kg LOD=0.010 mg/kg
COF/1, I-04 S. Agostino (FE), Emilia Romagna Region Italy	Potato Agata	1. n.i. 2. n.i. 3. 04/07/1996	Spray	1st: 0.0216 2nd: 0.0218 3rd: 0.0214 4th: 0.0206 5th: 0.0222	1st: 500 2nd: 500 3rd: 500 4th: 500 5th: 500	1st: 0.108 2nd: 0.109 3rd: 0.107 4th: 0.103 5th: 0.111	1st: 30/05/1996 2nd: 06/06/1996 3rd: 13/06/1996 4th: 20/06/1996 5th: 27/06/1996	1st: post-flower 2nd: tuber grow 3rd: tuber grow 4th: tuber grow 5th: leaf wilting	Tuber	< 0.05	7	HS (Harvest Study) Field code: COF/1/4I/P Analytical report: R 6124 Rain irrigation May: 10.4-30.3ºC June: 13-36.2ºC July: 12.7-34.9ºC GC-TSD; LOQ=0.05mg/kg LOD=0.010 mg/kg

COF/2, I-03 Cavazzana di Lusia (Rovigo) Veneto region Italy	Potato Jaerla	1. 03/02/1997 2. n.i. 3. 20/06/1997	Spray	1st: 0.0201 2nd: 0.0200 3rd: 0.0200 4th: 0.0201 5th: 0.0200	1st: 488 2nd: 521 3rd: 500 4th: 513 5th: 554	1st: 0.098 2nd: 0.104 3rd: 0.100 4th: 0.103 5th: 0.111	1st: 16/05/1997 2nd: 23/05/1997 3rd: 30/05/1997 4th: 06/06/1997 5th: 13/06/1997	1st: vegetative 2nd: pre-flowering 3rd: flowering 4th: post-flower 5th: tuber grow	Tuber	< 0.05	7	HS (Harvest Study) Field code: COF/2/3I/P Analytical report: R 7180 Sandy soil, sprinkler irrigation May: 5-32, mean 11.7-24.5ºC June 9-31, mean 15.6-25.9ºC GC-NPD; LOQ=0.05mg/kg

n.i.=not indicated

(a) According to EEC and Codex classifications (both) should be used
(b) Only if relevant
(c) High or low volume spaying, spreading, dusting *etc.*, overall, broadcast, type of equipment used must be indicated
(d) Year must be indicated
(e) BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4),
f) Minimum number of days after last application (Label pre-harvest interval, PHI, underline)
(g) Remarks may include: Climatic conditions; Reference to analytical method; Information concerning the metabolites included, the method of storage, storage stability, analysis date

From the cymoxanil DAR (S-EU)

Active substance (common name):	**Cymoxanil**	**Commercial Product (name):**	
Crop/crop group:	**Potato/Potatoes**	**Producer of commercial product:**	**Oxon**
Responsible body for reporting (name, address):	**Oxon** **Via Sempione 195- Pero (Milan)**	**Indoor/Glasshouse/Outdoor:**	**Outdoor**
Country:	**Italy**	**Other active substance in the formulation (common name and content):**	**chlorothalonil 375 g/l**
Content of active substance (g/kg or g/L):	**50 g/l**	**Residues calculated as:**	**Cymoxanil**
Formulation (*e.g.* WP):	**SC**		

1	2	3	4	5			6	7	8	9	10	11
Report No. Location (region)	Commodity / Variety	Date of 1. Sowing or Planting 2. Flowering 3. Harvest	Method of treatment	Application rate Per treatment			Dates of treatment(s) or no. of treatment(s) and last date	Growth stage at last treatment or date	Portion analyzed	Residues (mg/kg)	PHI (days)	Remarks:
	(a)	(b)	(c)	kg as/hL	Water (L/ha)	kg as/ha	(d)	(e)	(a)		(f)	(g)
COF/2, I-04 S. Giovanni Persiceto (Bologna), Emilia Romagna Region Italy	Potato Primura	1. n.i. 2. n.i. 3. 09/07/1997	Spray	1st: 0.0200 2nd: 0.0200 3rd: 0.0200 4th: 0.0200 5th: 0.0200	1st: 500 2nd: 500 3rd: 485 4th: 500 5th: 495	1st: 0.100 2nd: 0.100 3rd: 0.097 4th: 0.100 5th: 0.099	1st: 04/06/1997 2nd: 11/06/1997 3rd: 18/06/1997 4th: 25/06/1997 5th: 02/07/1997	1st: flowering 2nd: post-flower 3rd: post-flower 4th: tuber grow 5th: tuber grow	Tuber	< 0.05	7	HS (Harvest Study) Field code: COF/2/4I/P Analytical report: R 7180 Sandy loam, rain irrigation May: 3-32, mean 9.3-26.6ºC June: 10-34, mean 15-28.5ºC July: 9-35, mean 15.9-32.2ºC GC-NPD; LOQ=0.05mg/kg

