REGISTRATION REPORT

Part B

Section 6

Mammalian Toxicology

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The summaries and evaluations contained in this review report may be based on unpublished proprietary data submitted for the purpose of the assessment undertaken by the regulatory authority that prepared it.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authorities should not grant, amend, or renew a registration on the basis of the summaries and evaluation of unpublished proprietary data contained in this review report unless they have received the data on which the summaries and evaluation are based, either –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.
21st April 2021	Version revised by the applicant, highlighting in grey colour studies already evaluated during product authorisation and highlighting in green colour confirmatory-like studies which are under evaluation by the Latvia RMS for Zoxamide and concerned Member States in an interzonal procedure.
September 2021	Version evaluated by PL zRMS highlited in yellow
December 2021	Revised version, addressing the comments of MSs and comments and suplements of the applicant .

Table of Contents

6 Mammalian Toxicology (KCP 7)

This document summarises the toxicological data on 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, for authorisation in EU countries. Cymoxanil 33% + Zoxamide 33 % WG is a product on the EU market. It is a fungicide that has been jointly developed by the companies Gowan Crop Protection Ltd. (legal successor of the company Gowan Comercio Internacional e Servicos Limitada) and Sipcam Oxon S.p.A. (legal successor of the company OXON Italia S.p.A.). Cymoxanil 33% + Zoxamide 33 % WG is a fungicide, for which re-registration according to article 43 of regulation 1107/2009 is requested on behalf of Gowan Crop Protection Ltd., UK. 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.

This document is for the renewal of the authorisation of the product 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 for the parts which do not require a re-evaluation, including all assessments and data on cymoxanil.

The document is based on the Registration Report provided by the former zRMS UK/CRD, and Spanish INIA (zRMS) since UK is not part of EU anymore, and inhibits the evaluation results for product approval in the southern EU zone. Unchanged data from the previous version are highlighted in grey.

6.1 Summary

Table 6.1-1: Information on CYMOXANIL 33% + ZOXAMIDE 33% *

Product name and code	Cymoxanil 33% + Zoxamide 33% Trade name e.g. Lieto 66 WG, Lieto WG, Lieto GWN-9823 (Gowan's ownership) SIP 40936, SI 4656 (Sipcam Oxon's ownership)
Formulation type	Water dispersible granules [Code: WG]
Active substance(s) (incl. content)	Zoxamide, 330 g/kg Cymoxanil, 330 g/kg
Function	Fungicide
Product already evaluated as the 'representative formulation' during the approval of the active substance(s)	No

Product previously evaluated in another MS according to Uniform Principles	Yes Central zone: Austria, Belgium, Germany, The Netherlands, United Kingdom, Slovenia, Hungary, Romania, Check Republic, Ireland ⇨ with UK as former zRMS Southern zone: Spain, Greece, Croatia, France, Italy , Bulgaria, Portugal ⇨ with Spain as former zRMS

* Information on the detailed composition of CYMOXANIL 33% + ZOXAMIDE 33% / e.g. Lieto 66 WG can be found in the confidential dRR Part C.

Justified proposals for classification and labelling

According to the criteria given in Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008, the following classification and labelling with regard to toxicological data is proposed for the preparation:

Table 6.1-2: Justified proposals for classification and labelling for CYMOXANIL 33% + ZOXAMIDE 33% / e.g. Lieto 66 WG according to Regulation (EC) No 1272/2008

Hazard class(es), categories:	Acute toxicity, Cat. 4 Skin sensitization, Cat. 1 Reproductive toxicity, Cat. 2 Specific target organ toxicity (repeated exposure), Cat. 2
Hazard pictograms or Code(s) for hazard pictogram(s):	
Signal word:	Warning!
Hazard statement(s):	H302 - Harmful if swallowed H317 - May cause an allergic skin reaction H361fd – Suspected of damaging fertility. Suspected of damaging the unborn child H373 – May cause damage to organs (blood, thymus) through prolonged or repeated exposure H400 – Very toxic to aquatic life H410 – Very toxic to aquatic life with long-lasting effects
Precautionary statement(s):	P101- If medical advice is needed, have product container or label at hand. P102 - Keep out of reach of children. P201 - Obtain special instructions before use. P202 - Do not handle until all safety precautions have been read and understood P261 - Avoid breathing mist/vapours/spray. P264 - Wash hands thoroughly after handling. P270 - Do not eat, drink or smoke when using this product. P272 - Contaminated work clothing should not be allowed out of the workplace P273 - Avoid release to the environment, if this is not the intended use. P280 - Wear protective gloves/protective clothing/~~eye protection/face protection.~~ P301+P312 - IF SWALLOWED: Call a POISON CENTER / doctor if you feel unwell P302+P352 IF ON SKIN: Wash with plenty of water and soap. P308+P313 - IF exposed or concerned: Get medical advice/attention P330 - Wash mouth P333 + P313 - If skin irritation or rash occurs: Get medical advice/attention P362+P364 Take off contaminated clothing and wash it before reuse. P333+P317 If skin irritation or rash occurs: Get medical help. P391 - Collect spillage P405 - Store locked up P501 - Dispose of contents/container in accordance with local/regional /national/international regulations..

Additional labelling phrases:	To avoid risks to man and the environment, comply with the instructions for use. [EUH401] Contains zoxamide and cymoxanil

Table 6.1-3: Summary of risk assessment for operators, workers, residents and bystanders for CYMOXANIL 33% + ZOXAMIDE 33%

	Result	PPE / Risk mitigation measures
Operators	Acceptable	Grapes field, tractor mounted spraying devices: Normal work wear +gloves M/L+A, hood and visor A Grapes, manual applications: Normal work wear +gloves M/L+A Potatoes field , tractor mounted spraying devices: Normal work wear M/L+A * Due to the product classification (H317), normal work wear is recommended during M/L+A and gloves are generally proposed for mixing loading and maintenance
Workers	Acceptable	Grapes: Normal work wear, 2d re-entry Potatoes open field: Normal work wear
Bystanders	Acceptable	None
Residents	Acceptable	None Grapes: 10 m buffer zone, DRT and min water volume 400 L/ha Potatoes: 2-3 m buffer zone (standard)

No unacceptable risk for operators, workers, bystanders and residents was identified when the product is used as intended and provided that the PPE/ risk mitigation measures as stated in **Table 6.1-3** are applied.

In general, due to the product classification (H317), normal work wear is recommended during mixing/loading and application and gloves are generally proposed for mixing/loading and maintenance maintenance.

As a standard rule, treated crops should not be re-entered before spray deposits have completely dried. For workers re-entering grape fields for tying, etc., a 2 days re-entering period needs to be ovbserved.

A summary of the critical uses and the overall conclusion regarding exposure for operators, workers and bystanders/residents is presented in the following table.

Table 6.1-4: Critical uses and overall conclusion of exposure assessment

1	2	3	4	5	6	7	8	9	10			
Use-No.*	**Crops and situation (e.g. growth stage of crop)**	**F, Fn, Fpn G, Gn, Gpn or I ******	**Application**		**Application rate**		**PHI (d)**	**Remarks:** **(e.g. safener/synergist (L/ha))** **critical gap for operator, worker, bystander or resident exposure based on [Exposure model]**	**Acceptability of exposure assessment**			
			Method / Kind **(incl. application technique *****)**	**Max. number (min. interval between applications)** **a) per use** **b) per crop/ season**	**Max. application rate kg as/ha** **a) a.s. 1** **b) a.s. 2**	**Water L/ha** **min / max**			**Operator**	**Worker**	**Bystander**	**Residents**
1-12	Potatoes (BBCH 21 until PHI)	F	Spraying, LCTM	3 (7-10);	0.1485 (zoxamide + cymoxanil)	200 - 1000	7					
13-16	Wine and table grapes (BBCH 14-89)	F	Spraying, HCTM HH	3 (7-10); 3 (7-10)	0.1485 (zoxamide + cymoxanil)	400 - 1000	28					

* 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
*** e.g. LC: low crops, HC: high crop, TM: tractor-mounted, HH: hand-held

Explanation for column 10 "Acceptability of exposure assessment"

A	Exposure acceptable without PPE / risk mitigation measures
R	Further refinement and/or risk mitigation measures required
N	Exposure not acceptable/ Evaluation not possible

Data gaps

Data gaps should be listed in the summary to give an overview (especially for cMS).

Noticed data gaps are:
- None.

6.2 Toxicological Information on Active Substance(s)

Table 6.2-1: Information on active substance(s)

Common Name	**Zoxamide**	**Cymoxanil**
CAS-No.	156052-68-5	57966-95-7
Classification and proposed labelling		
With regard to toxicological endpoints (according to the criteria in Reg. 1272/2008, as amended)	Skin Sens. 1, H317 Warning GHS07	Acute Oral Tox. cat.4; Skin Sens. cat.1; STOT RE cat.2; Repr. Cat.2 GHS07; GHS08 Warning H302 H317 H373 H361fd
Additional C&L proposal	--	--
Agreed EU endpoints		
AOEL systemic	0.3 mg/kg bw/d based on a 90-day dog study (60 % oral absorption) and a safety factor of 100	0.01 mg/kg bw/d (corrected for 75% oral absorption)
Reference	EFSA Peer Review Conclusion (2017) EFSA Journal 2017;15(9):4980 SANTE/10052/2018 Rev 2 23 March 2018	EFSA Scientific Report (2008) 167, 1-116
Acute Acceptable Operator Exposure Level (AAOEL)	Not allocated, not necessary.	Not allocated, not necessary.
Reference	EFSA Peer Review Conclusion (2017)	EFSA Scientific Report (2008) 167, 1-116
Conditions to take into account/critical areas of concern with regard to toxicology		
	None. *	• the operator and worker safety and ensure that conditions of use prescribe the application of adequate personal protective equipment;
Reference	Final EC Renewal Report (SANTE/10052/2018 rev. 2, 2018)	EFSA Scientific Report (2008) 167, 1-116

* The following points related to the toxicity of zoxamide are mentioned in the EC Renewal Report (2018), but have been addressed :

1. Toxicity of zoxamide tech. as manufactured

The following point was considered as a critical area of concern as reported in the EFSA Conclusions (2017) for zoxamide, but was addressed in the EC Renewal Report (2018) as follows:
The batches used in the (eco)toxicity studies were concluded as not being representative of both the old (existing) and the new applicants proposed technical specifications for the active substance (see Sections 2 and 5).
EFSA identified a critical area of concern because two impurities (RH-141288 and RH-149687) present in the new technical specification were not covered by the toxicological batches.
However, the RMS does not agree with such conclusion. Impurity RH-141288 is also a significant mammalian metabolite (although found only in faeces in rat metabolism) and also an intermediate in the metabolic pathway which gives rise to other metabolites via oxidation. Therefore, it can be considered that RH-141288 has been intrinsically tested in the toxicity studies with the parent zoxamide. It is recommended that the Member States check the specifications of the technical material before granting authorizations and in case this impurity is present, a study to assess the toxicity of RH-141288 is requested from applicants and shared amongst Member States. Impurity RH-149687 is formed by hydrolysis of a chlorinating reagent used in the manufacture of zoxamide and has been extensively tested in a full range of toxicological studies which are in the public domain and which show that RH-149687 is not of toxicological concern.

This critical area of concern has been addressed in Part C (Confidential information). A complete genotox data package with a current zoxamide tech. production batch spiked with impurities at worst-case amounts confirmed the non-genotoxicity of zoxamide technical and supports the safety of the actual manufacturing material.

2. Toxicological relevance of RH-141455

The following points could not be finalised as reported in the EFSA Conclusions (2017) for zoxamide, but was addressed in the EC Renewal Report (2018) as follows::

1. The groundwater relevance assessment for groundwater metabolite RH-141455 predicted to be in annual average recharge leaving the top 1 m soil layer at > 0.75 µg/L could not be finalised, whilst the consumer risk assessment from drinking water originating from groundwater cannot be completed, as the available data are insufficient to set a reference value to complete the consumer risk assessment (see Sections 2 and 4).

The predicted concentrations of metabolite RH-141455 in groundwater are above 0.1 µg/L in all scenarios for grape vines and in all scenarios except the Sevilla scenario for potatoes (both under the PELMO and PEARL models and assuming applications are made every year or every three years). RH-141455 occurs at concentrations above 0.75 µg/L in four-five out of the nine pertinent FOCUS groundwater scenarios in both representative uses. EFSA therefore concludes that toxicological data are needed for this metabolite, e.g. a repeated-dose toxicity study, according to the guidance "Assessments of the relevance of metabolites in groundwater" (SANCO, 2003). However, considering the overall weight of evidence, RH-141455 should not be considered a relevant metabolite according to step 3 of the available guidance on metabolites in groundwater and is very unlikely to pose any risk to consumers via groundwater. The available data show that:

1) RH-141455 lacks the haloketone toxophore (chemical group responsible for the main toxicity) and was found to have no fungicidal activity.

2) The EFSA expert meeting concluded that RH-141455 is unlikely to be genotoxic and is less toxic than the parent compound zoxamide, which already shows a low toxic profile.

3) Predicted concentration levels of RH-141455 are below 0.75 µg/L in 3 scenarios for grape vines and in 5 scenarios for potatoes.

4) At the Pesticide Peer Review meeting, experts concluded that, if the approach of the Thresholds of Toxicological Concern (TTC) is used, RH-141455 would be of class Kramer 3 (i.e. exposure threshold would be 1.5 µg/Kg b.w). Using conservative assumptions, the estimated exposure would be 0.17 µg/Kg b.w, which is an order of magnitude lower than the appropriate TTC.

Since RH-141455 is considered less toxic than zoxamide, following a conservative approach, the ADI of zoxamide could be used to assess the consumer risk for RH-141455. Doing so, the predicted intake of RH-141455 from drinking water would result 0.17 µg/kg (i.e. .0.034% of the ADI for a 60 kg person). This means that RH-141455 would have to be significantly more toxic than zoxamide to pose any risk to the consumers via drinking water.

This data point has been further addressed in chapter 6.3. Additioanal tox studies were provided. As a result,

metabolite RH-141455 is regarded as toxicologically non relevant.

zRMS: The justification provided is acceptable and metabolite RH-141455 can be considered as toxicologiocally non-relevant.

2. Stability of the isomer's composition of zoxamide and its isomeric metabolites

The following point could not be finalised as reported in the EFSA Conclusions (2017) for zoxamide, but was addressed in the EC Renewal Report (2018) as follows::

The human health and environmental risk assessment consequent to potential changes in the isomer composition for zoxamide and metabolites RH-127450, RH-163353, [RH-150721 human health only] could not be finalised (see Sections 2, 3, 4 and 5).

For all of the substances assessed as racemic mixtures (zoxamide, RH-127450, RH-163353 and RH-150721), the chiral carbon is chemically stable, therefore interconversion is highly unlikely. Moreover, the available metabolism and degradation data do not show any preferential metabolism of one isomer over another one in either mammals, plants or the environment. A soil degradation study completed after the peer review showed no difference in rate of degradation of the isomers of neither zoxamide nor the major soil metabolite 127450. Even making the worst case assumption (all toxicity residues in one isomer and residues in crops comprised of only this isomer), dietary exposure would still be less than 8.2% the ADI.

The soil degradation study has been provided in Part B Section 8 (Kercher, 2017; report no. AS520), the stability of the chiral carbon of zoxamide and its racemic metabolites has been demonstrated in a range of studies/commodities throughout the different sections (residues, efate, ecotox).

2. Toxicity of metabolites RH-141452, RH-141455 and RH-150721 for consumers

The following point could not be finalised as reported in the EFSA Conclusions (2017) for zoxamide, but was addressed in the EC Renewal Report (2018) as follows::

The consumer risk assessment could not be finalised due to a number of data gaps that likely have an impact on the assessment of residue levels and due to the pending toxicological evaluation of metabolites which are included in the residue definition for risk assessment and monitoring (see Section 3).

EFSA concludes that a dietary consumer risk assessment could not be completed, due to data gaps on the toxicity profile of metabolites RH-141452, RH-141455 and RH-150721. EFSA proposes for some crops provisional residue definitions including some of these metabolites.

However, the overall evidence available as regards metabolites RH-141452, RH-141455 shows that they are unlikely to pose any risk to consumers via dietary exposure:

1) Both metabolites have no fungicidal activity,

2) LD50 for zoxamide as well as for both these metabolites is > 5000 mg/kg bw.

Data available show that RH-141455 is unlikely to be genotoxic and results in an Ames test were negative for metabolite 141452 and that they are both less toxic than the parent compound.

4) Using a conservative approach, the ADI of zoxamide could be applied to RH-141452 and RH-141455, and estimates using the EFSA PRIMO model rev 2.0 and based on provisionally estimated occurrence levels in potatoes result in exposure levels ranging from 0 to 0.1% of the ADI.

The metabolite RH-150721 is only relevant for the use on grapes, which has been excluded from the 'supported uses' (Appendix II).

Moreover, these data gaps were identified only late in the peer review (the current residue definition only comprises zoxamide). The applicant could provide them for renewal of product authorization, in accordance with the criteria for 'category 4' laid down in the guidance SANCO/2010/13170 rev.14.7 October 2016.

This data point has been further addressed in chapter 6.3. Additioanal tox studies for RH-141452, RH-141455 and RH-150721 were provided.

As a result, metabolite RH-141455 is regarded as toxicologically non relevant.

Taking into account that RH-141452

- is not genotoxic,

- showed an acute oral toxicity of LC_{50} > 5000 mg/kg bw/d (see EFSA, 2017), which is above 2000 mg/kg bw/d, the dose for classification, and
- is predicted to have a similar toxicity as RH-141455 (which is regarded as toxicologically non relevant)

this metabolite can be regarded as toxicologically not relevant without a further proof (i.e. without further repeated dose toxicity studies).

Neither RH-141455 nor RH-141452 should be included in the residue definition for plants.

An ADI for RH-150721 has been proposed.

zRMS: The justification provided is acceptable and metabolite RH-141452 can be considered as toxicologiocally non-relevant.

6.3 Toxicological Evaluation of Plant Protection Product

A summary of the toxicological evaluation for CYMOXANIL 33% + ZOXAMIDE 33% is given in the following tables. Full summaries of studies on the product that have not been previously considered within an EU peer review process are described in detail in Appendix 2.

Table 6.3-1: Summary of evaluation of the studies on acute toxicity including irritancy and skin sensitisation for CYMOXANIL 33% + ZOXAMIDE 33% / E.g. Lieto 66 WG

Type of test, species, model system (Guideline)	Result	Acceptability	Classification (acc. to the criteria in Reg. 1272/2008)	Reference
LD_{50} oral, rat (OECD 401)	1469 mg/kg bw	YES	H302	xxx., 1999; report no. 99R-102
LD_{50} dermal, rat (OECD 402)	> 5000 mg/kg bw	YES	None	xxx, 1999; report no. 99R-103
LC_{50} inhalation (OECD 403)	> 4.4 mg/L air	YES	None	xxx, 1999; report no. 99R-106
Skin irritation, rabbit (OECD 404)	Non-irritant	YES	None	xxx, 1999; report no. 99R-104
Eye irritation, rabbit (OECD 405)	Non-irritant	YES	None	xxx, 1999; report no. 99R-105
Skin sensitisation, guinea pig (OECD 406, M&K)	Sensitising	YES	H317	xxx., 1999; report no. 99R-107
Supplementary studies for combinations of plant protection products	No data - not required		--	--

Table 6.3-2: Additional toxicological information relevant for classification/labelling of CYMOXANIL 33% + ZOXAMIDE 33% / e.g. Lieto 66 WG

	Substance (concentration in product, % w/w)	**Classification of the substance (acc. to the criteria in Reg. 1272/2008)**	**Reference**	**Classification of product (acc. to the criteria in Reg. 1272/2008)**
Toxicological properties of active substance(s) (relevant for classification of product)	Zoxamide tech. (33 % (w/w))	Skin Sens. 1, H317 (criteria ≥ 0.1 %)	Reg. 1272/2008 / MSDS**	Skin Sens. 1, H317
	Cymoxanil tech. (33 % (w/w))	Acute Tox. 4, H302 Skin Sens. 1, H 317 STOT RE 2, H373 (blood, thymus) Repr. 2, H361fd	6th ATP to Reg. 1272/2008 / MSDS**	Acute Tox. 4, H302 Skin Sens. 1, H317 STOT RE 2, H373 (blood, thymus) Repr. 2, H361fd
Toxicological properties of non-active substance(s) (relevant for classification of product)	Co-formulant 1 (CAS No. 8061-51-6, 20.8 % (w/w))*	--	MSDS**	None
	Co-formulant 2 (CAS No. 1332-58-7, 6.6 % (w/w))	--	MSDS**	None
	Co-formulant 3 (CAS No. 85586-07-8, CAS No. 80206-82-2; 2% (w/w))	Acute Tox. 4, H302 Skin Irrit. 2, H315 Eye Dam. 1, H318 Aquatic Chronic 3, H412	MSDS**	None +
	Co-formulant 4 (CAS No. 112926-00-8, 2 % (w/w))	--	MSDS**	None
Further toxicological information	--			

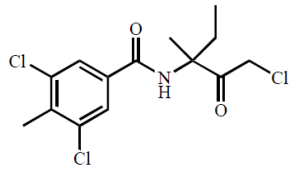
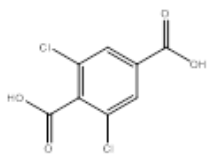
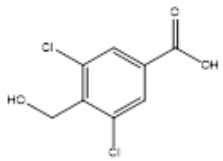
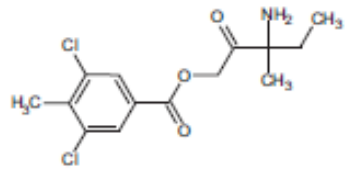
* Concentration range or concentration limit (e.g. 1-10% or > 1%) as provided in MSDS.
** Material safety data sheet (see Part C, Confidential information).
+ Justification for non-relevance of product classification from ingredient: The toxicological properties of the product have been studied; see results of acute tox studies in Table 6.3 1 in the dRR Part B Section 6.

Besides, the active substance zoxamide has been regarded during the AIR as follows (see EFSA Peer Review Conclusion, 2017):

Supplementary studies on the active substance	**Mechanism of action on Phytophthora capsici, tobacco, mouse lymphoma cells and isolated bovine tubulin:** Zoxamide was comparable in potency to carbendazim in inhibiting microtubule assembly and the growth of mouse lymphoma cells, and was considerably less active than colchicine and vinblastine. **Immunotoxicity:** zoxamide has no immunotoxic potential based on the available standard toxicity studies.
Endocrine disrupting properties	Zoxamide is unlikely to have endocrine disrupting properties.

However, additional data are submitted for metabolites of zoxamide, which may appear in processed commodities or for which EFSA (2017) has requested additional data. A summary of the available and new data is given in the following. The toxicological studies were evaluated during active ingredient renewal (AIR) on EU level (please refer to RAR, 2017 and EFSA Peer Review Conclusion, 2017), additionally requested studies by EFSA (2017) are summarised in this document. Only new studies are provided with this document.

Table 6.3-3: Structural formula of zoxamide and metabolites under discussion

			
Zoxamide	RH-141455	RH-141452	RH-150721

RH-141455 and RH-141452

The zoxamide metabolites RH-141452 and RH-141455 were identified as major metabolites (>10% TRR) in the potato metabolism study (xxx, PH and Spencer, WO, 1998, CA 6.1/02) but were not generally found in supervised field trials at levels >LOQ during AIR. Lower levels of RH-141452 were also found in the tomato and cucumber metabolism studies, and RH-141455 in the pea metabolism study. RH-141455 may occur in groundwater at levels > 0.1 µg/L.

RH-141452 and RH-141455 are formed by the hydrolysis of zoxamide to form the intermediate, RH-24549, which is then oxidised first to RH-141452 and then to RH-141455 (see rat metabolism Figure 2).

Both RH-141452 and RH-141455 are rat metabolites found at low levels in urine (xxx. 1998a, Report No: 94R-235, ER Ref No: 24.1). RH-141452 (designated M-17 in the rat metabolism study) was isolated from rat urine by acid/base extraction, and identified by HPLC and TLC by comparison with an authentic reference standard. Analysis by LC-MS gave a molecular weight consistent with RH-141452. The identity was confirmed by derivatisation (methylation with diazomethane) followed by GC/MSD analysis. RH-141452 (M-17) was estimated to account for 0.37% of the administered dose in the low dose female group. RH-141455 was not detected by this method.

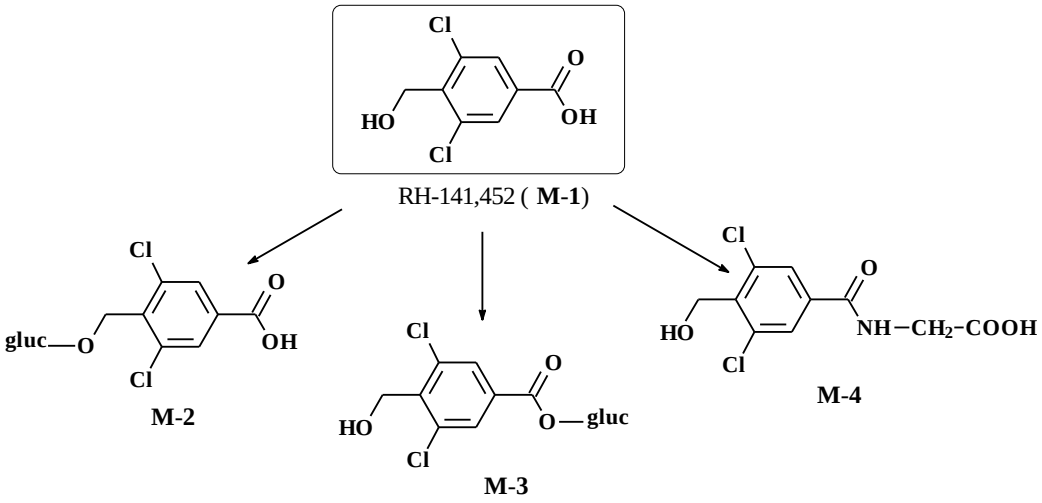
Both RH-141452 and RH-141455 were, however, found in rat urine using a non-radiolabelled residue method. Rat urine was diluted with water, acidified and extracted with ethyl acetate to separate the acidic components. After concentration and derivatization (methylation) with diazomethane, the metabolites were identified by GC/MSD and quantified using GC-ECD. Using this method, RH-141455 was found at

0.006% of dose in males and 0.004% of dose in females. RH-141452 was found at higher levels of 0.037% of dose in males and 0.034% of dose in females.
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 would be 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 xxx. 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. The metabolite pathways are illustrated as follows:

Figure 1: Metabolic pathway for RH-141452 in the rat



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 would be expected to be less toxic than parent zoxamide. In acute oral toxicity studies in male and female mice, the acute oral LD50 of RH-141452 and RH-141455 in male and female mice were both > 5000 mg/kg bw.

In genotoxicity testing, both metabolites gave negative results in the *Salmonella typhimurium* gene mutation assay (Ames test). In addition, RH-141455 gave negative results in an *in vitro* micronucleus test in human lymphocytes and also in an in vitro mutation test using mouse lymphoma L5178Y cells.

In addition, a comparison of the toxicological profile of zoxamide and two metabolites, RH-141452 and RH-141455 has been made using OECD QSAR Toolbox version 3.4.0.17 (Pellizzaro, M. and Da Silva, M., 2017; see RAR (2017)). This analysis also indicates that both metabolites are expected to have a lower toxicity than parent zoxamide.

However, EFSA (2017) requested *"Further genotoxic data are needed for metabolites RH-141452 and RH-150721, and further repeated dose toxicity studies in order to set reference values for RH-141452,*

RH-141455 and RH-150721 were not available (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see Section 2)." These data were provided as far as applicable and are summarised in the following. Full summaries of studies on the metabolite that have not previously been considered within an EU peer review process are additionally described in detail in Appendix 2 (A 2.11 Other/Special Studies).

Table 6.3-4: Summary of the results of toxicity studies for RH-141455

Type of test, species (Guideline)	Result	Acceptability	Reference*
Ames (OECD 471)	non-genotoxic	Yes	xxx 1998*; Report no. 98R-048
In vitro mutation test using mouse lymphoma L5178Y cells (OECD 476)	non-genotoxic	Yes	xxx, 2014*; Report no. FRK0049
In vitro micronucleus test in human lymphocytes (OECD 487)	non-genotoxic	Yes	xxx, 2014*; Report no. FRK0050
Acute oral mouse (OECD 401)	LD_{50} >5000	Yes	xxx, 1998*; Report no. 98R-047
14 d dietary toxicity in rats (OECD 407)	NOAEL >1000 mg/kg bw/d**	YES	xxx, 2020; Report no. U-19071
90 d dietary toxicity in rats (OECD 408) Limit test incl. 28 d recovery and plasma TK	NOAEL >1000 mg/kg bw/d ***	YES	xxx, 2020; Report no. U-19102

* indicates that a study was reviewed at EU level
** NOAEL = 15000 ppm, which is equivalent to 1123 and 1069 mg/kg body weight/day for the males and females, respectively.
***NOAEL = 16000 ppm, which is equivalent to 924 and 1119 mg/kg body weight/day for the males and females, respectively.

Table 6.3-5: Summary of the results of toxicity studies for RH-141452

Type of test, species (Guideline)	Result	Acceptability	Reference*
Ames test (OECD 471)	non-genotoxic	Yes	xxx. (1998)*; Report no. 98R-050
In vitro mammalian cell gene mutation test (HPRT-Locus) in Chinese Hamster V79 cells (OECD 476)	non-genotoxic	YES	xxx, 2020; Report no. 188620
In vitro mammalian micronucleus assay in Chinese Hamster V79 cells (OECD 487)	non-genotoxic	YES	xxx, 2019; Report no. 188616
Acute oral mouse (OECD 401)	LD_{50} >5000	Yes	xxx, 1998*; Report no. 98R-049

* indicates that a study was reviewed at EU level

As a result, metabolite RH-141455 is regarded as toxicologically non relevant.

Taking into account that RH-141452

- is not genotoxic,
- showed an acute oral toxicity of LC_{50} > 5000 mg/kg bw/d (see EFSA, 2017), which is above 2000 mg/kg bw/d, the dose for classification and

- is predicted to have a similar toxicity as RH-141455 (which is regarded as toxicologically non relevant)

this metabolite can be regarded as toxicologically not relevant without a further proof (i.e. without further repeated dose toxicity studies).

Neither metabolite should be included in the residue definition for plants.

zRMS: Based on presented data the metabolites of zoxamide RH-141455 and RH-141452 are considered as toxicologically not relevant in line with criteria given in Guidance on the assessment of the relevance of metabolites in groundwater of substances regulated under Council Directive 91/414/EEC (Sanco/221/2000 –rev.10- final 25 February 2003

RH-150721

RH-150721 (IUPAC name 3-amino-3-methyl-2-oxopentyl-3,5-dichloro-p-toluate, CAS No. 209809-78-9) is not a major metabolite in crops but forms a significant component of the residue in wine and appears >10% in a nature of hydrolysis study with zoxamide (study no. RB66JN) requested by EFSA (2017).

RH-150721 is a proposed intermediate in mammalian metabolism but was not isolated either from a rat metabolism study or in an *in vitro* comparative metabolism study using human, dog, rat and mouse hepatocytes. However, within the metabolic pathway in rats, reference is also made to the "aqueous hydrolysis study" (Reynolds, 1998). The study showed 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 metabolite RH-150721 is formed by elimination of hydrogen chloride, resulting in the transient cyclic metabolite RH-129151, followed by ring opening and further degradation.

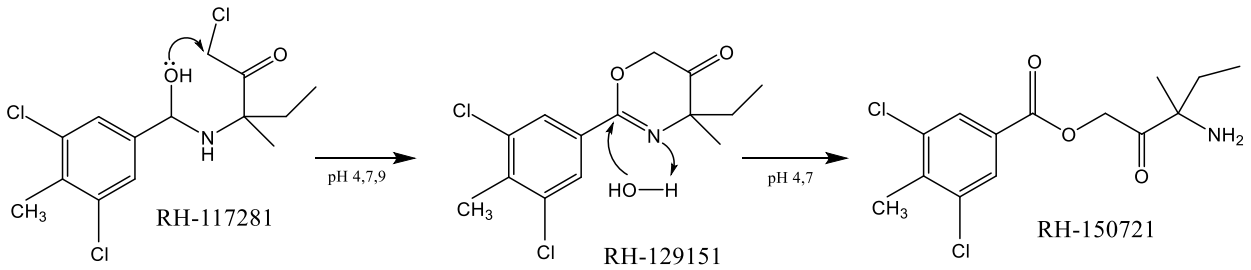


Figure 2: Formation of RH-150721

Additionally, stability 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. 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).

Other than a negative Ames test, no laboratory toxicological studies have been performed during AIR. However, QSAR analyses performed with both DEREK and the OECD Toolbox were available. According to both models, RH-150721 does not contain structural alerts for any significant toxicological hazards (in particular carcinogenicity, mutagenicity, reprotoxicity, acute toxicity or specific target organ toxicity) that are not also present in zoxamide.

The Derek Nexus QSAR analysis showed no active mutagenic or genotoxicity alerts (including clastogenicity), and only 2 other alerts common to the parent [116 Polyhalogenated aromatic = Carcinogenicity in

mammal is PLAUSIBLE (Extrapolation from alpha-2-mu-Globulin nephropathy) and 264 Polyhalogenated benzene = alpha-2-mu-Globulin nephropathy in mammal is EQUIVOCAL]. A summary of the OECD Toolbox report is presented in Volume 4 Part C of the RAR (2017). The OECD Toolbox analysis did not highlight any new areas of significant toxicological concern (in particular carcinogenicity, mutagenicity, reprotoxicity, acute toxicity or specific target organ toxicity) for RH-150721 over those identified for zoxamide.

However, EFSA (2017) requested "*Further genotoxic data are needed for metabolites RH-141452 and RH-150721, and further repeated dose toxicity studies in order to set reference values for RH-141452, RH-141455 and RH-150721 were not available (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see Section 2).*" These data were provided for RH-150721 and are summarised in the following. Full summaries of studies on the metabolite that have not previously been considered within an EU peer review process are additionally described in detail in Appendix 2 (A 2.11 Other/Special Studies).

Table 6.3-6: Summary of the results of toxicity studies for RH-150721

Type of test, species (Guideline)	Result	Acceptability	Reference*
Ames (OECD 471)	non-genotoxic	Yes	xxx, 2013*; Report no. 1549300
In vitro mammalian cell gene mutation assay in mouse lymphoma L5178Y cells (OECD 490)	non-genotoxic	Yes	xxx, 2017**; Report no. 171360
In vitro mammalian micronucleus assay in Chinese hamster V79 cells (OECD 487)	non-genotoxic	Yes	xxx 2017**; Report no. 171361
14 d dietary toxicity in rats (OECD 407)	NOAEL = 5000 ppm (equivalent to appr. 334 mg/kg bw/day in males and 382 mg/kg bw/day in females)	YES	xxx, 2020; Report no. U-19189
90 d dietary toxicity in rats (OECD 408) incl. 28 d recovery and plasma TK	NOAEL = 2000 ppm (111 mg/kg bw/d in males)	YES	xxx, 2020; Report no. U-19235

* indicates that a study was reviewed at EU level
** submitted to EFSA in 2017, but not yet reviewed

An *in vitro* micronucleus and a mouse lymphoma assay with the metabolite RH-150721 were submitted to EFSA in 2017, but not reviewed and summarised in the RAR. These studies of Schreib (2017, study no. 171360) and Donath (2017, study no. 171361) are summarised in Part B section 6 with negative results.

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. During the 14 days range finding study of xxx (2020, study no. U-19189), blood levels of RH-150721 could not be measured as the metabolism was too quick. There were no mortalities. Clinical signs were limited to thin appearance of females at 16000 ppm, which recovered after reduction of the top dose. Body weight and food consumption was reduced in the mid and high dose group, reaching statistical significance in males at the mid dose. There were no significant effects on haematology or urine analysis. Clinical chemistry effects were limited to the high dose and most likely linked to the strongly reduced food consumption in the initial phase of the study (BUN↓, total protein↓, albumin↓, globulin↓, phosphorus↓; ALT↑, ALP↑). Several organ weights were

slightly reduced vs. control, which was judged secondary to the low food consumption. The only histopathological finding that was not judged to be related to low food consumption was acinar hypertrophy of the salivary glands, a finding described before for other compounds as an adaptive change. During the performance of the 90 days dietary study on rats (xxx (2020, study no. U-19235), there were no mortalities, clinical signs, ophthalmoscopic changes, FOB effects or gross pathology findings. Body weight and food consumption were reduced in the high dose group, reaching statistical significance. During recovery, body weights improved but not to the level of control. Haematological effects were limited to the top dose, and mainly consisted of rather mild decreases of red blood cells, haemoglobin and haematocrit with increased MCV and MCH; RBC, MCH and MCV were not fully recovered after recovery. Due to the low magnitude, effects were not judged as adverse in nature. Clinical chemistry effects were identical to the effects seen in the 14 days study, correlate with the body weight, and were not considered adverse. Thyroid weights were decreased in the top dose, and T4 levels at the mid and top dose. The effects were considered adverse in the top dose as they correlated with minimal to mild colloid alteration in males only; effects on T4 were completely recovered to control level in both sexes after recovery period. Effects in the mid dose were not considered adverse due to the low magnitude and the absence of a histopathological correlate. Multiple organ weights were reduced at end of treatment, correlated with body weight, and recovered during recovery level; they were not considered adverse. Liver weights were minimally reduced at the end of recovery, however without a histopathological correlation and thus judged as being not adverse. Minimal to mild acinar hypertrophy of the salivary glands was found at the high dose, considered adaptive, but related to treatment. The NOAEL was set to 2000 ppm (111 mg/kg bw/d in males) based on reductions in body weight and food consumption, colloid changes in the thyroid and acinar hypertrophy of the salivary glands at the next higher dose level.

Full summaries of studies on the metabolite that have not previously been considered within an EU peer review process are additionally described in detail in Appendix 2 (A 2.11 Other/Special Studies).

zRMS: Based on presented data the metabolite of zoxamide RH-150721 is considered as toxicologically not relevant in line with criteria given in Guidance on the assessment of the relevance of metabolites in groundwater of substances regulated under Council Directive 91/414/EEC (Sanco/221/2000 –rev.10- final 25 February 2003

ADI for RH-150721

The toxicological profile of RH-150721 is mainly driven by body weight and food consumption 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). This proposal is based on the REACH guidance[1].