n.i.=not indicated

(a) According to EEC and Codex classifications (both) should be used
(b) Only if relevant
(c) High or low volume spaying, spreading, dusting *etc.*, overall, broadcast, type of equipment used must be indicated
(d) Year must be indicated
(e) BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4),
f) Minimum number of days after last application (Label pre-harvest interval, PHI, underline)
(g) Remarks may include: Climatic conditions; Reference to analytical method; Information concerning the metabolites included, the method of storage, storage stability, analysis date

A 2.2.4 Magnitude of residues in livestock

A 2.2.4.1 Livestock feeding studies

No new data / not relevant.

A 2.2.5 Magnitude of residues in processed commodities (industrial processing and/or household preparation)

A 2.2.5.1 Distribution of the residue in peel/pulp

Not relevant.

A 2.2.5.2 Processing studies on a core set of representative processes

No new data / not relevant.

A 2.2.6 Magnitude of residues in representative succeeding crops

Considering the available data dealing with nature of residues (see 7.2.2.2), no study dealing with magnitude of residues in succeeding crops is needed.

A 2.2.7 Other/Special Studies

The available data for the active substance sufficiently address aspects of the residue situation that might arise from the use of Cymoxanil 33% + Zoxamide 33% WG. Therefore, other special studies are not needed.

Appendix 3 Pesticide Residue Intake Model(s) (PRIMo)

A 3.1 EFSA Primo calculation

European Food Safety Authority

EFSA PRIMo revision 3.1; 2019/03/19

Zoxamide (F)

LOQs (mg/kg) range from: **0.01** to: **0.05**

Toxicological reference values

ADI (mg/kg bw/day):	**0.5**	ARfD (mg/kg bw):	**not necessary**
Source of ADI:		Source of ARfD:	
Year of evaluation:		Year of evaluation:	

Input values

Details - chronic risk assessment | Supplementary results - chronic risk assessment

Details - acute risk assessment/children | Details - acute risk assessment/adults

Comments:

Normal mode

Chronic risk assessment: JMPR methodology (IEDI/TMDI)

No of diets exceeding the ADI : ---

	Calculated exposure (% of ADI)	MS Diet	Expsoure (µg/kg bw per day)	Highest contributor to MS diet (in % of ADI)	Commodity / group of commodities	2nd contributor to MS diet (in % of ADI)	Commodity / group of commodities	3rd contributor to MS diet (in % of ADI)	Commodity / group of commodities	Exposure resulting from: MRLs set at the LOQ (in % of ADI)	Exposure resulting from: commodities not under assessment (in % of ADI)
TMDI/NEDI/IEDI calculation (based on average food consumption)	7%	NL toddler	32.74	4%	Spinaches	1%	Escaroles/broad-leaved endives	0.2%	Table grapes	0.4%	
	5%	IT adult	25.53	2%	Lettuces	0.9%	Other lettuce and other salad plants	0.6%	Spinaches	0.0%	
	4%	ES adult	22.35	3%	Lettuces	0.5%	Chards/beet leaves	0.4%	Spinaches	0.1%	
	4%	IT toddler	19.50	2%	Lettuces	0.7%	Other lettuce and other salad plants	0.4%	Chards/beet leaves	0.1%	
	4%	ES child	19.17	3%	Lettuces	0.5%	Spinaches	0.5%	Chards/beet leaves	0.1%	
	4%	GEMS/Food G10	18.29	2%	Lettuces	0.4%	Cress and other sprouts and shoots	0.3%	Spinaches	0.1%	
	3%	SE general	17.34	2%	Lettuces	0.4%	Spinaches	0.1%	Cucumbers	0.1%	
	3%	DE child	17.13	1%	Spinaches	0.5%	Lettuces	0.2%	Cucumbers	0.2%	
	3%	NL child	15.35	2%	Spinaches	0.6%	Escaroles/broad-leaved endives	0.4%	Lettuces	0.2%	
	3%	GEMS/Food G06	14.43	0.6%	Lettuces	0.4%	Watermelons	0.3%	Spinaches	0.1%	
	3%	GEMS/Food G08	13.58	1%	Lettuces	0.4%	Lamb's lettuce/corn salads	0.2%	Parsley	0.1%	
	3%	GEMS/Food G07	13.13	1%	Lettuces	0.2%	Spinaches	0.2%	Wine grapes	0.1%	
	2%	NL general	12.06	0.9%	Spinaches	0.6%	Escaroles/broad-leaved endives	0.6%	Lettuces	0.1%	
	2%	GEMS/Food G11	11.85	0.6%	Spinaches	0.5%	Lamb's lettuce/corn salads	0.5%	Lettuces	0.1%	
	2%	IE adult	11.26	0.8%	Spinaches	0.5%	Lettuces	0.3%	Melons	0.1%	
	2%	FR infant	11.01	2%	Spinaches	0.2%	Chards/beet leaves	0.2%	Courgettes	0.1%	
	2%	FR child 3 15 yr	10.20	0.7%	Other lettuce and other salad plants	0.6%	Spinaches	0.1%	Melons	0.1%	
	2%	FR adult	9.71	0.9%	Other lettuce and other salad plants	0.3%	Spinaches	0.3%	Wine grapes	0.1%	
	2%	DK child	8.96	0.8%	Lettuces	0.7%	Cucumbers	0.1%	Melons	0.1%	
	2%	FR toddler 2 3 yr	8.31	0.9%	Spinaches	0.2%	Other lettuce and other salad plants	0.1%	Parsley	0.1%	
	2%	GEMS/Food G15	8.27	0.7%	Lettuces	0.2%	Watermelons	0.2%	Parsley	0.1%	
	2%	DE women 14-50 yr	8.23	0.7%	Lettuces	0.3%	Spinaches	0.1%	Lamb's lettuce/corn salads	0.1%	
	1%	DE general	7.21	0.6%	Lettuces	0.3%	Spinaches	0.1%	Lamb's lettuce/corn salads	0.1%	
	1%	UK vegetarian	6.68	0.8%	Lettuces	0.2%	Spinaches	0.1%	Wine grapes	0.0%	
	1%	FI 6 yr	6.54	0.5%	Lettuces	0.3%	Spinaches	0.3%	Cucumbers	0.0%	
	1%	FI adult	6.15	0.8%	Lettuces	0.1%	Cucumbers	0.1%	Spinaches	0.1%	
	1%	FI 3 yr	6.13	0.4%	Cucumbers	0.4%	Spinaches	0.2%	Lettuces	0.0%	
	1%	PT general	5.48	0.6%	Lettuces	0.3%	Wine grapes	0.0%	Melons	0.1%	
	1%	UK adult	5.21	0.7%	Lettuces	0.1%	Wine grapes	0.1%	Spinaches	0.0%	
	1.0%	DK adult	4.86	0.5%	Lettuces	0.1%	Wine grapes	0.1%	Cucumbers	0.0%	
	0.7%	RO general	3.32	0.2%	Watermelons	0.2%	Wine grapes	0.1%	Cucumbers	0.1%	
	0.6%	LT adult	3.08	0.4%	Lettuces	0.2%	Cucumbers	0.0%	Tomatoes	0.0%	
	0.5%	UK toddler	2.63	0.2%	Spinaches	0.1%	Lettuces	0.0%	Cucumbers	0.1%	
	0.3%	PL general	1.68	0.1%	Lettuces	0.0%	Celery leaves	0.0%	Table grapes	0.0%	
	0.2%	UK infant	1.25	0.1%	Milk: Cattle	0.1%	Spinaches	0.0%	Tomatoes	0.1%	
	0.1%	IE child	0.58	0.0%	Lettuces	0.0%	Spinaches	0.0%	Sage	0.0%	

Conclusion:
The estimated long-term dietary intake (TMDI/NEDI/IEDI) was below the ADI.
The long-term intake of residues of Zoxamide (F) is unlikely to present a public health concern.