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

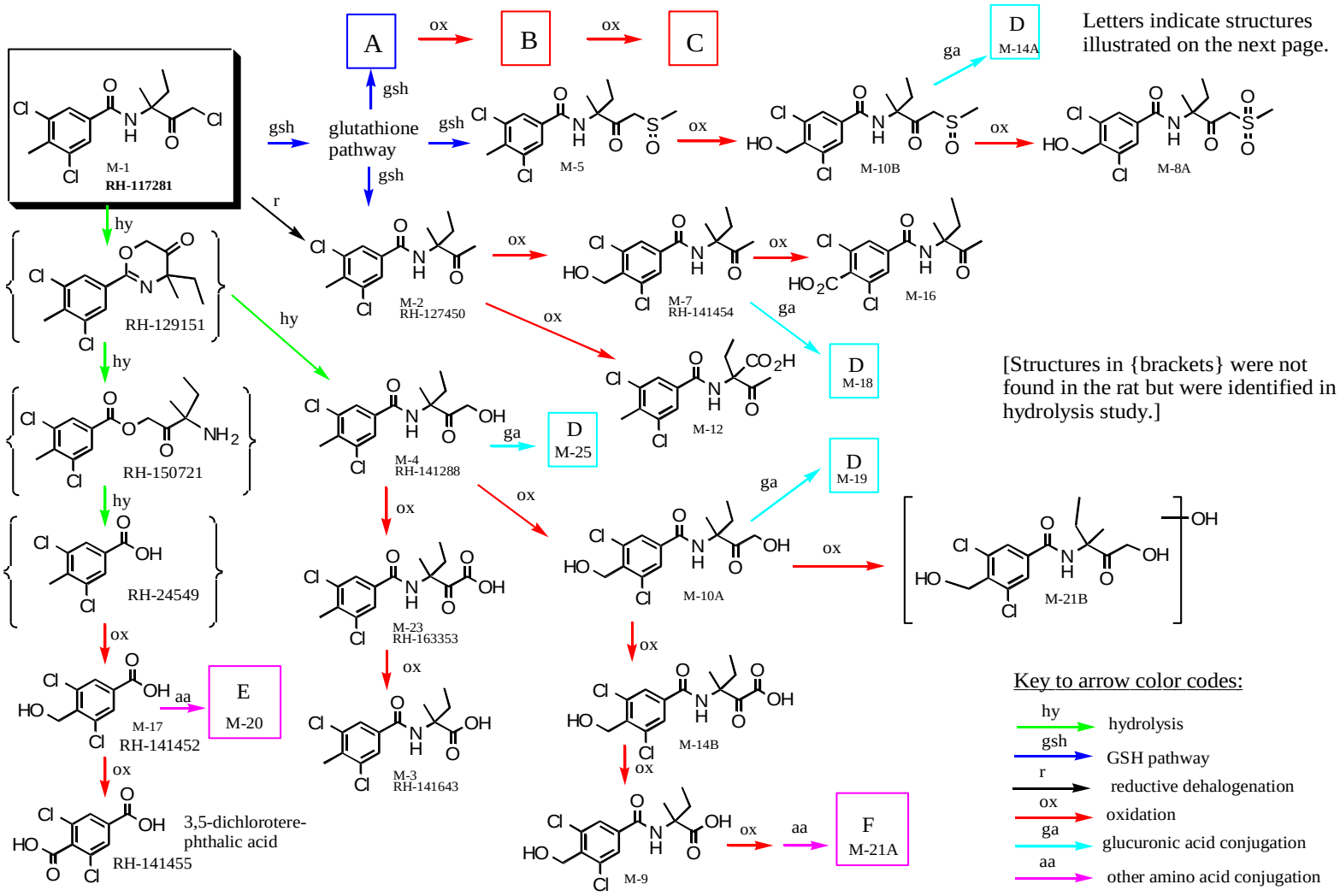
Safety factor : 100 (standard) x 2 (from 90 d study to chronic[2]) = 200

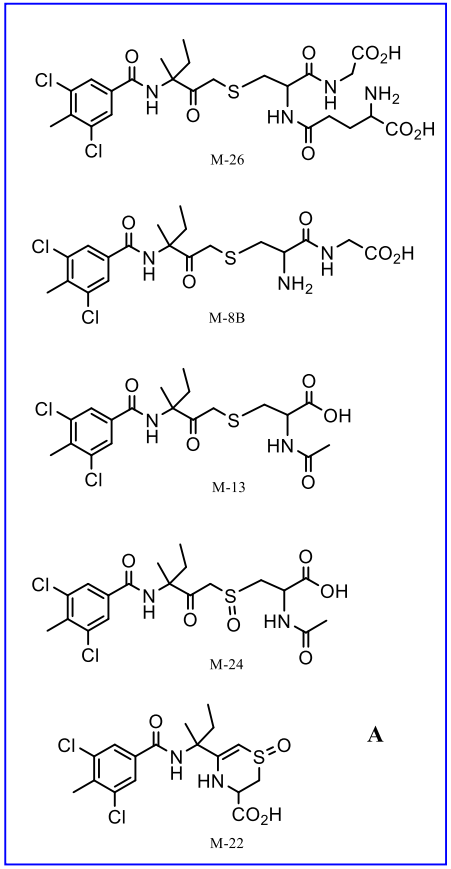
--> ADI = 0.56 mg/kg bw/d

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

zRMS: ADI for RH-150721 equal 0.56 mg/kg bw/d as proposed based on results of the presented studies is acceptable and can be used for risk assessment

Figure 3: Metabolic pathway for zoxamide in the rat

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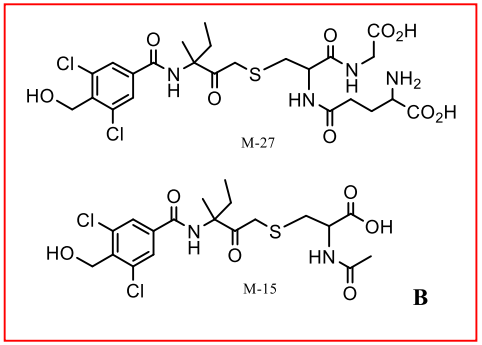
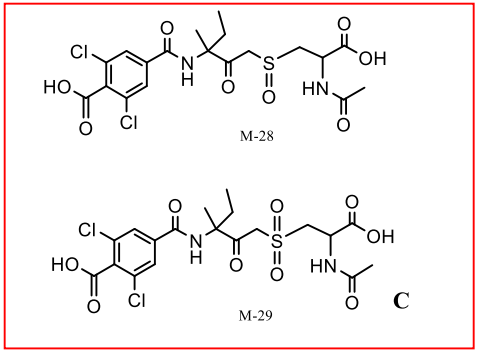
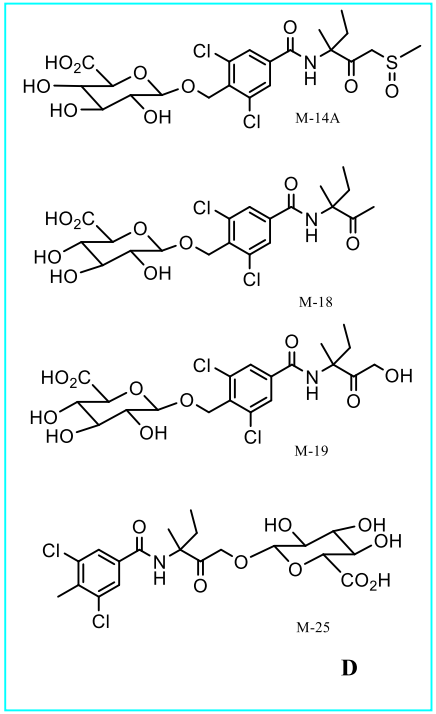
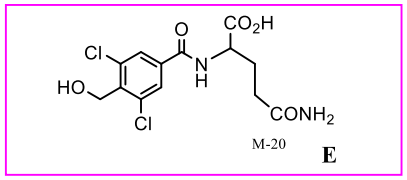
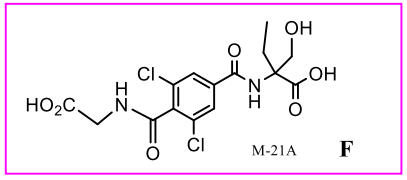
M-8B
M-13
M-24
M-22
A


M-27
M-15
B


M-28
M-29
C


M-14A
M-18
M-19
M-25
D


M-20
E


M-21A
F

RH-129151

Since RH-129151 appears >10% in a nature of hydrolysis study with zoxamide (study no. RB66JN) requested by EFSA (2017) and can therefore appear in processed commodities, an *in-vitro* genotox package has been performed for safety reasons and is provided in the following.

Table 6.3-7: Summary of the results of toxicity studies for RH-129151

Type of test, species (Guideline)	Result	Acceptability	Reference*
Ames (OECD 471)	non-genotoxic	YES	xxx, 2019; Report no. 188626
In vitro mammalian cell gene mutation test (HPRT-Locus) in Chinese Hamster V79 cells (OECD 476)	non-genotoxic	YES	xxx, 2020; Report no. 188628
In vitro mammalian micronucleus assay in Chinese Hamster V79 cells (OECD 487)	non-genotoxic	YES	xxx 2020; Report no. 188627

* indicates that a study was reviewed at EU level

Full summaries of studies on the metabolite that have not previously been considered within an EU peer review process are additionally described in detail in Appendix 2 (A 2.11 Other/Special Studies).

zRMS: Based on the negative results in the battery of in vitro mutagenicity tests a metabolite RH-129151 is considerd as not being mutagenic

RH-24549

Since RH-24549 appears >10% in a nature of hydrolysis study with zoxamide (study no. RB66JN) requested by EFSA (2017) and can therefore appear in processed commodities, an AMES test has been performed and is provided in the following. A complete in vitro genotox package is under preparation.

Table 6.3-8: Summary of the results of toxicity studies for RH-24549

Type of test, species (Guideline)	Result	Acceptability	Reference*
Ames (OECD 471)	non-genotoxic	YES	xxx, 2019 Report no. STUGC19AA1580-1

* indicates that a study was reviewed at EU level

Full summaries of studies on the metabolite that have not previously been considered within an EU peer review process are additionally described in detail in Appendix 2 (A 2.11 Other/Special Studies).

zRMS: Based on the negative results in the Ames a metabolite RH-24549 is considerd as not being mutagenic. It is noted that the other mutagenicity assays of RH-24549 are under preparation

6.4 Toxicological Evaluation of Groundwater Metabolites

The following data on metabolites with the potential to reach the groundwater in concentrations above 0.1 µg/L and requiring relevance assessment were submitted. Note that the relevance assessment of the metabolites is reported in Part B.10.

Zoxamide

The toxicological studies were evaluated during active ingredient renewal (AIR) on EU level (please refer to RAR, 2017 and EFSA Peer Review Conclusion, 2017). Studies additionally requested by EFSA (2017) are provided with this submission and are summarised in this document. Only the zoxamide metabolite RH-141455 (3,5-dichloro-4-carboxybenzoic acid) can appear > 0.1 µg/L.

Cymoxanil

All relevant toxicological data are summarised in the Draft Assessment Report (DAR) of Cymoxanil (2008).

6.4.1 RH-141455

The zoxamide metabolite RH-141455 (3,5-dichloro-4-carboxybenzoic acid) reached at maximum 8.4 % (day 14) in a soil metabolism study. It developed from the metabolite RH-24549 (FF = 0.5). Besides, it occurred in a surface water degradation study with zoxamide (pelagic test according to OECD guideline 309; van den Bosch, 2014) at max. amounts of 10.5 %. Thus, it can potentially reach groundwater/drinking water and is therefore addressed in the respective PEC calculations.
An overview on the results of the accepted toxicological studies for the groundwater metabolite RH-141455 is given in the following table. Full summaries of studies on the metabolite that have not previously been considered within an EU peer review process are additionally described in detail in Appendix 2 (A 2.11 Other/Special Studies).

Table 6.4-1: Summary of the results of toxicity studies for RH-141455

Type of test, species (Guideline)	Result	Acceptability	Reference*
Ames (OECD 471)	non-genotoxic	Yes	xxx, 1998*; Report no. 98R-048
In vitro mutation test using mouse lymphoma L5178Y cells (OECD 476)	non-genotoxic	Yes	xxx, 2014*; Report no. FRK0049
In vitro micronucleus test in human lymphocytes (OECD 487)	non-genotoxic	Yes	xxx 2014*; Report no. FRK0050
Acute oral mouse (OECD 401)	LD_{50} >5000	Yes	xxx, 1998*; Report no. 98R-047
14 d dietary toxicity in rats (OECD 407)	NOAEL >1000 mg/kg bw/d**	YES	xxx, 2020; Report no. U-19071
90 d dietary toxicity in rats (OECD 408) Limit test incl. 28 d recovery and plasma TK	NOAEL >1000 mg/kg bw/d ***	YES	xxx, 2020; Report no. U-19102

* indicates that a study was reviewed at EU level
** NOAEL = 15000 ppm, which is equivalent to 1123 and 1069 mg/kg body weight/day for the males and females, respectively.
***NOAEL = 16000 ppm, which is equivalent to 924 and 1119 mg/kg body weight/day for the males and females, respectively.

As a result, the metabolite RH-141455 can be regarded as toxicologically not relevant in groundwater.

6.4.2 Cymoxanil metabolite

No metabolites >0.1 µg/L were identified for Cymoxanil or. No further evaluation is required.

6.5 Dermal Absorption (KCP 7.3)

Dermal absorption values for zoxamide and cymoxanil are available from an *in vitro* dermal penetration study with the formulated product CYMOXANIL 33% + ZOXAMIDE 33% WG (Prisk C., 2020; report no. 38683). In this study the undiluted preparation (containing nominally 330 g/kg of each zoxamide and cymoxanil) applied as a paste as well as the highest intended field dilution of CYMOXANIL 33% + ZOXAMIDE 33% WG (containing 0.1155 g a.s./L of each zoxamide and cymoxanil) have been studied. The endpoints for the risk assessment are summarised in the following table, they have been evaluated in accordance to the EFSA Guidance on Dermal Absorption (2017).

Table 6.5-1: Dermal absorption rates for active substances in CYMOXANIL 33% + ZOXAMIDE 33%

	Zoxamide		Cymoxanil	
	Value	**Reference**	**Value**	**Reference**
Concentrate (330 g a.s./kg)	0.21 %	Prisk, L., 2018 Report no. 38683 New study reported in Appendix 2.	0.13 %	Prisk, L., 2018 Report no. 38683 New study reported in Appendix 2.
Dilution (0.1155 g a.s./L)	5.9 %		35 %	

6.5.1 Justification for proposed values - zoxamide

Proposed dermal absorption rates for zoxamide are based on a dermal absorption study on a formulation identical to CYMOXANIL 33% + ZOXAMIDE 33%. The study results are summarised in the following table. The full summary of the study on the dermal absorption of zoxamide/CYMOXANIL 33% + ZOXAMIDE 33% that have not previously been evaluated within an EU peer review process are described in detail in Appendix 2.

Table 6.5-2: Summary of the results of submitted dermal absorption studies for zoxamide

Test	**Concentrate**	**Spray dilution (0.1155 g a.s./L)**	**Formulation in study**	**Acceptability of study**	**Justification provided on representativity of study formulation for current product**	**Acceptability of justification**	**Reference***
In vitro (human) - zoxamide	0.21 %	5.9 %	CYMOXANIL 33% + ZOXAMIDE 33%	YES	Not required. Test is performed on CYMOXANIL 33% + ZOXAMIDE 33%.	YES	Prisk, L., 2018 Report no. 38683

* indicates that a study was reviewed at EU level

Dermal absorption values for the active substances from the *in vitro* study on human skin (Prisk L., 2018; Report no. 38683) were evaluated according to the EFSA guidance on dermal absorption (2017).

Table 6.5-3: Zoxamide - dermal absorption *in vitro*, human skin (Prisk, 2018; Report no. 38683)

	DA w/o *str. corn.*[1]	DA incl. *str. corn.*[2]	DA > 75% in 12 h
No of replicates	7 (=> factor according to table 1 of the EFSA 2017 = **0.92**) 8 (=> factor according to table 1 of the EFSA 2017 = **0.84**)		
Concentrate 330 g/kg	0.03 ± 0.02%*	**0.15 ± 0.07%**	no
Field dil. 0.1155 g/L	3.17 ± 1.31*	**4.92 ± 1.14%**	no
DA conc. acc. to EFSA 2017	0.15% + 0.07% x 0.92 = 0.2144% → **0.21%**		
DA dil. acc. to EFSA 2017	4.92% + 1.14% x 0.84 = 5.8775% → **5.9%**		

* SD > 25% of mean
[1] absorption = receptor fluid + receptor chamber washes + skin sample (excluding all tape strips)
[2] absorption = receptor fluid + receptor chamber washes + skin sample (excluding tape strips 1 and 2)

6.5.2 Justification for proposed values – cymoxanil

Proposed dermal absorption rates for zoxamide are based on a dermal absorption study on a formulation identical to CYMOXANIL 33% + ZOXAMIDE 33%. The study results are summarised in the following table. The full summary of the study on the dermal absorption of zoxamide/CYMOXANIL 33% + ZOXAMIDE 33% that have not previously been evaluated within an EU peer review process are described in detail in Appendix 2.

Table 6.5-4: Summary of the results of submitted dermal absorption studies for Cymoxanil

Test	**Concentrate**	**Spray dilution (1:1500)**	**Formulation in study**	**Acceptability of study**	**Justification provided on representativity of study formulation for current product**	**Acceptability of justification**	**Reference***
In vitro (human) - Cymoxanil	0.13 %	35 %	CYMOXANIL 33% + ZOXAMIDE 33%	YES	Not required. Test is performed on CYMOXANIL 33% + ZOXAMIDE 33%.	YES	Prisk, L., 2018 Report no. 38683
Transferred Dried Surface Residue from leaves							
In vitro (human) - Cymoxanil	5.7%		Field dilution of nominally 0.1155 g a.s./L water (i.e. actually 0.1185 g/L cymoxanil and 0.1075 g/L zoxamide) applied as a dry residue.	YES	Not required.	YES	Maas W., 2020: Report no. 20211644

* indicates that a study was reviewed at EU level

Dermal absorption values for the active substances from the *in vitro* study on human skin (Prisk L., 2018; Report no. 38683) were evaluated according to the EFSA guidance on dermal absorption (2017).

Table 6.5-5: Cymoxanil - dermal absorption *in vitro*, human skin (Prisk, 2018; Report no. 38683)

	DA w/o *str. corn.*[1]	DA incl. *str. corn.*[2]	DA > 75% in 12 h
No of replicates	8 (=> factor according to table 1 of the EFSA 2017 = **0.84**)		
Concentrate 330 g/kg	-	**0.10 ± 0.04%**	no
Field dil. 0.1155 g/L	**27.17 ± 9.01***		yes
DA conc. acc. to EFSA 2017	0.10% + 0.04% x 0.84 = 0.1336% → **0.13%**		
DA dil. acc. to EFSA 2017	27.17% + 9.01% x 0.84 = 34.7384% → **35%**		

[1] absorption = receptor fluid + exposed skin + receptor chamber wash + tape strips 3-20 (*stratum corneum*)

6.6 Exposure Assessment of Plant Protection Product (KCP 7.2)

Table 6.6-1: Product information and toxicological reference values used for exposure assessment

Product name and code	CYMOXANIL 33% + ZOXAMIDE 33% WG	
Formulation type	WG	
Category	Fungicide	
Active substance(s) (incl. content)	**Zoxamide** 330 g/kg	**Cymoxanil** 330 g/kg
AOEL systemic	0.3 mg/kg bw/d	0.01 mg/kg bw/d
Inhalation absorption	100 %	100 %
Oral absorption	60 %	75 %
Dermal absorption	Concentrate: 0.21 % Dilution: 5.9 % (dilution: 0.1155 g/L) (based on product CYMOXANIL 33% + ZOXAMIDE 33% WG)	Concentrate: 0.13% Dilution: 35 % (dilution: 0.1155 g/L) (based on product CYMOXANIL 33% + ZOXAMIDE 33% WG)

6.6.1 Selection of critical use(s) and justification

The critical GAPs used for the exposure assessment of the plant protection product are shown in **Table 6.1-4**. A list of all intended uses within the zone/ EU is given in Part B, Section 0.

Justification

The critical GAP uses represent the highest single application rates (i.e. 0.45 kg prod./ha) with max. numbers of applications (i.e. 3 for grapes and potatoes) at minimum application intervals (i.e. 7 days) and with a minimum spray volume of 200 L/ha.

Surrogate critical application scenarios were selected :

Grapes (max. 3x 0.45 kg product/ha in 200 L/ha water at 7 days interval)

- Tractor-mounted airblast sprayer, upwards
- Manual, hand-held application (using a tank sprayer with a lance or a Knapsack sprayer)

Potatoes open field (max. 3x 0.45 kg product/ha in 200 L/ha water at 7 days interval)

- Tractor-mounted boom sprayer, downwards
- Manual, hand-held application (using a tank sprayer with a lance or a Knapsack sprayer)

6.6.2 Operator exposure (KCP 7.2.1)

6.6.2.1 Estimation of operator exposure

A summary of the exposure model(s) used for estimation of operator exposure to the active substances during application of CYMOXANIL 33% + ZOXAMIDE 33% according to the critical use(s) is presented in Table 6.6-2. Detailed calculations are in Appendix 3.

According to the EC guidance document (2017; SANTE-10832-2015 rev. 1.7), consideration of acute exposure should only be made where an Acute Acceptable Operator Exposure Level (AAOEL) has been established during an approval, review or renewal evaluation of an active substance, i.e. no acute operator, worker and bystander exposure assessments can be performed with the OPEX models where no AAOEL has been set. For zoxamide (see EFSA Peer Review Conclusion, 2017) no AAOEL was set. For cymoxanil (see EFSA Peer EFSA Peer Review Conclusion (2013) no AAOEL was set.

Table 6.6-2: Exposure models for intended uses

Critical use(s)	**Grapes (max. 3x 0.45 kg product/ha in 200 L/ha, interval of 7 days)** **Potatoes field (max. 3x 0.45 kg product/ha in 200 L/ha, interval of 7 days)**
Model(s)	EFSA model : Guidance on the assessment of exposure of operators, workers, residents and bystanders in risk assessment for plant protection products; EFSA Journal 2014;12(10):3874 Calculator version: 30/03/2015

Comments on the operator exposure model(s)

Estimation of operator exposure according to the EFSA model

The EFSA operator exposure model (2015) is based on standard figures for different parameters. Models are available for the estimation of exposure to liquid and solid pesticide formulations using a tractor-mounted and hand-held sprayer for application in field crops. The following points are of particular importance when considering the estimates:

- The standard operator is assumed to weigh 60 kg.
- The work rate for the GAP uses chosen is 8 hours and 10 ha per day (grapes, vehicle mounted application), 4 ha per day (grapes, handheld application using tank sprayers with lances), 1 ha per day (grapes, Knapsack sprayer).
- Normal working clothing of operators is assumed to have a penetration factor of 10%.
- The active substances are of low volatility (i.e. vapour pressure $< 5 \times 10^{-3}$ Pa).

Table 6.6-3: Estimated operator exposure

		Zoxamide		Cymoxanil	
Model data	**Level of PPE**	**Total absorbed dose (mg/kg/day)**	**% of systemic AOEL**	**Total absorbed dose (mg/kg/day)**	**% of systemic AOEL**
Grapes - Tractor-mounted airblast sprayer					
Application rate		3 x 0.1485 kg a.s./ha		3 x 0.1485 kg a.s./ha	
EFSA model (AOEM; 75th percentile) Body weight: 60 kg	Potential exposure	0.0202	6.74	0.1094	1093.68
	Work wear (arms, body and legs covered) M/L and A	0.0075	2.49	0.0340	339.98
	Work wear (arms, body and legs covered) and gloves M/L and A, hood and visor A	0.0023	0.78	0.0038	38.33
Grapes - Manual, hand-held sprayer, upwards spraying					
Application rate		3 x 0.1485 kg a.s./ha		3 x 0.1485 kg a.s./ha	
EFSA model (AOEM; 75th percentile) Body weight: 60 kg	Potential exposure	0.0590	19.68	0.3429	3429.22
	Work wear (arms, body and legs covered) M/L and A	0.0043	1.44	0.0185	184.69
	Work wear (arms, body and legs covered) and gloves M/L and A	0.0026	0.85	0.0083	82.66
Grapes - Manual, Knapsack sprayer, upwards spraying					
Application rate		3 x 0.1485 kg a.s./ha		3 x 0.1485 kg a.s./ha	
EFSA model (AOEM; 75th percentile) Body weight: 60 kg	Potential exposure	0.0465	15.51	0.2706	2706.2
	Work wear (arms, body and legs covered) M/L and A	0.0027	0.89	0.0106	106.27
	Work wear (arms, body and legs covered) and gloves M/L and A	0.0018	0.60	0.0072	72.22
Potatoes - Tractor-mounted, boom spayer, downwards spraying					
Application rate		3 x 0.1485 kg a.s./ha		3 x 0.1485 kg a.s./ha	
EFSA model (AOEM; 75th percentile) Body weight: 60	Potential exposure	0.0033	1.10	0.0116	116.07
	Work wear (arms, body and legs covered) M/L and A	0.0025	0.84	0.0080	80.06

		Zoxamide		Cymoxanil	
Model data	**Level of PPE**	**Total absorbed dose (mg/kg/day)**	**% of systemic AOEL**	**Total absorbed dose (mg/kg/day)**	**% of systemic AOEL**
kg					

Based on the above calculations, the operator exposure for the intended GAP uses of CYMOXANIL 33% + ZOXAMIDE 33% is below the limit of 100% AOEL for single active ingredients and combined (additive) effects if the following PPEs are taken into account:

Grapes (max. 3x 0.45 kg product/ha in 200 L/ha water at 7 days interval)

- Tractor-mounted airblast sprayer, upwards
  ⇨ Work wear and gloves during mixing/loading and application. Hood and visor during application
- Manual, hand-held application incl. Knapsack
  ⇨ Work wear and gloves during mixing/loading and application

Potatoes (max. 3x 0.45 kg product/ha in 200 L/ha water at 7 days interval)

- Tractor-mounted boom sprayer, downwards
  ⇨ Work wear during mixing/loading and application

Assuming the skin sensitizing properties of the formulated product CYMOXANIL 33% + ZOXAMIDE 33%, gloves are generally proposed for mixing loading and maintenance and normal working clothing for application.

zRMS:
Grapes - Tractor-mounted airblast sprayer
The systemic exposure of operator applying Cymoxanil 33% + Zoxamide 33% WG Lieto 66 WG) to high crops (grapes) at dose of 0.45 kg of product/ha (0.1485 kg of each a.s./ha), using tractor-mounted airblast sprayer and wearing protective gloves and work clothing (long sleeved shirt, long trousers, sturdy boots) during mixing/loading and application and in addition hood and visor during application calculated with the EFSA AOEM amounted for zoxamide 0.78 % of AOEL, while for cymoxanil 38.33% of AOEL.
Thus, the estimated exposure of operator wearing protective gloves and work clothing (long sleeved shirt, long trousers, sturdy boots) during mixing/loading and application and in addition hood and visor during application, to each of the two active substances of Cymoxanil 33% + Zoxamide 33% WG (Lieto 66 WG) using appropriate assumptions and AOEM model demonstrates that such a systemic exposure is well below respective AOELs set in EU for these active substances, and, when these protective equipments are worn, a a combined exposure (sum of exposures to all active substances) expressed as percentage of their AOELs is also below 100%, thus is not posing an unacceptable systemic health risk.

Grapes - Manual, hand-held sprayer, upwards spraying
The estimated exposure of operator applying Cymoxanil 33% + Zoxamide 33% WG (Lieto 66 WG) to high crops (grapes) at dose of 0.45 kg of product/ha (0.1485 kg of each a.s./ha) using hand-held sprayer and wearing protective gloves and work clothing (long sleeved shirt, long trousers, sturdy boots) during mixing/loading and application to each of the two active substances of Cymoxanil 33% + Zoxamide 33% WG (Lieto 66 WG) calculated with the EFSA AOEM amounted for zoxamide 0.85 % of AOEL, while for cymoxanil 82.66% of AOEL. Thus, a a combined exposure (sum of exposures to all active substances) expressed as percentage of their AOELs is also below 100%, thus is not posing an unacceptable systemic health risk.

Grapes - Manual, Knapsack sprayer, upwards spraying
The estimated exposure of operator applying Cymoxanil 33% + Zoxamide 33% WG (Lieto 66 WG) to high crops (grapes) at dose of 0.45 kg of product/ha (0.1485 kg of each a.s./ha) using knapsack sprayer and wearing protective gloves and work clothing (long sleeved shirt, long trousers, sturdy boots) during mixing/loading and application to each of the two active substances of Cymoxanil 33% + Zoxamide 33% WG (Lieto 66 WG) calculated with the EFSA AOEM amounted for zoxamide 0.60 % of AOEL, while for cymoxanil 72.22% of AOEL. Thus, a a combined exposure (sum of exposures to all active substances) expressed as percentage of their AOELs is also below 100%, thus is not posing an unacceptable systemic health risk.

Potatoes - Tractor-mounted, boom spayer, downwards spraying
The estimated exposure of operator applying Cymoxanil 33% + Zoxamide 33% WG (Lieto 66 WG) to low crops (potatoes) at dose of 0.45 kg of product/ha (0.1485 kg of each a.s./ha) using tractor-mounted boom spayer and wearing work clothing (long sleeved shirt, long trousers, sturdy boots) during mixing/loading and application to each of the two active substances of Cymoxanil 33% + Zoxamide 33% WG (Lieto 66 WG) calculated with the EFSA AOEM amounted for zoxamide 0.84 % of AOEL, while for cymoxanil 80.06% of AOEL. Thus, a a combined exposure (sum of exposures to all active substances) expressed as percentage of their AOELs is also below 100%, thus is not posing an unacceptable systemic health risk.

Given the toxicological properties and classification of the formulation Cymoxanil 33% + Zoxamide 33% WG (Lieto 66 WG) according to Regulation 1272/2008/EC), as Skin Sens..1, H317, Repr. 2; H361fd and STOT RE 2; wearing protective gloves and protective clothing is recommended when handling the concentrate.

6.6.3 Measurement of operator exposure

Since the operator exposure estimations carried out indicated that the acceptable operator exposure level (AOEL) will not be exceeded under conditions of intended uses when considering the above mentioned personal protective equipment (PPE), a study to provide measurements of operator exposure is not necessary and was therefore not performed.

6.6.4 Worker exposure (KCP 7.2.3)

6.6.4.1 Estimation of worker exposure

A summary of the model(s) used for estimation of worker exposure to the active substances during application of CYMOXANIL 33% + ZOXAMIDE 33% according to the critical use(s) is presented in Table 6.6-4. Outcome of the estimation is presented in Table 6.6-5. Detailed calculations are in Appendix 3.

According to the EC guidance document (2017; SANTE-10832-2015 rev. 1.7), consideration of acute exposure should only be made where an Acute Acceptable Operator Exposure Level (AAOEL) has been established during an approval, review or renewal evaluation of an active substance, i.e. no acute operator, worker and bystander exposure assessments can be performed where no AAOEL has been set – as for zoxamide (see EFSA Peer Review Conclusion, 2017) and Cymoxanil (see EFSA Peer EFSA Peer Review Conlcusion (2013).

Table 6.6-4: Exposure models for intended uses

Critical use(s)	**Grapes (max. 3x 0.45 kg product/ha in 200 L/ha, interval of 7 days)** **Potatoes field (max. 3x 0.45 kg product/ha in 200 L/ha, interval of 7 days)**
Model(s)	EFSA model : Guidance on the assessment of exposure of operators, workers, residents and bystanders in risk assessment for plant protection products; EFSA Journal 2014;12(10):3874 Calculator version: 30/03/2015

Comments on the worker exposure model(s)

Estimation of operator exposure according to the EFSA model

The EFSA operator exposure model (2015) is based on standard figures for different parameters. Models are available for the estimation of exposure to liquid and solid pesticide formulations using a tractor-mounted and hand-held sprayer for application in field crops. The following points are of particular importance when considering the estimates:

- The standard worker is assumed to weigh 60 kg.
- A work rate of 2 hours is considered as default value for inspection tasks (evt. also irrigation), a work rate of 8 hours for harvesting and crop maintanance.
- An initial dislodgeable foliar residue (DFR) of 3 µg a.s./cm^2 foliage/kg was considered for zoxamide. Specific DFR values from available studies were used for cymoxanil.
- Normal working clothing and gloves are assumed to have a penetration factor of 10%.
- A default DT_{50} value of 30 days for a.s. degradation on/in plants is considered for zoxamide. For cymoxanil, DT_{50} values from relevant field studies are considered directly, to decrease the number of tables/scenarios used.
- The active substances are of low volatility (i.e. vapour pressure < 5 x 10^{-3} Pa).

Surrogate critical worker exposure scenarios were selected as follows :

- **Grapes –** Tying after application of max. 3x 0.45 kg product/ha at 7 days interval
- **Potatoes** - re-entry tasks for inspections

Where the crops (wine grapes, potatoes) are harvested mechanically, re-entry of workers for harvesting is not a relevant exposure path.

In all re entry situations, the low volatility of zoxamide (< 1.3 x 10^{-5} Pa [25°C]) and cymoxanil (1.50 x 10-4 Pa [20°C]) means that any additional exposure from inhalation of vapours that might occur would be negligible. The major route for exposure during re-entry is thus the contact with residues via the skin. Exposure from inhalation is not a relevant path for worker re-entry in the open field. The extent of potential exposure to the active substances varies with the re-entry time and the task undertaken.

Refinement(s)

Substance specific DFR and DT_{50} value(s) for residues dissipation

Dislodgeable Foliar Residues (DFR values) on leaves were studied following the applications of a cymoxanil containig formulation on grapevine and potato (see detailed summaries Appendix 4). The folllowing values were obtained and used for the risk assessment:

	DFR after last application	**DT_{50}**

	(worst-case)	
	µg/cm² kg a.s./ha	Days for RA (Days, trial result)
Grapes	1.6	1 (0.93)
Potato	2	1 (0.9535)

A general DT50 on leaves of around 1 day for cymoxanil is also confirmed by a number of data on different kind of leaves, as reported in the Cymoxanil RAR (2020) for the pending evaluation.
Since re-entry activities involve the contact with the dry residue on the leaves, the use of the worst-case dermal absorption value from the spray dilution can be considered an extreme worst case. A dermal absorption value to be used for re-entry activities was derived from the study by Maas W. (2020) summarised in Appendix 2. In the study, a more realistic situation concerning contact with dry cymoxanil residue during re-entry operations has been concidered. The final dermal absorption value derived in agreement with EFSA guidance on Dermal Absorption (2017) is 5.7%. This value has been considered to refine the re-entry part of resident adult and child exposure where needed.

Alternative refinements are proposed, maintaining the worst-case dermal absorption value for Cymoxanil of 35% for the diluted product:

1) Use of specific transfer coefficient (TC) in vineyard of 4861 cm²/h and 24% on the hands using gloves for actual exposure (gloves & covered body/workwear) as a further alternative procedure based on Baugher (Baugher D.G., 2005) [3]from BROWSE Work Package 2 Report (2014). For this assessment, 3d re-entry are considered sufficient to prevent any risk from re-entry (see below)
2) Use of specific transfer coefficient (TC) in vineyards of 3500 cm²/h in the absence of gloves as reported by UK authorities-CRD (Dawick et al., 2020)[4] For this assessment, 2d re-entry are considered sufficient to prevent any risk from re-entry (see below)

Table 6.6-5: Estimated worker exposure

		Zoxamide		**Cymoxanil**	
Model data	**Level of PPE**	**Total absorbed dose (mg/kg/day)**	**% of systemic AOEL**	**Total absorbed dose (mg/kg/day)**	**% of systemic AOEL**
Grapes - tying					
Application rate		3 x 0.1485 kg a.s./ha		3 x 0.1485 kg a.s./ha	
EFSA model	Potential exposure	0.2707	90.22	0.3353	3352.59
Cymoxanil: DFR after application	Work wear (arms, body and legs covered)	0.0911	30.37	0.1129	1128.71

[3] *Baugher D.G. (2005).* Penetration of Clothing by Dislodgeable Foliar Residues of Pesticides During Agricultural Occupational Re-entry - Redacted Draft Final. From BROWSE WP report: https://secure.fera.defra.gov.uk/browse/software/documentation/model_documentation_wp2_final.pdf
[4] Dawick, H., Hamey, p., MacDonald, A., Chan, J. Stevens, M., Childs, K. and SaintMart, J. (2020). Proposals for new transfer coefficient (TC) value for worker re-entry activities in vineyards. Bystander Resident Orchard (BROV) Re-entry Project report. Health Safety Executive.

		Zoxamide		Cymoxanil	
Model data	**Level of PPE**	**Total absorbed dose (mg/kg/day)**	**% of systemic AOEL**	**Total absorbed dose (mg/kg/day)**	**% of systemic AOEL**
1.6 µg/cm^2 kg a.s./ha, DT_{50} 1 day	Work wear (arms, body and legs covered). Cymoxanil dry residue DA= 5.7%	0.0911	30.37	0.0184	183.82
	Work wear (arms, body and legs covered). Cymoxanil dry residue DA= 5.7% and 2d re-entry.	0.0911#	30.37#	0.00460	45.95
	Work wear (arms, body and legs covered). Cymoxanil dry residue dermal absorption = 5.7% Assumed level of DFR on re-entry on second day after application 1.6 µg/cm^2 kg a.s./ha /2= 0.8 µg/cm^2 kg a.s./ha			0.0091191	91.19
	Work wear (arms, body and legs covered) and gloves. TC 4861 cm²/h (BROWSE Project), Assumed re-entry 3 days after application			0.00683	68.33
	Work wear (arms, body and legs covered) TC 3500 cm²/h (BROV Project), Assumed re-entry 2 days after application			0.00978	97.78
Potatoes – 2 hours work (inspection/irrigation) +					
Application rate		3 x 0.1575 kg a.s./ha		3 x 0.105 kg a.s./ha	
EFSA model	Potential exposure	0.0282	9.4	0.0437	436.54%
Cymoxanil: DFR after application 2 µg/cm2 kg a.s./ha, DT50 1 day	Work wear (arms, body and legs covered)	0.0032	1.05	0.0049	48.89%

* values representative for all grape scenarios
+ worst-case scenario for potato and grape
# worst-case for zoxamide

zRMS has calculated below with EFSA model the worker exposure assuming 5.7% dermal absorption of cymoxanil from a dry residue on leaves and DRF on second day after application of 0.8 µg/cm^2 kg a.s./ha

taking into account DT_{50} of 1 day. The resulting exposure of worker wearing work wear with arms, body and legs covered and re-entering the treated crops on second day after application amounted 91,19% of cymoxanil AOEL, which confirms that a worker exposure re-entering a treated grapes on second day after application is below AOEL. According the calculation of applicant using a moving time window approach in determining time weighted average concentrations on leaves according to formula provided in Appendix H: EFSA Journal 2009; 7(12):1438 the worker exposure on second day after application would result in 45.95% of AOEL).

Worker exposure from residues on foliage for Reboot		
Crop type	Grapes	
Indoor or outdoor	Outdoor	
Application method	Upward spraying	
Application equipment	Vehicle-mounted	
Worker's task	Hand harvesting	
Main body parts in contact with foliage	Hand and body	
Application rate of active substance	0,1485	kg a.s./ha
Number of applications	1	
Interval between multiple applications	365	days
Half-life of active substance	1	days
Multiple application factor	1,0	
Dermal absorption of the product	0,13%	
Dermal absorption of the in-use dilution	5,70%	
Dislodgeable foliar residue (i_AppRate*i_DFR)	0,1188	µg a.s./cm^2
Working hours	8	hr
Dermal transfer coefficient - Total potential exposure	30000	cm^2/hr
Dermal transfer coefficient - arms, body and legs covered	10100	cm^2/hr
Dermal transfer coefficient - hands, arms, body and legs covered	no TC available for this assessment	cm^2/hr
Inhalation transfer coefficient for automated applications	NA	ha/hr*10^(-3)
Inhalation transfer coefficient for cutting ornamentals	NA	ha/hr*10^(-3)
Inhalation transfer coefficient for sorting / bundling ornamentals	NA	ha/hr*10^(-3)

1. Total

	Potential exposure	Work wear - arms, body and legs covered	Working wear and gloves
Total systemic exposure (mg a.s./day)	1,6251840	0,5471453	no TC available for this assessment
Total systemic exposure per kg body weight (mg/kg bw/day)	0,0270864	0,0091191	
% of RVNAS	270,86%	91,19%	

Based on the above calculations, the worker exposure for the intended GAP uses of CYMOXANIL 33% + ZOXAMIDE 33% is below the limit of 100% AOEL for single active ingredients and combined (additive) effects if the following label restrictions are taken into account:

Grapes (max. 3x 0.45 kg product/ha in 200 L/ha water at 7 days interval) - Hand harvesting and maintenance:

- ⇨ Work wear (arms, body and legs covered) during all worker re-entry tasks, 2d re-entry due to cymoxanil.

Potatoes (max. 3x 0.45 kg product/ha in 200 L/ha water at 7 days interval)

- ⇨ Work wear (arms, body and legs covered) during all worker re-entry tasks

Assuming the skin sensitizing properties of the formulated product CYMOXANIL 33% + ZOXAMIDE 33%, normal working clothing is generally proposed for all worker re-entry tasks.

6.6.4.2 Refinement of generic DFR value (KCP 7.2)

Dislodgeable Foliar Residues (DFR values) on leaves were studied following the applications of a cymoxanil containig formulation on grapevine and potato (see detailed summaries Appendix 4). The folllowing values will be used for Risk Assessment

	DFR after last application (worst case)	DT_{50}
	µg/cm² kg a.s./ha	Days for RA (Days, trial result)
Grapes	1.6	1 (0.93)
Potato	2	1 (0.9535)

6.6.4.3 Measurement of worker exposure

Since the worker exposure estimations carried out indicated that the acceptable operator exposure level (AOEL) will not be exceeded under conditions of intended uses when considering the above-mentioned PPE, a study to provide measurements of worker exposure is not necessary and has not been performed.

zRMS:
Application on grapes
The exposure of worker not wearing PPE but wearing a work clothing (long sleeved shirt, long trousers) and entering for 8 hours for tying or hand harvesting on second day after application a field of grapes treated with formulation Cymoxanil 33% + Zoxamide 33% WG (Lieto 66 WG) 3 x 0.45 kg product/ha (0.1485 kg of each a.s./ha) in 200 L/ha water at 7 days interval as foreseen in GAP, calculated with the EFSA AOEM is below a respective AOEL for both active substances. However, entering such a field on the day of application will create an inacceptable health risk due to excessive exposure of worker to Cymoxanil, being above its AOEL. Therefore, re-entering for 8 hours for tying or hand harvesting fields of grapes treated with Lieto 66 WG WG by workers is only allowed on second day after treatment. Re-entering on third day or later will further reduce an exposure of worker due to short half-live of cymoxanil residue on leaves.

Application on potatoes
The exposure of worker not wearing PPE but wearing a work clothing (long sleeved shirt, long trousers) and entering for 2 hours for inspection a field of potatoes treated with formulation Cymoxanil 33% + Zoxamide 33% WG (Lieto 66 WG) 3 x 0.45 kg product/ha (0.1485 kg of each a.s./ha) in 200 L/ha water at 7 days interval as foreseen in GAP, calculated with the EFSA AOEM is below a respective AOEL for both active substances. Also a sum of exposures to these active substance expressed as percentage of their AOELs is also below 100%, therefore the application of product Cymoxanil 33% + Zoxamide 33% WG (Lieto 66 WG) does not pose an unacceptable risk to the health of worker for its intended use within good agricultural practice. Re-entering fields of potatoes treated with Cymoxanil 33% + Zoxamide 33% WG (Lieto 66 WG) by workers for inspection is only allowed when the spray solution has been fully dried out..

6.6.5 Bystander and resident exposure (KCP 7.2.2)

6.6.5.1 Estimation of bystander and resident exposure

Table 6.6-6 shows the exposure model(s) used for estimation of bystander and resident exposure to zoxamide and Cymoxanil. Outcome of the estimation is presented in Table 6.6-7. Detailed calculations are in Appendix 3.