A 3.2 TMDI calculation - Cymoxanil

EFSA Primo rev. 2

cymoxanil

Prepare workbook for refined calculations

Undo refined calculations

Status of the active substance:		Code no.	
LOQ (mg/kg bw):		proposed LOQ:	
Toxicological end points			
ADI (mg/kg bw/day):	**0.013**	ARfD (mg/kg bw):	**0.08**
Source of ADI:		Source of ARfD:	
Year of evaluation:		Year of evaluation:	

Explain choice of toxicological reference values.

The risk assessment has been performed on the basis of the MRLs collected from Member States in April 2006. For each pesticide/commodity the highest national MRL was identified (proposed temporary MRL = pTMRL).
The pTMRLs have been submitted to EFSA in September 2006.

Chronic risk assessment

TMDI (range) in % of ADI minimum - maximum: 2 - 9

No of diets exceeding ADI: ---

Highest calculated TMDI values in % of ADI	MS Diet	Highest contributor to MS diet (in % of ADI)	Commodity / group of commodities	2nd contributor to MS diet (in % of ADI)	Commodity / group of commodities	3rd contributor to MS diet (in % of ADI)	Commodity / group of commodities	pTMRLs at LOQ (in % of ADI)
9.1	WHO Cluster diet B	4.7	Tomatoes	3.3	Table and wine grapes	1.0	Potatoes	
7.7	PT General population	4.3	Table and wine grapes	2.1	Potatoes	1.4	Tomatoes	
7.4	FR all population	6.3	Table and wine grapes	0.7	Tomatoes	0.4	Potatoes	
5.0	WHO cluster diet E	2.8	Table and wine grapes	1.5	Potatoes	0.8	Tomatoes	
4.4	DE child	2.0	Table and wine grapes	1.5	Tomatoes	1.0	Potatoes	
4.4	NL child	2.3	Potatoes	1.2	Table and wine grapes	1.0	Tomatoes	
4.0	WHO cluster diet D	1.6	Potatoes	1.6	Tomatoes	0.8	Table and wine grapes	
3.8	WHO regional European diet	1.7	Tomatoes	1.5	Potatoes	0.6	Table and wine grapes	
3.5	WHO Cluster diet F	1.3	Potatoes	1.1	Table and wine grapes	1.0	Tomatoes	
3.5	DK adult	2.3	Table and wine grapes	0.6	Tomatoes	0.6	Potatoes	
3.5	FR toddler	1.9	Potatoes	1.2	Tomatoes	0.3	Table and wine grapes	
3.3	IE adult	1.8	Table and wine grapes	0.9	Potatoes	0.6	Tomatoes	
3.2	PL general population	1.4	Tomatoes	1.3	Potatoes	0.5	Table and wine grapes	
3.1	SE general population 90th percentile	1.6	Potatoes	1.2	Tomatoes	0.3	Table and wine grapes	
3.0	NL general	1.3	Table and wine grapes	1.1	Potatoes	0.7	Tomatoes	
3.0	UK Adult	1.7	Table and wine grapes	0.7	Tomatoes	0.5	Potatoes	
2.9	UK vegetarian	1.4	Table and wine grapes	1.0	Tomatoes	0.5	Potatoes	
2.7	IT kids/toddler	2.2	Tomatoes	0.3	Potatoes	0.2	Table and wine grapes	
2.7	UK Toddler	1.3	Potatoes	0.9	Tomatoes	0.4	Table and wine grapes	
2.3	ES child	1.5	Tomatoes	0.7	Potatoes	0.1	Table and wine grapes	
2.3	ES adult	1.2	Tomatoes	0.7	Table and wine grapes	0.4	Potatoes	
2.2	IT adult	1.8	Tomatoes	0.2	Potatoes	0.2	Table and wine grapes	
2.2	LT adult	1.2	Potatoes	1.0	Tomatoes	0.0	Table and wine grapes	
2.0	DK child	0.9	Potatoes	0.8	Tomatoes	0.3	Table and wine grapes	
1.9	FR infant	1.6	Potatoes	0.2	Tomatoes	0.1	Table and wine grapes	
1.9	UK Infant	1.3	Potatoes	0.6	Tomatoes	0.1	Table and wine grapes	
1.6	FI adult	0.7	Tomatoes	0.5	Table and wine grapes	0.5	Potatoes	

Conclusion:
The estimated Theoretical Maximum Daily Intakes (TMDI), based on pTMRLs were below the ADI.
A long-term intake of residues of cymoxanil is unlikely to present a public health concern.