According to the EC guidance document (2017; SANTE-10832-2015 rev. 1.7), consideration of acute exposure should only be made where an Acute Acceptable Operator Exposure Level (AAOEL) has been established during an approval, review or renewal evaluation of an active substance, i.e. no acute operator, worker and bystander exposure assessments can be performed where no AAOEL has been set. For zoxamide (see EFSA Peer Review Conclusion, 2017) no AAOEL was set. (see EFSA Peer Review Conclusion, 2008) no AAOEL was set.

Table 6.6-6: Exposure models for intended uses

Critical use(s)	**Grapes (max. 3x 0.45 kg product/ha in 200 L/ha, interval of 7 days)** **Potatoes field (max. 3x 0.45 kg product/ha in 200 L/ha, interval of 7 days)**
Model(s)	EFSA model : Guidance on the assessment of exposure of operators, workers, residents and bystanders in risk assessment for plant protection products; EFSA Journal 2014;12(10):3874 Calculator version: 30/03/2015

Estimation of bystander exposure

Only resident exposure is provided since, according to EFSA Guidance on the assessment of exposure of operators, workers, residents and bystanders in risk assessment for plant protection products (EFSA Journal 2014;12(10):3874): "*No bystander risk assessment is required for PPPs that do not have significant acute toxicity or the potential to exert toxic effects after a single exposure. Exposure in this case will be determined by average exposure over a longer duration, and higher exposures on one day will tend to be offset by lower exposures on other days. Therefore, exposure assessment for residents also covers bystander exposure*." This is the case for zoxamide and cymoxanil.

Estimation of residents (child and adult) exposure according to the EFSA model

The EFSA (2015) exposure model is based on standard figures for different parameters. Models are available for the estimation of several contamination routes of residents (young children and adults) via spray drift (at the time of application), vapour (may occur after a plant protection product has been applied), surface deposits and entry into treated crops.

The following points are of particular importance when considering the estimates:

- Resident exposure is assumed to be 2 hours (default for resident on lawn; dermal, surface deposits), 0.25 hours (dermal, entry into treated crops) and 24 hours (inhalation from vapour).
- The standard weight of an adult is 60 kg, the standard weight of a child (less than three years old) is 10 kg.
- The active substances are of low volatility (i.e. vapour pressure < 5 x 10^{-3} Pa).
- A default DT_{50} value of 30 days for a.s. degradation on/in plants was considered for zoxamide. DT_{50} value of 1 day as derived from available field studies (see Appendix 4) was used for cymoxanil.

For residents, maintaining the worst-case dermal absorption value for Cymoxanil of 35% for the diluted product also for re-entry instead of CYM DA dry Residue 5.7%, an alternative refinement is proposed, based on a limitation of minimal water volumes to 475 L/ha, still within the registered GAP, in addition to DRT and 10 m buffer zone.

For further details, please refer to the model.

Table 6.6-7: Estimated bystander and resident exposure

Estimated bystander and resident exposure

	Zoxamide		Cymoxanil	
Model data	**Total absorbed dose (mg/kg/day)**	**% of systemic AOEL**	**Total absorbed dose (mg/kg/day)**	**% of systemic AOEL**
Grapes - Tractor-mounted airblast sprayer (representative 'worst-case') Application rate: 3x 0.45 kg product/ha applied at a minimum interval of 7 days				
Residents - Tier 1 (standard) assumptions[1] – all pathways (mean)				
Residents (adult) Distance: 5 m Body weight: 60 kg	0.0042	1.4	0.0156	155.57
Residents (children) Distance: 5 m Body weight: 10 kg	0.0084	2.8	0.0289	289.35
Residents - Tier 2 refinement – all pathways (mean)				
Residents (adult) Distance: 10 m Tractor mounted with DRT Water vol. 400 L/min CYM DA dry Residue 5.7% Body weight: 60 kg	0.0042 *	1.4 *	~~0.0039~~ 0.0078	~~38.67~~ 76.79
Residents (adult) Distance: 10 m Tractor mounted with DRT Water vol. 475 L/min Body weight: 60 kg			0.0051	50.99
Residents (adult) Distance: 5 m Tractor mounted with DRT CYM DA dry Residue 5.7% Water vol. 400 L min Body weight: 10 kg Table A 46A			0.0024	24.27
Residents (children) Distance: 10 m Tractor mounted with DRT Water vol. 400L min CYM DA dry Residue 5.7% Body weight: 10 kg	0.0084 *	2.8 *	0.0078	76.79
Residents (children) Distance: 10 m Tractor mounted with DRT Water vol. 475 L min Body weight: 10 kg			0.0099	98.88
Residents (children) Distance: 5 m Tractor mounted with DRT CYM DA dry Residue 5.7% Water vol. 400 L min Body weight: 10 kg Table A 46A			0.0051	51.04

Potatoes - Tractor-mounted boom sprayer (representative 'worst-case') Application rate: 3x 0.45 kg product/ha applied at a minimum interval of 7 days				
Residents - Tier 1 (standard) assumptions[1] – all pathways (mean)				
Residents (adult) Distance: 2-3 m Body weight: 60 kg	0.0022	0.72	0.0039	38.96
Residents (children) Distance: 2-3 m Body weight: 10 kg	0.0051	1.71	0.0102	102.42
Residents - Tier 2 refinements– all pathways (mean)				
Residents (children) Distance: 2-3 m CYM DA dry Residue 5.7% Body weight: 10 kg	0.0051 *	1.71 *	0.0063	63.09
Residents (children) Distance: 2-3 m Body weight: 10 kg 250 L min	0.0051 *	1.71 *	0.0095	94.73

[1] Risk for residents calculated with EFSA exposure model.
* Considering Tier 1 assumptions for zoxamide.

The calculations according to the EFSA model demonstrate that residents of any age are not at risk during and after application of CYMOXANIL 33% + ZOXAMIDE 33% on grapes and potatoes in the field. The effects of single active ingredients and combined (additive) effects, calculated for safety reasons, are within the limits of 100 % AOELs for all scenarios. A bystander risk assessment is not required for PPPs that do not have significant acute toxic effects or the potential to exert toxic effects after a single exposure – which is the case for zoxamide and cymoxanil.

6.6.5.2 Measurement of bystander and/or resident exposure

Since the resident exposure estimations indicate acceptable exposure levels for zoxamide, Cymoxanil and combined (additive) effects, which were calculated for safety reasons, a study to provide measurements of bystander/resident exposure is not necessary and was therefore not performed.

zRMS:
Application on grapes
The exposure of resident (adult and child) to each of the active substance (Cymoxanil and Zoxamide) of a formulation Cymoxanil 33% + Zoxamide 33% WG (Lieto 66 WG) applied 3 times per season at 7 days interval at dose of 0.45 kg product/ha (0.1485 kg of each a.s./ha) in 200 L/ha water as foreseen in GAP on grapes, using tractor-mounted airblast sprayer, calculated with the EFSA AOEM demonstrates that such a exposure to Cymoxanil for adult resident is equal respectively to 155.6 % and for child residents 289.35%, while to Zoxamide for adult resident is equal respectively to 1.4 % and for child residents 2,8% % of respective AOEL. Such exposure to Cymoxanil creates an unacceptable health risk to residents therefore the refinement of exposure was needed using appropriate risk management measures such as an increases of the distance from the source or using drift reduction technology. .
Increasing distance of residents from the source of spray to 10m, using tractor-mounted airblast sprayer with drift reduction technology and increasing amount of water for product dilution to 400L reduced the exposure to Cymoxanil for adult resident to 76.79% and for child residents to 76.79% of AOEL.

When amount of water was further increased to 475L, assuming dermal absorption of cymoxanil of 0.13% for concentrate and 35% for dilution a Cymoxanil absorption for adult resident was equal respec-

tively to 50.99% % and for child residents 98.88% (Table A 48)
In case of assuming 5 m non-spray buffer and dermal absorption from dried residues of cymoxanil on leaves 5.7% the exposure of resident (adult and child) to Cymoxanil from a formulation Cymoxanil 33% + Zoxamide 33% WG (Reboot) applied 3 times per season at 7 days interval at dose of 0.45 kg product/ha (0.1485 kg of each a.s./ha) in 400 L/ha water as foreseen in GAP on grapes, using tractor-mounted air-blast sprayer, calculated with the EFSA AOEM demonstrates that such a exposure to Cymoxanil for adult resident is equal respectively to 24.27 % and for child residents 51.04% (table A 46A),

Therefore it is concluded that exposure of adult and child residents to both active substances (Cymoxanil and Zoxamide) of a formulation Cymoxanil 33% + Zoxamide 33% WG (Lieto 66 WG WG) applied 3 times per season at 7 days interval at dose of 0.45 kg product/ha (0.1485 kg of each a.s./ha) in 400 L/ha water as foreseen in modified GAP on grapes is acceptable and does not create a health risk for residents if a 10 m buffer zone of a minimum 10 m is obeyed and airblast sprayer is equipped with drift reduction technology (DRF).

Application on potatoes
The exposure of resident (adult and child) to each of the active substance (Cymoxanil and Zoxamide) of a formulation Cymoxanil 33% + Zoxamide 33% WG (Lieto 66 WG) applied 3 times per season at 7 days interval at dose of 0.45 kg product/ha (0.1485 kg of each a.s./ha) in 200 L/ha water as foreseen in GAP potatoes, using tractor-mounted boom sprayer and assuming a dermal absorption of Cymoxanil from a diluted formulation as 35%, calculated with the EFSA AOEM demonstrates that such a exposure to Cymoxanil for adult resident is equal respectively to 39 % and for child residents 102.4%, while to Zoxamide for adult resident is equal respectively to 0.72 % and for child residents 1.71 % % of respective AOEL. Such exposure to Cymoxanil creates an unacceptable health risk to child residents therefore the refinement of exposure was needed.
Taking into account that dermal absorption of Cymozanil from dry residue on leaves is 5.7%, and not 35 % as from diluted product resulted in reduction of the exposure to Cymoxanil for adult resident to 17.1 % and for child residents to 63 % of AOEL.
Therefore it is concluded that exposure of adult and child residents to both active substances (Cymoxanil and Zoxamide) of a formulation Cymoxanil 33% + Zoxamide 33% WG (Lieto 66 WG WG) applied 3 times per season at 7 days interval at dose of 0.45 kg product/ha (0.1485 kg of each a.s./ha) in 200 L/ha water as foreseen in GAP potatoes, using tractor-mounted boom sprayer on grapes is acceptable and does not create a health risk for adult and child residents.

No bystander acute exposure estimation is required since no acute acceptable operator exposure value (AAOEL) has be set for any of these active substances: Cymoxanil and Zoxamide. Therefore, as indicated in the EU guidance (SANTE-10832-2015 rev. 1.7; 24 January 2017), no unacceptable risk is expected for bystanders due to short-term single exposure to Cymoxanil and Zoxamide as a result of application of Cymoxanil 33% + Zoxamide 33% WG (Lieto 66 WG WG) with accordance with intended use within good agricultural practice.

6.6.6 Combined exposure

6.6.6.1 Exposure Assessment of zoxamide and Cymoxanil in CYMOXANIL 33% + ZOXAMIDE 33%

Note: The combined toxicological effect of these active substances has not been investigated with regard to repeated dose toxicity.

The product contains different active substances. Thus, 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."

Nevertheless, combined effects from zoxamide and cymoxanil have been taken into account for operators, workers, bystanders and residents of any age in the risk calculations above, assuming additive effects in a first-tier approach: to sum up the component exposures (as % of AOEL). The different modes of action or mechanisms/targets of toxicity was not considered. As a result, no harmful effects are foreseeable (please refer to the table below) even for this unrealistic worst-case appraoch, when considering dose and effect additivity.

In the table below, the respective Hazard Quotients (HQs) are calculated by assessing the exposure according to appropriate models and dividing the individual exposure levels by the respective systemic AOEL. This is equivalent to the predicted exposure as % of systemic AOEL. The Hazard Index (HI) is the sum of the individual HQs.

Table 6.6-8: Chronic risk assessment from combined exposure

Application scenario	Active Ingredient	Estimated exposure / AOEL (HQ)
Operators Grapes – Tractor-mounted airblast sprayer (3x 0.45 kg prod./ha), **workwear[1] and gloves M/L and A and hood and visor A**	Zoxamide	< 0.01
	Cymoxanil	0.38
	Cumulative risk Operators (HI)	**< 0.39**
Operators Grapes – Manual, hand-held sprayer (3x 0.45 kg prod./ha), **workwear[1] and gloves M/L and A**	Zoxamide	< 0.01
	Cymoxanil	0.83
	Cumulative risk Operators (HI)	**< 0.84**
Operators Grapes – Manual, Knapsack sprayer (3x 0.45 kg prod./ha), **workwear[1] and gloves M/L and A**	Zoxamide	< 0.01
	Cymoxanil	0.72
	Cumulative risk Operators (HI)	**< 0.73**
Operators Potatoes – Tractor-mounted airblast sprayer (3x 0.45 kg prod./ha), **workwear[1]**	Zoxamide	< 0.01
	Cymoxanil	0.80
	Cumulative risk Operators (HI)	**< 0.81**
Workers Grapes, tying (3x 0.45 kg prod./ha), **workwear[1] and 2d re-entry**	Zoxamide	0.30
	Cymoxanil	0.46
	Cumulative risk Workers (HI)	**0.76**
Workers Potatoes, Inspection / irrigation (3x 0.45 kg prod./ha), **workwear[1]**	Zoxamide	0.01
	Cymoxanil	0.49
	Cumulative risk Workers (HI)	**0.50**
Resident – Adult - Grapes (3x 0.45 kg prod./ha)	Zoxamide	0.01
	Cymoxanil	0.39
	Cumulative risk Resident – Adult (HI)	**0.40**
Resident – Child – Grapes (3x 0.45 kg prod./ha)	Zoxamide	0.03
	Cymoxanil	0.77
	Cumulative risk Resident – Child (HI)	**0.80**

Application scenario	Active Ingredient	Estimated exposure / AOEL (HQ)
Resident – Adult – Potatoes [2] (3x 0.45 kg prod./ha)	Zoxamide	< 0.01
	Cymoxanil	0.40
	Cumulative risk Resident – Adult (HI)	**< 0.41**
Resident – Child – Potatoes [2] (3x 0.45 kg prod./ha)	Zoxamide	0.02
	Cymoxanil	0.63
	Cumulative risk Resident – Child (HI)	**0.65**

[1] Normal work wear = Coverall or long-sleeved jacket and trousers made of cotton or cotton/polyester
[2] Worst-case scenario for all crops for inspection tasks (evt. irrigation)

All uses are acceptable (i.e. HI < 1) with the above-mentioned measures, even if combined (additive) effects of the active substances are considered.

zRMS: Health risks of operators, workers and residents due to combined exposure to both active substances are acceptable provided that indicated risk managet measures are applied

Appendix 1 Lists of data considered in support of the evaluation

Tables considered not relevant can be deleted as appropriate.

MS to blacken authors of vertebrate studies in the version made available to third parties/public.

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	Vertebrate study Y/N	Owner
KCP 7.1.1	xxx	1999	RH – 7281/Cymoxanil 66% DG Blend (1:1) Acute oral toxicity study in male and female rats xxx, Report No. 99R – 102 GLP Not published	Y	GWI
KCP 7.1.2	xxx	1999	RH – 7281/Cymoxanil 66% DG Blend (1:1) Acute dermal toxicity study in male and female rats xxx, Report No. 99R – 103 GLP Not published	Y	GWI
KCP 7.1.3	xxx	1999	RH – 7281/Cymoxanil 66% DG Blend (1:1) Acute inhalation toxicity study in rats Rohm and Haas Company, USA, Report No. 99R – 106 GLP Not published	Y	GWI
KCP 7.1.4	xxx	1999	RH – 7281/Cymoxanil 66% DG Blend (1:1) Skin irritation study in rabbits xxx, Report No. 99R – 104 GLP Not published	Y	GWI
KCP 7.1.5	xxx	1999	RH – 7281/Cymoxanil 66% DG Blend (1:1) Eye irritation study in rabbits xxx, USA, Report no. 99R – 105 GLP Not published	Y	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
KCP 7.1.6	xxx	1999	RH – 7281/Cymoxanil 66% DG Blend (1:1) Dermal sensitization study in guinea pigs xxx Report no. 99R – 107 GLP Not published	Y	GWI
KCP 7.2/01	Jullian E.	2014	Quantification of dislodgeable foliar residues following five applications of Vitene Ultra to grapevines in northern France and Italy, 2013 Report number: S13-01291 Laboratory: Eurofins Agroscience Services, Ltd. Owner: SIPCAM OXON S.p.A GLP: Y Published: N	N	Sipcam Oxon S.p.A.
KCP 7.2/02	Jullian E.	2014	Quantification of dislodgeable foliar residues following Six applications of Vitene Ultra to potato in the United Kingdom, 2013 Report number: S13-01293 Laboratory: Eurofins Agroscience Services, Ltd. Owner: SIPCAM OXON S.p.A GLP: Y Published: N	N	Sipcam Oxon S.p.A.
KCP 7.3/01	Prisk, L.	2018	The i*n vitro* percutaneous absorption of radiolabelled Cymoxanil and radiolabelled zoxamide in the concentrate and one in-use dilution through human skin Gowan Crop Protection Ltd., UK & Oxon Italia SpA Charles River Laboratories Edinburgh Ltd., UK, Report No. 38683 GLP Not published	N	GWI Sipcam Oxon S.p.A.
KCP 7.3/02	Maas W.,	2020	The i*n vitro* percutaneous absorption of radiolabelled cymoxanil from a transferred dried surface residue through human split-thickness skin Sipcam Oxon S.p.A, Italy Charles River Laboratories Den Bosch BV, The Netherlands Report No. 20211644, GLP, Not published	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
KCP 7.4 (KCA 5.8.1)	Schreib, G.	2019	Reverse mutation assay using bacteria (*Salmonella typhimurium* and *Escherichia coli*) with RH-24549 Gowan Crop Protection Ltd, UK Eurofins BioPharma Product Testing Munich GmbH, Germany, Report No. STUGC19AA1580-1 GLP Not published	N	GWI
KCP 7.4 (KCA 5.8.1)	Schreib, G.	2019	Reverse mutation assay using bacteria (*Salmonella typhimurium* and *Escherichia coli*) with RH-129151 Gowan Crop Protection Ltd, UK Eurofins BioPharma Product Testing Munich GmbH, Germany, Report No.188626 GLP Not published	N	GWI
KCP 7.4 (KCA 5.8.1)	Voges, Y.	2020	*In vitro* mammalian cell gene mutation test (HPRT-locus) in Chinese Hamster V79 cells with RH-129151 Gowan Crop Protection Ltd, UK Eurofins BioPharma Product Testing Munich GmbH, Germany, Report No. 188628 GLP Not published	N	GWI
KCP 7.4 (KCA 5.8.1)	Voges, Y.	2020	*In vitro* Mammalian cell gene mutation test (HPRT-Locus) in Chinese Hamster V79 Cells with RH-141452 Gowan Crop Protection Ltd, UK Eurofins BioPharma Product Testing Munich GmbH, Germany, Report No. 188620 GLP Not published	N	GWI
KCP 7.4 (KCA 5.8.1)	Schreib, G.	2017	*In vitro* mammalian cell gene mutation assay (Thymidine Kinase Locus/$TK^{+/-}$) in mouse lymphoma L5178Y cells with RH-150721 Gowan Crop Protection Ltd, UK Eurofins BioPharma Product Testing Munich GmbH, Germany, Report No. 171360 GLP Not published	N	GWI
KCP 7.4 (KCA 5.8.1)	Donath, C.	2020	*In vitro* Mammalian micronucleus assay in Chinese Hamster V79 cells with RH-129151 Gowan Crop Protection Ltd, UK Eurofins BioPharma Product Testing Munich GmbH, Germany, Report No. 188627	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		
KCP 7.4 (KCA 5.8.1)	Donath, C.	2019	*In vitro* mammalian micronucleus assay in Chinese Hamster V79 cells with RH-141452 Gowan Crop Protection Ltd, UK Eurofins BioPharma Product Testing Munich GmbH, Germany, Report No. 188616 GLP Not published	N	GWI
KCP 7.4 (KCA 5.8.1)	Donath, C.	2017	*In vitro* mammalian micronucleus assay in Chinese Hamster V79 cells with RH-150721 Gowan Crop Protection Ltd, UK Eurofins BioPharma Product Testing Munich GmbH, Germany, Report No. 171361 GLP Not published	N	GWI
KCP 7.4 (KCA 5.8.1)	xxx	2020	RH-141455: 2-day oral dietary pharmacokinetic study in Sprague Dawley rats Gowan Crop Protection Ltd, UK xxx, Report No. U-19044 No GLP Not published	Y	GWI
KCP 7.4 (KCA 5.8.1)	xxx	2020	RH-141455: 14-day oral dietary dose range finding study in Sprague Dawley rats Gowan Crop Protection Ltd, UK xxx, Report No. U-19071 No GLP Not published	Y	GWI
KCP 7.4 (KCA 5.8.1)	xxx	2020	RH-141455: 90-day oral dietary toxicity study with toxicokinetics and 28-day recovery period in Sprague Dawley rats Gowan Crop Protection Ltd, UK xxx, Report No. U-19102 GLP Not published	Y	GWI
KCP 7.4	xxx	2020	RH-150721: 2-day oral dietary pharmacokinetic study in Sprague Dawley rats	Y	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 5.8.1)			Gowan Crop Protection Ltd, UK xxx, Report No. U-19134 No GLP Not published		
KCP 7.4 (KCA 5.8.1)	xxx	2020	RH-150721: 14-day oral dietary dose range finding study in Sprague Dawley rats Gowan Crop Protection Ltd., UK xxx, Report No. U-19189 GLP Not published	Y	GWI
KCP 7.4 (KCA 5.8.1)	xxx	2020	RH-150721**:** 90-day oral dietary toxicity study and 28-day recovery period in Sprague Dawley rats Gowan Crop Protection Ltd, UK xxx, Report No. U-19235 GLP Not published	Y	GWI
KCP 7.4 (KCA 5.8.1)	Strupp, Ch.	2020	Zoxamide metabolite RH-150721 - proposal of ADI setting for dietary risk assessment Gowan Crop Protection Ltd, UK, Report No. CS13072020 No GLP Not published	N	GWI

GWI – Gowan Crop Protection Ltd.

Green shaded = confirmatory-like studies which are under evaluation by the RMS for Zoxamide in an interzonal procedure.

Grey shaded = data / reference already provided 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 5.1.1	xxx	2013	A study to compare the metabolite profile of 14C-zoxamide in cryopressed human, dog, rat and mouse hepatocytes. xxx, Report No. CXR1237, October 29, 2013 GLP Not published	N	GWI
KCA 5.1.1	xxx	2014	A study to investigate the presence of a specific metabolite of zoxamide, RH-150721, in analytical data from previous study CXR1237 xxx, Report No. CXR1416, July 17, 2014 GLP Not published	N	GWI
KCA 5.1.1	xxx	1998	14C-RH-117,281: Pharmacokinetic and metabolism study in rats xxx, Report No. 94R-235; ER Ref No. 24.1; US Ref No. 94R-235, GLP Not published	Y	GWI
KCA 5.1.1	xxx	1998	Distribution of 14C-RH-117,281 to the bone marrow of mice xxx, Report No. 97R-173, October 15, 1998, ER Ref No. 24.2 GLP Not published	Y	GWI
KCA 5.1.1	xxx	2001	Identification of RH-139432 from zoxamide (RH-117281) rat pharmacokinetic study samples xxx, Report No. 34-00-105, February 26, 2001, ER Ref No. 45.3 GLP Not published	Y	GWI
KCA 5.2.1	xxx	1996	RH-117,281 technical: acute oral toxicity study in male and female rats xxx, Report No. 95R-268, June 26, 1996, ER Ref No. 1.3 GLP Not published	Y	GWI
KCA 5.2.1	xxx.	1998	RH-117,281 technical: Acute oral toxicity study in male and female mice	Y	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
			xxx, Report No. 98R-165, December 4, 1998, ER Ref No. 24.3 GLP Not published		
KCA 5.2.2	xxx	1996	RH-117,281 technical: Acute dermal toxicity study in male and female rats xxx, Report No. 95R-269, ER Ref No. 1.4 GLP Not published	Y	GWI
KCA 5.2.3	xxx	1996	RH-117,281 Technical: acute inhalation toxicity study in rats xxx., Report No. 95R-266, ER Ref No. 2.2, US Ref No. 95R-266 GLP Not published	Y	GWI
KCA 5.2.4	xxx	1996	RH-117,281 technical: skin irritation study in rabbits xxx., Report No. 95R-270, ER Ref No. 1.5, US Ref No. 95R-270 GLP Not published	Y	GWI
KCA 5.2.5	xxx	1996	RH-117,281 Technical: eye irritation study in rabbits xxx., Report No. 95R-271, ER Ref No. 1.6, US Ref No. 95R-271 GLP Not published	Y	GWI
KCA 5.2.6	xxx	1997	Dermal sensitization study of RH-117,281 Technical in guinea pigs - maximization test Covance Laboratories, Report No. 6228-112 xxx, Report No. 95RC-170, ER Ref No. 4.2, US Ref No. 95RC-170 GLP No published	Y	GWI
KCA 5.2.6	xxx		RH-117,281 Technical: Delayed contact hypersensitivity study in guinea pigs xxx. Report No. 97R-074, ER Ref No. 23.2, US Ref No. 97R-074 GLP Not published	Y	GWI
KCA 5.2.6	xxx	1998	RH-117,281 Technical: Delayed contact hypersensitivity (dilution) study in guinea pigs	Y	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
			xxx. Report No. 98R-154, ER Ref No. 24.4, US Ref No. 98R-154 GLP Not published		
KCA 5.2.7	xxx	2014	Zoxamide: Cytotoxicity assay *in vitro* with Balb/c 3T3 cells: Natural red test at simultaneous irradiation with artificial sunlight xxxx, Report No. 1641200 GLP Not published	N	GWI
KCA 5.3.1	xxx	1996	RH-117,281 Technical: four-week range-finding toxicity study in dogs xxx, Report No. 94R-234, April 15, 1996, ER Ref No. 2.3 GLP Not published	Y	GWI
KCA 5.3.2	xxx	1996	RH-117,281: three-month dietary toxicity study in mice xxx., Report No. 94R-075, February 9, 1996, ER Ref No.5.3 GLP Not published	Y	GWI
KCA 5.3.2	xxx	1996	RH-117,281: three-month dietary toxicity/neurotoxicity study in rats xxx., Report No. 94R-233, March 22, 1996, ER Ref No. 3.1 GLP Not published	Y	GWI
KCA 5.3.2	xxx	1997	RH-117,281 Technical: three-month dietary toxicity study in dogs xxx, Report No. 96R-030, October 8, 1997, ER Ref No. 9.1 GLP Not published	Y	GWI
KCA 5.3.2	xxx	1998	RH-117,281 Technical: one-year chronic dietary toxicity study in dogs xxx, Report No. 95R-277, June 9, 1998, ER Ref No. 25.1 GLP Not published	Y	GWI
KCA 5.3.3	xxx	1998	RH-117,281 Technical: twenty-eight-day dermal toxicity study in rats	Y	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
			xxx., Report No. 97R-075, October 16, 1998, ER Ref No. 23.3 GLP Not published		
KCA 5.4.1	xxx	1996	RH-117,281 Technical: *Salmonella Typhimurium* gene mutation assay (Ames test) xxx., Report No. 95R-262, October 25, 1996, ER Ref No. 2.7 GLP Not published	N	GWI
KCA 5.4.1	xxx	1998	RH-117,281: Test for chemical induction of chromosome aberrations in cultured Chinese Hamster ovary (CHO) cells xxx., Study No. 616/20-D5140 xxx., Report No. 96RC-125, December 1998, ER Ref No. 23.6 GLP Not published	N	GWI
KCA 5.4.1	xxx	1994	RH-117,281: Test for chemical induction of gene mutation at the HGPT locus in cultured Chinese Hamster ovary cells with and without metabolic activation. xxx, Report No. 0282-2510 xxx., Report No. 94RC-077, November 16, 1994, ER Ref No. 23.4 GLP Not published	N	GWI
KCA 5.4.2	xxx	1996	RH-117,281 Technical: micronucleus assay in CD-1 mouse bone marrow cells xxx., Report No. 95R-264, October 29, 1996, ER Ref No. 1.9 GLP Not published	N	GWI
KCA 5.4.2	xxx	1998	Distribution of 14C-RH-117,281 to the bone marrow of mice xxx, Report No. 97R-173, July 24, 1998, ER Ref No. 24.2 GLP Not published	Y	GWI
KCA 5.4.2	xxx	1998	Mechanism of action of the oomycete fungicides RH-54032 and RH-117281 on *Phytophthora capsici*, tobacco, mouse lymphoma cells and isolated bovine tubulin	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
			xxx., Report No. 98R-1098, November 30, 1998, ER Ref No. 23.5 GLP Not published		
KCA 5.5	xxx	1998	RH-117,281 Technical: 24-month dietary chronic/oncogenicity study in rats xxx, Report No. 417-505 xxx, Report No. 94RC-236, November 3, 1998, ER Ref No. 21.1 GLP Not published	Y	GWI
KCA 5.5	xxx.	1998	RH-117,281 Technical: Eighteen-month dietary oncogenicity study in mice (photomicrographs) xxx., Report No. 96R-094, September 24, 1998, ER Ref No. 20.1 GLP Not published	Y	GWI
KCA 5.6.1	xxx.	1998	RH-117,281 Technical: Two generation reproductive toxicity study in rats xxx., Report No. 95R-272, December 10, 1998, ER Ref No. 26.1 GLP Not published	Y	GWI
KCA 5.6.2	xxx	1995b	RH-7281 Technical: oral (gavage) developmental toxicity study in rats xxx., Report No. 94R-079, September 15, 1995, ER Ref No. 6.1 GLP Not published	Y	GWI
KCA 5.6.2	xxx	1997	RH-117,281 Technical: oral (gavage) developmental study in rabbits xxx., Report No. 95R-267, April 8, 1997; ER Ref No. 8.2 GLP Not published	Y	GWI
KCA 5.7.1	xxx	1997	RH-117,281 Technical: acute oral (gavage) neurotoxicity study in rats xxx., Report No. 95R-182, January 6, 1997, ER Ref No. 10.1 GLP Not published	Y	GWI
KCA 5.7.1	xxx	1996	RH-117,281: three-month dietary toxicity/neurotoxicity study in rats	Y	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
			xxx, Report No. 94R-233, March 22, 1996, ER Ref No. 3.1 GLP Not published		
KCA 5.7.1	xxx	1995	Carbaryl and DDT: Neurotoxicity evaluation of positive control substances in rats xxx., Report No. 94R-224, ER Ref No. 27.5 GLP Not published	Y	GWI
KCA 5.7.1	xxx	1995	d-Amphetamine and Chlorpomazine: Motor activity assessment of positive control substances in rats xxx., Report No. 94R-225, ER Ref No. 27.6 GLP Not published	Y	GWI
KCA 5.7.1	xxx	1995	Triethyltin and Acrylamide: Neurotoxicity evaluation of positive control substances in rats xxx., Report No. 94R-211; ER Ref No. 28.1 GLP Not published	Y	GWI
KCA 5.8.1	xxx	2014	RH-141455: *In vitro* mutation test using mouse lymphoma L5178Y xxx Report No. FRK0049, July 8, 2014 GLP Not published	N	GWI
KCA 5.8.1	xxx	2014	RH-141455: *In vitro* micronucleus test in human lymphocytes xxx, Report No. FRK0050, July 8, 2014 GLP Not published	N	GWI
KCA 5.8.1	xxx	2013	RH-150,721: *Salmonella typhimurium* reverse mutation assay xxx. 1549300, October 7, 2013 GLP Not published	N	GWI
KCA 5.8.1	xxx	2013	DEREK evaluation of the toxicities of zoxamide and metabolite RH-150,721 xxx., Study No: FRK0046, May 17, 2013	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 5.8.1	xxx	1998	14C-RH-141,452: Rat metabolism study, Tier I testing xxx., Report No. RPT00410 xxx, Report No. 97RC-154, November 19, 1998, ER Ref No: 27.1 GLP Not published	Y	GWI
KCA 5.8.1	xxx	1998	RH-141,452: Acute oral toxicity study in male and female mice xxx, Report No. 98R-049, September 24, 1998, ER Ref No. 25.2 GLP Not published	Y	GWI
KCA 5.8.1	xxx	1998	RH-141,452: *Salmonella typhimurium* gene mutation assay (Ames test) xxx., Report No. 98R-050, October 1, 1998, ER Ref No. 25.3 GLP Not published	Y	GWI
KCA 5.8.1	xxx	1998	14C-RH-141,455: Rat metabolism study, Tier I testing xxx., Report No. 98RC-017, ER Ref No. 27.2 xxx, Report No. RPT00411 GLP Not published	Y	GWI
KCA 5.8.1	xxx	1998	RH-141,455: Acute oral toxicity study in male and female mice xxx Report No. 98R-047, September 24, 1998, ER Ref No. 27.3 GLP Not published	Y	GWI
KCA 5.8.1	xxx	1998	RH-141,455: *Salmonella typhimurium* gene mutation assay (Ames test) xxx Report No. 98R-048, September 23, 1998, ER Ref No. 27.4 GLP Not published	Y	GWI

GWI – Gowan Crop Protection Ltd.

For cymoxanil it is referred to the data available in the DAR (2003) and its addenda as well as the here submitted study/studies via Letter of Access of the company Sipcam Oxon SpA.

The following tables are to be completed by MS

List of data submitted by the applicant and not relied on

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

List of data relied on not submitted by the applicant but necessary for evaluation

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

Appendix 2 Detailed evaluation of the studies relied upon

A 2.1 Statement on bridging possibilities

Please refer to the dRR Part C for information on the formulated product (developmental formulation) for used for the 6-pack. These studies were already evaluated by the zRMS (Spain) during product authorisation as highlighted in grey.

A 2.2 Acute oral toxicity (KCP 7.1.1)

Comments of zRMS (UK/CRD and Spanish INIA):	Study acceptable. Agreed endpoint: CLP: "Cymoxanil 33% + Zoxamide 33% WG" is classified as "H302: Harmful if swallowed" based on available data.

Reference:	**KCP 7.1.1/01**
Report	xxx, 1999: RH – 7281/Cymoxanil 66% DG Blend (1:1) Acute oral toxicity study in male and female rats xxx, Report No. 99R–102, GLP, Not published
Guideline(s):	OECD 401 EEC B. l US EPA OPPTS 870.1100
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test material (Lot/Batch No.)	RH – 7281/Cymoxanil 66% DG Blend (1:1) / CYMOXANIL 33% + ZOXAMIDE 33% WG (EG 3860) Cymoxanil: 33% (nominal), 34.7 % (analysed) Zoxamide: 33% (nominal); 33.8% (analysed)
Species	Crl:CD®BR rats
No. of animals (group size)	3 groups of 6 rats/sex
Dose(s)	500, 2000 and 5000 mg/kg bw
Exposure	Once by gavage
Vehicle/Dilution	Distilled water
Post exposure observation period	14 days
Remarks	None

Three groups of six males and six females of Crl:CD®BR rats were gavaged with distilled water suspension of test substance RH – 7281 Cymoxanil 66% DG Blend (1:1) - Lot code EG 3860, (CYMOXANIL 33% + ZOXAMIDE 33% WG, analytical content Cymoxanil 34.7% , Zoxamide 33.8%, see part C for details) at 500, 2000 and 5000 mg/kg body weight.
Observations were performed for signs of illness and other reactions to treatment at 1, 2 and 4 hours after dosing and once daily thereafter for 14 days. Descendents were necropsied as well as surviving rats.

Results and discussions

Findings:

Mortality:
After 14 days no deaths were observed in the 500 mg/kg group. Nine of the twelve rats died at 2000 mg/kg. All rats died at 5000 mg/kg by 4 hours post dosing.

Clinical observations:
Ataxia, tremors, limbs splayed, exaggerated gait, nystagmus, leaning, lacrimation, low body posture) were observed among survivors and decedents in both sexes at 2000 mg/kg. Survivors at 2000 mg/kg were normal by day 8 post-dosing. In males and/or females at 500 mg/kg, ataxia, low body posture, tremors, and one incidence of straub tail were noted beginning at 1 hr post-dosing.
Rats at 500 mg/kg recovered from these signs by 24 hrs post-dosing. Salivation was noted at 5000 mg/kg prior to death. Additional clinical signs noted included: passiveness in both sexes at 500 and 2000 mg/kg and scant or no faeces in both sexes at 2000 mg/kg. Rats recovered from these signs by day 8.

Body Weight:
No effects on body weights of survivors were observed.

Necropsy:
Necropsy of survivors didn't show any gross changes.

Deficiences:
None.

Conclusion/endpoint:

The acute oral LD_{50} in rats was calculated to be 1469 mg/kg with 95% confidence limits of 814 to 2070 mg/kg. The product should be classified as R 22 'harmful if swallowed' according to Directive 67/548/EC. According to Regulation (EC) No 1272/2008 the respective classification/hazard statement is: Acute oral toxicity: Category 4, H302 "Harmful if swallowed".

A 2.3 Acute percutaneous (dermal) toxicity (KCP 7.1.2)

Comments of zRMS (UK/CRD and Spanish INIA):	Study acceptable. Agreed endpoint: No classification and labelling is triggered for the formulation "Cymoxanil 33% + Zoxamide 33% WG" regarding acute percutaneous (dermal) toxicity based on available data.

Reference **KCP 7.1.2/01**

Report xxx, 1999: RH – 7281/Cymoxanil 66% DG Blend (1:1) Acute dermal toxici-

	ty study in male and female rats xxx, Report No. 99R – 103, GLP, Not published
Guideline(s):	OECD 402 EEC B. 3 US EPA OPPTS 870.1200
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test material (Lot No.)	RH – 7281/Cymoxanil 66% DG Blend (1:1) / CYMOXANIL 33% + ZOXAMIDE 33% WG (EG 3860) Cymoxanil: 33% (nominal), 34.7 % (analysed) Zoxamide: 33% (nominal); 33.8% (analysed)
Species	Crl:CD®BR rats
No. of animals (group size)	6 rats/sex
Dose(s)	5000 mg/kg bw
Exposure	24 hours (dermal, semi-occlusive)
Vehicle/Dilution	the test substance was moistened with distilled water (2:1 w/v)
Post exposure observation period	14 days
Remarks	None

A group of 6 males and 6 females of Crl:CD®BR rats was exposed to a single dose of 5000 mg/kg body weight of RH – 7281 Cymoxanil 66% DG Blend (1:1) - Lot code EG 3860, (CYMOXANIL 33% + ZOXAMIDE 33% WG, analytical content Cymoxanil 34.7%, Zoxamide 33.8%, see part C for details) for 24 hours. Animals were observed at 1, 2 and 4 hours after dosing and once daily thereafter for 14 days. Body weights were recorded and necropsy was performed on survivors.

Results and discussions

Findings:

Mortality:
No deaths were noted over the observation period.

Clinical observations and dermal responses:
Skin effects were observed from day 1 and continued through day 14.

Body Weight:
There were no apparent body weight effects.

Necropsy:
Necropsy revealed no gross changes.

Deficiencies:
None.

Conclusion/endpoint:

The acute dermal LD_{50} for RH – 7281 Cymoxanil 66% DG Blend (1:1) was greater than 5000 mg/kg. According to both Directive 67/548/EC and Regulation (EC) No 1272/2008, the product does not require classification and labelling for dermal toxicity.

A 2.4 Acute inhalation toxicity (KCP 7.1.3)

Comments of zRMS (UK/CRD and Spanish INIA):	Study acceptable. Agreed endpoint: No classification and labelling is triggered for the formulation "Cymoxanil 33% + Zoxamide 33% WG" regarding inhalation toxicity based on available data.

Reference:	**KCP 7.1.1/01**
Report	xxx, 1999: RH – 7281/Cymoxanil 66% DG Blend (1:1) Acute inhalation toxicity study in rats xxx, Report No. 99R–106, GLP, Not published
Guideline(s):	OECD 403 EEC B. 2 US EPA OPPTS 870.1300
Deviations:	At the time they were exposed, the males and females were approximately 7 weeks old. This deviation from the protocol (animals will be 8-12 weeks at exposure initiation) had no adverse impact on the study outcome.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test material (Lot No.)	RH – 7281/Cymoxanil 66% DG Blend (1:1) / CYMOXANIL 33% + ZOXAMIDE 33% WG (EG 3860) Cymoxanil: 33% (nominal), 34.7 % (analysed) Zoxamide: 33% (nominal); 33.8% (analysed)
Species	Crl:CD®BR rats
No. of animals (group size)	6 rats/sex/dose
Concentration(s)	4.4 mg/L air (MMAD of 7.0 ± 0.2µm and GSD of 2.3 ± 0.1 µm)
Exposure	4 hours (nose only)
Vehicle/Dilution	None
Post exposure observation period	14 days
Remarks	None

Six males and six females rats were exposed to a single 4 hours nose-only inhalation of RH – 7281 Cymoxanil 66% DG Blend (1:1) - Lot code EG 3860, (CYMOXANIL 33% + ZOXAMIDE 33% WG, analytical content Cymoxanil 34.7% , Zoxamide 33.8%, see part C for details) aerosol concentration of 4.4 mg/L of air, with MMAD of 7.0 ± 0.2 µm and GSD of 2.3 ± 0.1. Rats were observed for signs of illness or reaction to treatment at 4 hours after dosing and once daily thereafter for 14 days. Body weights were recorded before the exposure and on days 7 and 14.

Results and discussions

Findings:

Mortality:
No mortalities were observed over the 14-day study period.