A 3.3 IESTI calculation – Cymoxanil

EFSA PRIMo rev. 2

Acute risk assessment /children						Acute risk assessment / adults / general population					
The acute risk assessment is based on the ARfD. For each commodity the calculation is based on the highest reported MS consumption per kg bw and the corresponding unit weight from the MS with the critical consumption. If no data on the unit weight was available from that MS an average European unit weight was used for the IESTI calculation. In the **IESTI 1** calculation, the variability factors were 10, 7 or 5 (according to JMPR manual 2002), for lettuce a variability factor of 5 was used. In the **IESTI 2** calculations, the variability factors of 10 and 7 were replaced by 5. For lettuce the calculation was performed with a variabilty factor of 3. **Threshold MRL** is the calculated residue level which would leads to an exposure equivalent to 100 % of the ARfD.											
Unprocessed commodities	**No of commodities for which ARfD/ADI is exceeded (IESTI 1):**		---	**No of commodities for which ARfD/ADI is exceeded (IESTI 2):**		---	**No of commodities for which ARfD/ADI is exceeded (IESTI 1):**		---	**No of commodities for which ARfD/ADI is exceeded (IESTI 2):**		---
	IESTI 1	***)**	****)**	**IESTI 2**	***)**	****)**	**IESTI 1**	***)**	****)**	**IESTI 2**	***)**	**)
	Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)	Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)	Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)	Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)
	16.4	Table grapes	0.2 / -	16.4	Table grapes	0.2 / -	7.9	Table grapes	0.2 / -	7.9	Table grapes	0.2 / -
	14.5	Tomatoes	0.2 / -	10.5	Tomatoes	0.2 / -	5.9	Wine grapes	0.2 / -	5.9	Wine grapes	0.2 / -
	9.6	Potatoes	0.05 / -	6.9	Potatoes	0.05 / -	3.8	Tomatoes	0.2 / -	3.1	Tomatoes	0.2 / -
	1.9	Wine grapes	0.2 / -	1.9	Wine grapes	0.2 / -	1.9	Potatoes	0.05 / -	1.5	Potatoes	0.05 / -
	No of critical MRLs (IESTI 1)		---				**No of critical MRLs (IESTI 2)**			---		
Processed commodities	**No of commodities for which ARfD/ADI is exceeded:**		---				**No of commodities for which ARfD/ADI is exceeded:**		---			
			*****)**						*****)**			
	Highest % of ARfD/ADI	Processed commodities	pTMRL/ threshold MRL (mg/kg)				Highest % of ARfD/ADI	Processed commodities	pTMRL/ threshold MRL (mg/kg)			
	8.2	Grape juice	0.2 / -				1.0	Wine	0.2 / -			
	4.4	Tomato juice	0.2 / -				0.5	Tomato (preserved-	0.2 / -			
	0.9	Potato puree (flakes)	0.05 / -				0.1	Raisins	0.2 / -			
	0.1	Wine	0.2 / -				0.1	Potato uree (flakes)	0.05 / -			
	0.1	Grapes (raisins)	0.2 / -				0.1	Fried potatoes	0.05 / -			
*) The results of the IESTI calculations are reported for at least 5 commodities. If the ARfD is exceeded for more than 5 commodities, all IESTI values > 90% of ARfD are reported. **) pTMRL: provisional temporary MRL ***) pTMRL: provisional temporary MRL for unprocessed commodity												
Conclusion: For cymoxanil IESTI 1 and IESTI 2 were calculated for food commodities for which pTMRLs were submitted and for which consumption data are available. No exceedance of the ARfD/ADI was identified for any unprocessed commodity. For processed commodities, no exceedance of the ARfD/ADI was identified.												

Appendix 4 Additional information provided by the applicant

None.