Clinical observations:
No clinical signs of toxicity.

Body weight:
No effects on body weight.

Necropsy:
Necropsy revealed no gross changes.

Deficiencies:
None.

Conclusion/endpoint:

The acute inhalation LC_{50} was greater than 4.4 mg/L of air. According to both Directive 67/548/EC and Regulation (EC) No 1272/2008 the product does not require classification and labelling for inhalation toxicity.

A 2.5 Skin irritation (KCP 7.1.4)

Comments of zRMS (UK/CRD and Spanish INIA):	Study acceptable. Agreed endpoint: No classification and labelling is triggered for the formulation "Cymoxanil 33% + Zoxamide 33% WG" regarding skin irritation based on available data.

Reference	**KCP 7.1.4/01**
Report	xxx, 1999: RH – 7281/Cymoxanil 66% DG Blend (1:1) Skin irritation study in rabbits xxx, Report No. 99R–104, GLP, Not published
Guideline(s):	OECD 404 EEC B. 4 US EPA OPPTS 870.2500
Deviations:	No
GLP:	Yes

Acceptability: Yes

Duplication (if vertebrate study) No

Materials and methods

Test material (Lot/Batch No.)	RH – 7281/Cymoxanil 66% DG Blend (1:1) / CYMOXANIL 33% + ZOXAMIDE 33% WG (EG 3860) Cymoxanil: 33% (nominal), 34.7 % (analysed) Zoxamide: 33% (nominal); 33.8% (analysed)
Species	Rabbit, New Zealand White
No. of animals (group size)	3 males
Initial test using one animal	No
Exposure	0.5 g (4 hours, semi-occlusive)
Vehicle/Dilution	the test substance (0.5 g) was moistened with 0.25 mL of distilled water (2: 1 w/v)
Post exposure observation period	14 days
Remarks	None

Skin irritation of RH – 7281 Cymoxanil 66% DG Blend (1:1) - Lot code EG 3860, (CYMOXANIL 33% + ZOXAMIDE 33% WG, analytical content Cymoxanil 34.7%, Zoxamide 33.8%, see part C for details) was assessed in New Zealand White rabbits. 0.5 g of test substance was moistened with 0.25 ml of distilled water and topically applied to the shaved intact skin of three male rabbits; the application sites were semi-occluded for 4 hours. Skin irritation was evaluated according to Draize criteria at 1, 24, 48, 72 hour and at 7 and 14 days after patch removal. Skin reactions, signs of systemic toxicity and mortality were also observed.

Results and discussions

Findings:

Mortality:
No mortality occurred.

Clinical signs:
There were no treatment-related clinical signs.

Dermal responses:
Very slightly to well-defined erythema was observed in all rabbits from 1 hour to 72 hours. Well-defined erythema was still evident in a single animal on day 7, but no longer evident by day 14. Slight to moderate oedemas were noted in two animals at 1 hour and slight oedema was still present in one animal at 24 hours.

Table A 1: Individual and mean skin irritation scores in rabbits treated with RH – 7281 Cymoxanil 66% DG Blend (1:1)

Observations (range of scores; normal score)	Animal No.	Time						Mean score 24-72 h
		1 hour	24 hours	48 hours	72 hours	7 days	14 days	

Erythema	99-27023	2 a	2 a	2 a	1 a	0 a	0	1.7
	99-27025	2 a	2 a	2 a	2 a	2 a	0	2
	99-27026	2 a	2 a	2 a	1 a	0 a	0	1.7
Edema	99-27023	0	0	0	0	0	0	0.0
	99-27025	3	2	0	0	0	0	0.7
	99-27026	2	0	0	0	0	0	0.0

a = Tan-stained

Deficiencies:
None.

Conclusion/endpoint:

According to both Directive 67/548/EC and Regulation (EC) No 1272/2008 the product does not require classification and labelling for dermal irritation.

A 2.6 Eye irritation (KCP 7.1.5)

Comments of zRMS (UK/CRD and Spanish INIA):	Study acceptable. Agreed endpoint: No classification and labelling is triggered for the formulation "Cymoxanil 33% + Zoxamide 33% WG" regarding eye irritation based on available data.

Reference	**KCP 7.1.5/01**
Report	xxx, 1999d: RH – 7281/Cymoxanil 66% DG Blend (1:1) Eye irritation study in rabbits xxx, Report No. 99R–105, GLP, Not published
Guideline(s):	OECD 405 EEC B. 5 US EPA OPPTS 870.2400
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test material (Lot No.)	RH – 7281/Cymoxanil 66% DG Blend (1:1) / CYMOXANIL 33% + ZOXAMIDE 33% WG (EG 3860) Cymoxanil: 33% (nominal), 34.7 % (analysed) Zoxamide: 33% (nominal); 33.8% (analysed)
Species	Rabbit, New Zealand White
No. of animals (group size)	3 males

Initial test using one animal	No
Exposure	0.1 g (single instillation in conjunctival sac)
Irrigation (time point)	After the 24-hr observation, each eye (treated and control) was irrigated with 0.9% saline for approximately 60 seconds.
Vehicle/Dilution	0.9% saline solution
Post exposure observation period	72 hours
Remarks	None

Eye irritation of RH – 7281 Cymoxanil 66% DG Blend (1:1) - Lot code EG 3860, (CYMOXANIL 33% + ZOXAMIDE 33% WG, analytical content Cymoxanil 34.7%, Zoxamide 33.8%, see part C for details) was assessed in New Zealand White rabbits. A dose of 0.1 g was applied into the conjunctival sac of three male rabbits. After 24- hrs observation each eye (treated and control) was irrigated with 0.9% saline solution.
The cornea, iris and conjunctiva of the treated and control eyes were examined at 1, 24, 48 and 72 hours after dosing. The degree of eye irritation was evaluated according to the Draize criteria.

Results and discussions

Findings:

Mortality:
No mortality occurred.

Clinical signs:
There were no treatment-related clinical signs.

Ocular responses:
Corneal effects were present in a single rabbit at 1 hour after the treatment, in all rabbits after 24 hours and were no longer evident at 48 hours. No iridal effects were observed during the study, conjunctival effects were noted in all rabbits at 1 hour and continued trough 48 hours. No ocular effects were evident at 72 hours.

Table A 2: Individual and mean eye irritation scores in rabbits treated with RH–7281 Cymoxanil 66% DG Blend (1:1)

Rabbit number	Parameter		Time after instillation				Mean irritation score*
			1 hour	24 hours	48 hours	72 hours	
99-27046[a,b]	Cornea	Opacity	0	1	0	0	0.33
	Iris	--	0	0	0	0	0.0
	Conjunctiva	Redness	1	2	1	0	1.0
		Chemosis	1	1	0	0	0.33
99-27048[a]	Cornea	Opacity	1	1	0	0	0.33
	Iris	--	0	0	0	0	0.0
	Conjunctiva	Redness	1	1	1	0	0.67
		Chemosis	2	1	0	0	0.33
99-27052[a,b]	Cornea	Opacity	0	1	0	0	0.33
	Iris	--	0	0	0	0	0.0
	Conjunctiva	Redness	1	1	1	0	0.67
		Chemosis	2	1	0	0	0.33

[a]fur around eye stained with test material, [b] test material in eye
* mean of 24, 28 and 72 hours

Deficiencies:
None.

Conclusion/endpoint:

According to both Directive 67/548/EC and Regulation (EC) No 1272/2008, the product does not require classification and labelling for eye irritation.

A 2.7 Skin sensitisation (KCP 7.1.6)

Comments of zRMS (UK/CRD and Spanish INIA):	Study acceptable. Agreed endpoint: Based on the animal data available, classification according to CLP criteria with Skin Sen.1B; H317 (Xi; R43 according to Directive 67/548/EEC) is warranted.

Reference	**KCP 7.1.6/01**
Report	xxx, 1999: RH – 7281/ Cymoxanil 66% DG Blend (1:1) Dermal sensitization study in guinea pigs xxx, Report No. 99R–107, GLP, Not published
Guideline(s):	OECD 406 EEC B. 6 US EPA OPPTS 870.2600
Deviations:	Duplicate analytical samples of the vehicles (saline and Freund's Complete Adjuvant solution) used for the intradermal injection and topical challenge phases of this study were inadvertently not taken.
GLP:	Yes

Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test material (Lot No.)	RH – 7281/Cymoxanil 66% DG Blend (1:1) / CYMOXANIL 33% + ZOXAMIDE 33% WG (EG 3860) Cymoxanil: 33% (nominal), 34.7 % (analysed) Zoxamide: 33% (nominal); 33.8% (analysed)
Species	Guinea pigs
No. of animals (group size)	Test substance group: 20 male guinea pigs Irritation control group: 10 male guinea pigs Positive control group: 10 male guinea pigs Irritation control for positive control group: 5 male guinea pigs
Range finding:	No
Exposure (concentration(s), no. of applications)	Intradermal induction: animals in Group 1 (RH-72811Cymoxanil 66% DG; 20 animals) received an induction dose of duplicate intradermal injections on the shoulder area of 1:1 Freund's Complete Adjuvant/sterile saline, a 10% (w/w) suspension of RH-72811Cymoxanil 66% DG in sterile saline, and a 10% (w/w) suspension of RH-72811Cymoxanil 66% DG in 1:1 Freund's Complete Adjuvant/sterile saline; animals in Group 2 (Irritation Control; 10 animals) received intradermal injections of 1:1 Freund's Complete Adjuvant/sterile saline, sterile saline alone, and a 50% (v/v) suspension of sterile saline in 1:1 Freund's Complete Adjuvant/sterile saline; animals in Group 3 (Positive Control; 10 animals) received intradermal injections of 1:1 Freund's Complete Adjuvant/sterile saline, a 5% (w/v) suspension of hexylcinnamaldehyde in mineral oil, and a 5% (w/v) suspension of hexylcinnamaldehyde in 1:1 Freund's Complete Adjuvant/sterile saline; animals in group 4 (Irritation Control for Positive Control; 5 animals) received intradermal injections of 1:1 Freund's Complete Adjuvant/sterile saline, mineral oil alone, and a 50% (v/v) suspension of mineral oil in 1:1 Freund's Complete Adjuvant/sterile saline. Topical induction: On Day 8, either RH-7281/Cymoxanil 66% DG (Group I), sterile saline (Group 2), hexylcinnamaldehyde (Group 3), or mineral oil (Group 4) was applied to saturation as a topical induction dose over the intradermal injection sites of the animals in the respective groups. Challenge: Two weeks after the topical induction application, all animals received a topical challenge dose. A 25% (w/w) suspension of RH-72811Cymoxanil 66% DG in sterile saline was applied to the right side and the control material (sterile saline) was applied to the left side of each animal in Groups 1 and 2. A 25% (w/v) suspension of hexylcinnamaldehyde in mineral oil was applied to the right side and mineral oil alone was applied to the left side of each animal in

	Groups 3 and 4.
Vehicle	suspension of hexylcinnamaldehyde
Pre-treatment prior to topical application	Yes (10% w/w sodium lauryl sulfate (SLS) in petrolatum.)
Reliability check	None
Remarks	None

Skin sensitization potential of RH – 7281 Cymoxanil 66% DG Blend (1:1) was assessed on guinea pigs by intradermal injection and topical application via the Magnusson – Kligman Maximization Test.
20 animals were placed in group 1 and received an induction dose through intradermal injections of RH – 7281 Cymoxanil 66% DG Blend (1:1) - Lot code EG 3860, (CYMOXANIL 33% + ZOXAMIDE 33% WG, analytical content Cymoxanil 34.7%, Zoxamide 33.8%, see part C for details). 10 animals were used as irritation control, other 10 as positive control (with a suspension of hexylcinnamaldehyde) and 5 as irritation control for positive control. A topical treatment followed on day 8 over the intradermal injection sites. Two weeks after the topical induction application all animals received a topical challenge dose.

Results and discussions

Findings:

Mortality:
There was no mortality.

Body weight:
Body weight was unaffected.

Skin responses:
The challenge sites were examined for dermal reactions at 24 and 48 hours after patch removal.
13 of the 20 animals in group 1 exhibited dermal reactions to the challenge application of 25% (w/w) RH – 7281 Cymoxanil 66% DG in sterile saline.

The vehicle (negative) control revealed no skin reactions.

Table A 3: Skin responses after challenge with RH–7281 Cymoxanil 66% DG Blend (1:1)

Treatment	Time post challenge	Number of animals score 0	Number of animals score 1	Number of animals score 2	Number of animals score 3	% of sensitised animals
Negative control	24h 48h	10 10	0 0	0 0	0 0	0 0
Positive control	24h 48h	1 3	3 7	6 0	0 0	90 70
Test substance	24h 48h	8 7	11 12	1 1	0 0	60 65

Negative control = solvent of study test substance.
Positive control = hexylcinnamaldehyde at 5% w/v in mineral oil.
Score 0 = no visible changes; score 1 = discrete or patchy erythema; score 2 = moderate and confluent edema; score 3 = intense erythema and swelling

Conclusion/endpoint:

RH – 7281 Cymoxanil 66% DG Blend (1:1) is a sensitizer under the conditions of this study, and should be classified as R 43 "may cause sensitization by skin contact" according to Directive 67/548/EC. According to Regulation (EC) No 1272/2008 the respective classification/hazard statement is: Skin Sensitization category 1B, H317 "May cause allergic skin reaction.

A 2.8 Supplementary studies for combinations of plant protection products (KCP 7.1.7)

No studies necessary.

A 2.9 Data on co-formulants (KCP 7.4)

A 2.9.1 Material safety data sheet for each co- formulant

Information regarding material safety data sheets of the co-formulants can be found in the confidential part of the dossier (Registration Report - Part C).

A 2.9.2 Available toxicological data for each co-formulant

Information regarding material safety data sheets of the co-formulants can be found in the confidential part of the dossier (Registration Report - Part C).

A 2.10 Studies on dermal absorption (KCP 7.3)

The following study has been performed with the formulated product CYMOXANIL 33% + ZOXAMIDE 33% WG (with nominal concentrations of 330 g/kg cymoxanil and 330 g/kg zoxamide) and its highest in-field dilution (0.1155 kg a.s./1000 L water = 0.1155 g/L of each cymoxanil and zoxamide). A summary of the study design and results, as requested by the EFSA Guidance on Dermal Absorption (EFSA Journal 2012, 10(4):2665) is presented.

Comments of zRMS (PL) :	The study performed according to relevant OECD guidelines and in GLP conditions is acceptable and results may be used for risk assessment. <u>Cymoxanil</u> The final mean dermal absorption values of Cymoxanil from the concentrated formulation was 0.10% ± 0.04, and from the diluted formulation (1:1000) 27.17% ± 9.01%. However, according to recommendation given in EU Guidance for Dermal Absorption (EFSA Journal 2017;15(6):4873) to address variability between replicates dermal absorption should be calculated as follows: Absorption (mean value) + ks, where s is the sample standard deviation. Multiplication factor (k) for 8 replicates is equal 0.84. Therefore a dermal absorption of Cymoxanil for concentrate (330 g/kg g/L) = mean value + ks = 0.1 + 0.84 x 0.04 = 0.13 % and for diluted product (0.1155 g/L)= mean value + ks = 27.17 % + 0.84 x 9.01 = 27.17 + 7.57= 35% Therefore for Cymoxanil these values (0.13 % for concentrate and 35% for dilution) should be used in this part for exposure and risk assessment.

	The final mean dermal absorption values of Zoxamide from the concentrated formulation was 0.15 ± 0.07%, and from the diluted formulation (1:1000) 4.92 ± 1.14%. However, according to recommendation given in EU Guidance for Dermal Absorption (EFSA Journal 2017;15(6):4873) to address variability between replicates dermal absorption should be calculated as follows: Absorption (mean value) + ks, where s is the sample standard deviation. Multiplication factor (k) for 8 replicates is equal 0.84, and for 7 replicates - 0.92. Therefore a dermal absorption of Zoxamide for concentrate (330 g/kg g/L) = mean value + ks = 0.15 + 0.92 x 0.07 = 0.21 % and for diluted product (0.1155 g/L)= mean value + ks = 4.92 % + 0.84 x 1.14 = 5.9% Therefore these values (0.21 %) for concentrate and 5.9% for dilution) should be used in this part for exposure and risk assessment.

Reference	**KCP 7.3/01**
Report	Prisk, L., 2018: The *In Vitro* percutaneous absorption of radiolabelled cymoxanil and radiolabelled zoxamide in the concentrate and one in-use dilution through human skin Gowan Crop Protection Ltd., UK and Sipcam Oxon S.p.A, Italy Charles River Laboratories Edinburgh Ltd., UK, Report No. 38683, GLP, Not published
Guideline(s):	OECD 428 (2004) OECD 28 (2004) OECD 156 (2011) SANCO/222/2000 rev. 7 (2004) EFSA (2012) Guidance on Dermal Absorption
Deviations:	The protocol stated that samples with mass balance outwith 90-110% will be rejected from the mean ± SD. Four samples dosed with the test preparation 4 (spray dilution with [14C]-Zoxamide) had a mass balance greater than 110%. These cells have been accepted based on the OECD No. 28 guideline, which states that for volatile test items recovery boundaries 100 ± 20% may be acceptable. Therefore, this deviation was regarded to have no impact on the integrity of the study.
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test material (Lot/Batch No.)	[acetyl-2-^{14}C]-Cymoxanil Lot/Batch No. XXIII/39/D/1 Specific activity: 7.921 MBq/mg (214 µCi/mg) Radiochemical purity: 99.09%
	[Phenyl-UL-^{14}C]-Zoxamide Lot/Batch No. CFQ43062 Specific activity: 5.15 MBq/mg (47 mCi/mmol or 139 µCi/mg)

	Radiochemical purity: 99.5 %
Samples	split-thickness skin membranes
Human skin membrane	Breast and abdomen/back skin from different donors (male and female, aged 29 to 72 years old)
Exposure	8 hours
Dilution	1. Undiluted WG formulation (CYMOXANIL 33% + ZOXAMIDE 33%) with nominal concentrations of 330 g/kg cymoxanil and 330 g/kg zoxamide (i.e. 363 g/kg cymoxanil [182 g/kg in saline formulation] and 377 g/kg zoxamide [190 g/kg in saline formulation]) 2. Field dilution of nominally 0.1155 g a.s./L water (i.e. actually 0.1185 g/L cymoxanil and 0.1075 g/L zoxamide)
Post exposure time	24 hours
Remarks	None

Results and discussions

The results of the *in vitro* percutaneous penetrations are summarised in the following tables.

Table A 4: Summary of the mean results

Test Preparation	**1**	**2**	**3**	**4**
	Concentrate	**Spray Dilution**	**Concentrate**	**Spray Dilution**
Active Substance	[^{14}C]-Cymoxanil		[^{14}C]-Zoxamide	
Target Concentration Concentration by Radioactivity No. of Replicates	330 g/kg 355 g/kg n = 8	0.1155 g/L 0.1185 g/L n = 8	330 g/kg 355 g/kg n = 7	0.1155 g/L 0.1247 g/L n = 8
	% Applied Dose (mean ± SD)			
Amount in Skin Wash	57.99 ± 30.89	20.52 ± 5.61	47.15 ± 8.15	17.68 ± 6.11
Dislodgeable Dose 8 h	101.90 ± 1.56	65.72 ± 9.83	99.03 ± 6.96	101.84 ± 9.78
Total Dislodgeable Dose	102.14 ± 1.50	65.77 ± 9.82	100.02 ± 6.65	104.84 ± 9.54
Amount in Tape Strips 1-2 (*Stratum Corneum*)	0.05 ± 0.05	0.29 ± 0.17	0.16 ± 0.10	1.13 ± 0.60
Amount in Tape Strips 3-20 (Stratum Corneum)	0.04 ± 0.03	1.64 ± 0.47	0.11 ± 0.06	1.75 ± 0.76
Total Unabsorbed Dose	102.28 ± 1.51	67.81 ± 9.47	100.31 ± 6.61	107.73 ± 9.09
Amount in Receptor Fluid	0.04 ± 0.01	24.31 ± 8.36	0.01 ± <0.01	1.95 ± 1.13
Amount in Receptor Chamber Wash	0.01 ± 0.01	0.74 ± 0.43	<0.01 ± <0.01	0.19 ± 0.06
Total Absorbed Dose	0.04 ± 0.02	25.05 ± 8.66	0.01 ± 0.01	2.13 ± 1.17
Exposed Skin	0.02 ± 0.01	2.12 ± 0.57	0.02 ± 0.02	1.04 ± 0.44
Dermal Delivery	0.06 ± 0.02	**27.17 ± 9.01**	0.03 ± 0.02	3.17 ± 1.31
Potentially Absorbable Dose	**0.10 ± 0.04**	28.81 ± 9.31*	**0.15 ± 0.07**	**4.92 ± 1.14**
Total Recovery	102.34 ± 1.52	94.98 ± 1.65	100.34 ± 6.61	110.91 ± 9.76
	µg equiv./cm^2 (mean ± SD)	**ng equiv./cm^2 (mean ± SD)**	**µg equiv./cm^2 (mean ± SD)**	**ng equiv./cm^2 (mean ± SD)**

Amount in Skin Wash	1057 ± 563	243 ± 66.5	866 ± 150	190 ± 65.7
Dislodgeable Dose 8 h	1858 ± 28.4	779 ± 117	1820 ± 128	1095 ± 105
Total Dislodgeable Dose	1863 ± 27.3	779 ± 116	1838 ± 122	1127 ± 103
Amount in Tape Strips 1-2 (Stratum Corneum)	0.97 ± 0.89	3.40 ± 2.07	3.01 ± 1.87	12.1 ± 6.43
Amount in Tape Strips 3-20 (Stratum Corneum)	0.73 ± 0.47	19.4 ± 5.55	2.08 ± 1.17	18.8 ± 8.14
Total Unabsorbed Dose	1865 ± 27.6	804 ± 112	1843 ± 122	1158 ± 97.7
Amount in Receptor Fluid	0.69 ± 0.26	288 ± 99.1	0.13 ± 0.07	20.9 ± 12.1
Amount in Receptor Chamber Wash	0.11 ± 0.23	8.78 ± 5.09	0.07 ± 0.07	2.00 ± 0.61
Total Absorbed Dose	0.80 ± 0.30	297 ± 103	0.20 ± 0.13	22.9 ± 12.6
Exposed Skin	0.34 ± 0.17	25.2 ± 6.70	0.39 ± 0.32	11.2 ± 4.69
Dermal Delivery	1.14 ± 0.40	**322 ± 107**	0.59 ± 0.29	34.1 ± 14.1
Potentially Absorbable Dose	**1.87 ± 0.73**	341 ± 110*	**2.67 ± 1.32**	**52.9 ± 12.3**
Total recovery	1866 ± 27.7	1126 ± 19.6	1844 ± 121	1192 ± 105
	ng equiv./cm²/h (mean ± SD)	**ng equiv./cm²/h (mean ± SD)**	**ng equiv./cm²/h (mean ± SD)**	**ng equiv./cm²/h (mean ± SD)**
Maximal flux (ng equiv./cm²/h) [a]	197.56 ± 59.77	98.29 ± 63.94	18.69 ± 27.49	1.71 ± 0.98
Mean absorption into the receptor fluid within the first half of the study (t0.5)	72%	96%	56%	70%
>75% Absorbed in Receptor Fluid in the First Half of the Study	No	Yes	No	No

Dislodgeable dose 8 h = amount in skin wash (8 h) + tissue swab (8 h) + pipette tip (8 h). For Test Preparation 4, this also included filters (8 h). Total dislodgeable dose = amount in donor wash + tissue swab (24 h) + dislodgeable dose (8 h). For Test Preparation 4, this also included trap (24 h) + filters (24 h). Total unabsorbed dose = total dislodgeable dose + *stratum corneum* + unexposed skin. Total absorbed dose = amount in the receptor fluid + receptor chamber wash. Exposed skin = exposed area of skin after tape stripping. Dermal delivery = exposed skin + total absorbed dose. Potentially absorbable dose = amount in the receptor fluid + exposed skin + receptor chamber wash + tape strips 3-20 (*stratum corneum)*.

* = absorption was complete for this test preparation (EFSA, 2012 and 2017); therefore, tape strip material is not required to be included in the final dermal absorption value.

[a] = The flux (absorption rate) was calculated by dividing the amount of material absorbed into the receptor fluid by the number of hours for a ng equiv./cm2/h value. Maximum flux presented in the above table is the highest absorption rate calculated for the test preparation.

As confirmed by the EFSA Guidance Document (2017), the first 2 tape strips represent active substance material that will not become available due to desquamation. Only if absorption is essentially complete at the end of the study (i.e. after 24 hours sampling period), all tape strips can be excluded from calculation of the absorbable dose fraction. The resulting study endpoints for the formulation concentrate and the field dilution of cymoxanil and zoxamide are presented in **bold** in the below tables.

In this connection, absorption is regarded as complete when > 75% of the amount that has permeated into the receptor fluid at the end of the sampling (i.e. after 24 h) has reached the receptor fluid during the first half of the sampling period (i.e. after 12 hours). The mean relative permeation into the receptor fluid occurring within half of the sampling period (t0.5) was calculated from individual replicate data according to recommendations of the EFSA Guidance Document (2017).

Table A 5: Tabulated summary according to the Guidance on Dermal Absorption (EFSA Journal, 2012, 10(4): 2665), taking into account further definitions and requirements of EFSA (2107) - Cymoxanil

Test Preparation No.	**1**	**2**

Dilution	Formulation Concentrate	Field Dilution
Nominal Concentration of Active Substance	330 g/kg	0.1155 g/L
Nominal Concentration of Active Substance in the Solid Formulation/Saline Paste	165 g/kg	N/A
Name of Test Item	**Cymoxanil**	
Name of Formulation	GWN-9823	
Type of Formulation	Water dispersible granule (WG)	
Vehicle Used	N/A	Tap water
Application Rate (Test Preparation)	10 mg/cm^2	10 µL/cm2
Actual Amount Applied (Test Item)	1829 µg equiv./cm^2	1.18 µg equiv./cm2
Skin Preparation Used	Split-Thickness (dermatomed) skin (350-400 µm)	
Skin Sample Source	Abdomen, Abdomen & Back	Abdomen, Abdomen & Back, Breast
Donor Species	Human	Human
Exposure Time	8 h	8 h
Sampling Duration (Time of Last Sample)	24 h	24 h
Type of Diffusion Cell	Static	Static
Receptor Fluid Composition	Phosphate buffered saline containing polyoxyethylene 20-oleyl ether (PEG, *ca* 6%, w/v), sodium azide (*ca* 0.01%, w/v), streptomycin (*ca* 0.1 mg/mL) and penicillin (*ca* 100 units/mL), pH 7.3-7.4.	
Adequate Solubility of Test Item in Receptor Fluid Confirmed	Yes	Yes
Type of Tape Used	Scotch® Tape	
Group Size/Number of Replicates	8	8
Amount in Skin Sample Washes (%, mean ± SD)	57.99 ± 30.89	20.52 ± 5.61
Amount in 8 h Tissue Swabs (%, mean ± SD)	43.89 ± 29.72	45.19 ± 13.48
Amount in Receptor Fluid and Receptor Chamber Wash (%, mean ± SD)	0.04 ± 0.02	25.05 ± 8.66
Amount in Tape Stripped Skin Samples (%, mean ± SD)	0.02 ± 0.01	2.12 ± 0.57
Amount in Tape Strips 3 to 20 (%, mean ± SD)	0.04 ± 0.03	1.64 ± 0.47
Amount in Tape Strips 1 + 2 (%, mean ± SD)	0.05 ± 0.05	0.29 ± 0.17
Amount in Receptor Fluid and Stripped Skin Membrane (%, mean ± SD)	0.06 ± 0.02	27.17 ± 9.01
Amount in Receptor Fluid, Stripped Skin Membrane and Tape Strips 3-20 (%, mean ± SD)	0.10 ± 0.04	28.81 ± 9.31
Mean absorption into the receptor fluid within the first half of the study (t0.5)	72%	96%
>75% Absorbed in Receptor Fluid in the First Half of the Study	No	Yes
Therefore, the Amount Absorbed According to EFSA Defination[1] (%, mean ± SD)	**0.10 ± 0.04**	**27.17 ± 9.01**
Total Recovery (%, mean ± SD)	102.34 ± 1.52	94.98 ± 1.65

[1] According to EFSA definitions: absorbed dose = receptor fluid + receptor chamber washes + skin sample (excluding all tape strips) if absorption is complete (>75% of absorption occurred in the first half of the study) or absorbed dose = receptor fluid + receptor chamber washes + skin sample (excluding tape strips 1 and 2) if absorption is incomplete (<75% of absorption occurred in the first half of the study).

Table A 6: Tabulated summary according to the Guidance on Dermal Absorption (EFSA Journal, 2012, 10(4): 2665), taking into account further definitions and requirements of EFSA (2107) - Zoxamide

Test Preparation No.	**3**	**4**
Dilution	Formulation Concentrate	Field Dilution
Concentration of Active Substance in the Formulation	330 g/kg	0.1155 g/L
Nominal Concentration of Active Substance in the Solid Formulation/Saline Paste	165 g/kg	N/A
Name of Test Item	**Zoxamide**	
Name of Formulation	GWN-9823	
Type of Formulation	Water dispersible granule (WG)	
Vehicle Used	N/A	Tap water
Application Rate (Test Preparation)	10 mg/cm^2	10 µL/cm^2
Actual Amount Applied (Test Item)	1834 µg equiv./cm^2	1.07 µg equiv./cm2
Skin Preparation Used	Split-Thickness (dermatomed) skin (350-400 µm)	
Skin Sample Source	Abdomen	Abdomen
Donor Species	Human	Human
Exposure Time	8 h	8 h
Sampling Duration (Time of Last Sample)	24 h	24 h
Type of Diffusion Cell	Static	Static
Receptor Fluid Composition	Phosphate buffered saline containing polyoxyethylene 20-oleyl ether (PEG, *ca* 6%, w/v), sodium azide (*ca* 0.01%, w/v), streptomycin (*ca* 0.1 mg/mL) and penicillin (*ca* 100 units/mL), pH 7.4	
Adequate Solubility of Test Item in Receptor Fluid Confirmed	Yes	Yes
Type of Tape Used	Scotch® tape	
Group Size/Number of Replicates	7	8
Amount in Skin Sample Washes (%, mean ± SD)	47.15 ± 8.15	17.68 ± 6.11
Amount in 8 h Tissue Swabs (%, mean ± SD)	51.77 ± 12.11	84.02 ± 11.67
Amount in Receptor Fluid and Receptor Chamber Wash (%, mean ± SD)	0.01 ± 0.01	2.13 ± 1.17
Amount in Tape Stripped Skin Samples (%, mean ± SD)	0.02 ± 0.02	1.04 ± 0.44
Amount in Tape Strips 3 to 20 (%, mean ± SD)	0.11 ± 0.06	1.75 ± 0.76
Amount in Tape Strips 1 + 2 (%, mean ± SD)	0.16 ± 0.10	1.13 ± 0.60
Amount in Receptor Fluid and Stripped Skin Membrane (%, mean ± SD)	0.03 ± 0.02	3.17 ± 1.31
Amount in Receptor Fluid, Stripped Skin Membrane and Tape Strips 3-20 (%, mean ± SD)	0.15 ± 0.07	4.92 ± 1.14
Mean absorption into the receptor fluid within the first half	56%	70%

of the study (t0.5)		
>75% Absorbed in Receptor Fluid in the First Half of the Study	No	No
Therefore, the Amount Absorbed According to EFSA Definition [1] (%, mean ± SD)	**0.15 ± 0.07**	**4.92 ± 1.14**
Total Recovery (%, mean ± SD)	100.34 ± 6.61	110.91 ± 9.76

[1] According to EFSA definitions: absorbed dose = receptor fluid + receptor chamber washes + skin sample (excluding all tape strips) if absorption is complete (>75% of absorption occurred in the first half of the study) or absorbed dose = receptor fluid + receptor chamber washes + skin sample (excluding tape strips 1 and 2) if absorption is incomplete (<75% of absorption occurred in the first half of the study)

Conclusion

In conclusion, following topical application of [^{14}C]-Cymoxanil in Test Preparation 1 (330 g/kg) and Test Preparation 2 (0.1155 g/L) to human skin *in vitro*, the absorbed dose was 0.04% (0.80 µg equiv./cm^2) and 25.05% (297 ng equiv./cm^2) of the applied dose, respectively. The dermal delivery was 0.06% (1.14 µg equiv./cm^2) and 27.17% (322 ng equiv./cm^2) of the applied dose, respectively. The potentially absorbed dose was 0.10% (1.87 µg equiv./cm^2) of the applied dose for Test Preparation 1. The mass balance was 102.34% (1866 µg equiv./cm^2) and 94.98% (1126 ng equiv./cm2) of the applied dose, respectively. The final dermal absorption values, as defined per EFSA Guidance (2012 and 2017), were 0.10% ± 0.04% and 27.17% ± 9.01% of the applied dose for [^{14}C]-Cymoxanil in Test Preparation 1 and Test Preparation 2, respectively.

Following topical application of [^{14}C]-Zoxamide in Test Preparation 3 (330 g/kg) and Test Preparation 4 (0.1155 g/L) to human skin in vitro, the absorbed dose was 0.01% (0.20 µg equiv./cm^2) and 2.13% (22.9 ng equiv./cm^2) of the applied dose, respectively. The dermal delivery was 0.03% (0.59 µg equiv./cm^2) and 3.17% (34.1 ng equiv./cm^2) of the applied dose, respectively. The potentially absorbed dose was 0.15% (2.67 µg equiv./cm^2) and 4.92% (52.9 ng equiv./cm^2) of the applied dose, respectively. The mass balance was 100.34% (1844 µg equiv./cm2) and 110.91% (1192 ng equiv./cm2) of the applied dose, respectively. The final dermal absorption values, as defined per EFSA Guidance (2012 and 2017), were 0.15% ± 0.07% and 4.92% ± 1.14% of the applied dose for [^{14}C]-Zoxamide in Test Preparation 3 and Test Preparation 4, respectively.

(Prisk L. 2018)

To refine the exposure to Cymoxanil derived by re-entry in the field the following study has been performed with the formulated product CYMOXANIL 33% + ZOXAMIDE 33% WG at its highest in-field dilution (0.1155 kg a.s./1000 L water = 0.1155 g/L of each cymoxanil and zoxamide) administered as dried residue. This dose level reflects potential exposure to a dried surface residue, as can be found on foliage as a consequence of using the (diluted) Cymoxanil-containing product. For re-entry workers, exposure to a dried surface residue occurs post-application during re-entry tasks such as crop maintenance, crop inspection and hand harvesting.
A summary of the study design and results, as requested by the EFSA Guidance on Dermal Absorption (EFSA 2017) is presented.

Comments of zRMS (PL) :	The study performed according to relevant OECD guidelines is acceptable and the results may be used for risk assessment. Cymoxanil The final mean dermal absorption values of Cymoxanil from a Dried Surface Residue was 3.20% ± 2.94% However, according to recommendation given in EU Guidance for Dermal Absorption (EFSA Journal 2017;15(6):4873) to address variability between replicates dermal absorption should be calculated as follows: Absorption (mean value) + ks, where s is the sample standard deviation. Multiplication factor (k) for 8 replicates is equal 0.84. Therefore a dermal absorption of Cymoxanil from a dried surface residue = mean value + ks = 3.2 + 0.84 x 2.94 = 5.7 % . Therefore for dermal absorption of cymoxanil from a dried surface residue a value of 5.7% should be used in this part for exposure and risk assessment.

Reference	**KCP 7.3/02**
Report	Maas W., 2020: The *in vitro* Percutaneous Absorption of Radiolabelled Cymoxanil from a Transferred Dried Surface Residue through Human Split-Thickness Skin Sipcam Oxon S.p.A, Italy Charles River Laboratories Den Bosch BV, The Netherlands Report No. 20211644, GLP, Not published
Guideline(s):	OECD 428 (2004) OECD 28 (2004) OECD 156 (2011) SANCO/222/2000 rev. 7 (2004) EFSA (2017) Guidance on Dermal Absorption
Deviations:	None.
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test material (Lot/Batch No.)	[acetyl-2-^{14}C]-Cymoxanil Lot/Batch No. XXV/66/A/1 Specific activity: 6.897 MBq/mg Radiochemical purity: 99.53%
Samples	split-thickness skin membranes
Human skin membrane	Breast and abdomen skin from different donors (female, aged 38 to 60 years old)
Receptor fluid composition	phosphate buffered saline (PBS) containing polyoxyethylene 20 oleyl ether (6%, w/v), sodium azide (0.01%, w/v), streptomycin (0.1 mg/mL) and penicillin G (100 units/mL).
Exposure	8 hours
Dilution	Field dilution of nominally 0.1155 g a.s./L water (i.e. actually

	0.1185 g/L cymoxanil and 0.1075 g/L zoxamide) applied as a dry residue.
Experimental design	The transfer efficiency of the dried surface residue to the skin was determined preliminarly. The mean transfer efficiency result was used to determine the volume of dose formulation to be transferred to the PTFE-septa to be used in the main dermal absorption experiment in order to reach the target dose. The dose preparation, was distributed over a tetrafluoroethylene (PTFE)-coated septum and air-dried overnight leaving a dried residue, containing [14C]Cymoxanil and non-volatile formulation ingredients on the PTFE-septum. Transfer of the dry residue to the wetted skin was done by placing the PTFE septum on the skin surface.
Post exposure time	24 hours
Remarks	None

Results and discussions

The results of the *in vitro* percutaneous penetrations are summarised in the following tables.

Table A 7: Summary of the mean results

Test Preparation	**Spray Dilution, dried**
Active Substance	[^{14}C]-Cymoxanil
Target Concentration No. of Replicates	0.1155 g/L n = 8
	% Applied Dose (mean ± SD)
Amount in Donor Wash	0.080 ± 0.054
Dislodgeable Dose 8 h	95.1 ± 3.2
Total Dislodgeable Dose	95.2 ± 3.2
Amount in Tape Strips 1-2 (*Stratum Corneum*)	0.048 ± 0.040
Amount in Tape Strips 3-20 (Stratum Corneum)	0.122 ± 0.1
Total Unabsorbed Dose	95.4 ± 3.1
Amount in Receptor Fluid	2.99 ± 2.70
Amount in Receptor Chamber Wash	0.056± 0.056
Total Absorbed Dose	3.05 ± 2.73
Exposed Skin	0.153 ± 0.244
Dermal Delivery	3.20 ± 2.94
Potentially Absorbable Dose	**3.32 ± 3.03**
Total Recovery	98.6 ± 0.6
	ng equiv./cm^2 (mean ± SD)
Applied dose	0.9± 0.20
Amount in Donor Wash	0.001 ± 0.000
Dislodgeable Dose 8 h	0.85 ± 0.18

Total Dislodgeable Dose	0.85 ± 0.18
Amount in Tape Strips 1-2 (Stratum Corneum)	0.0005 ± 0.0005
Amount in Tape Strips 3-20 (Stratum Corneum)	0.0015 ± 0.0015
Total Unabsorbed Dose	0.85 ± 0.18
Amount in Receptor Fluid	0.029 ± 0.032
Amount in Receptor Chamber Wash	0.001 ± 0.001
Total Absorbed Dose	0.029 ± 0.032
Exposed Skin	0.0016 ± 0.0029
Dermal Delivery	0.031 ± 0.035
Potentially Absorbable Dose	0.032 ± 0.036
Total recovery	0.88 ± 0.20
	ng equiv./cm^2/h (mean ± SD)
Maximal flux (ng equiv./cm^2/h) [a]	4.39 ± 4.91
Mean absorption into the receptor fluid within the first half of the study (t0.5)	96.2 ± 1.3
>75% Absorbed in Receptor Fluid in the First Half of the Study	Yes
	3.20 + (0.84*2.94) = 5.7%

Dislodgeable dose 8 h = amount in skin wash (8 h) + tissue swab (8 h) + pipette tip (8 h). For Test Preparation 4, this also included filters (8 h). Total dislodgeable dose = amount in donor wash + tissue swab (24 h) + dislodgeable dose (8 h). For Test Preparation 4, this also included trap (24 h) + filters (24 h). Total unabsorbed dose = total dislodgeable dose + *stratum corneum* + unexposed skin. Total absorbed dose = amount in the receptor fluid + receptor chamber wash. Exposed skin = exposed area of skin after tape stripping. Dermal delivery = exposed skin + total absorbed dose. Potentially absorbable dose = amount in the receptor fluid + exposed skin + receptor chamber wash + tape strips 3-20 (*stratum corneum)*.

* = absorption was complete for this test preparation (EFSA, 2012 and 2017); therefore, tape strip material is not required to be included in the final dermal absorption value.

[a] = The flux (absorption rate) was calculated by dividing the amount of material absorbed into the receptor fluid by the number of hours for a ng equiv./cm2/h value. Maximum flux presented in the above table is the highest absorption rate calculated for the test preparation.

As confirmed by the EFSA Guidance Document (2017), the first 2 tape strips represent active substance material that will not become available due to desquamation. Only if absorption is essentially complete at the end of the study (i.e. after 24 hours sampling period), all tape strips can be excluded from calculation of the absorbable dose fraction. The resulting study endpoint for the formulation concentrate and the field dilution as dried residue of cymoxanil is presented in **bold** in the above table.

In this connection, absorption is regarded as complete when > 75% of the amount that has permeated into the receptor fluid at the end of the sampling (i.e. after 24 h) has reached the receptor fluid during the first half of the sampling period (i.e. after 12 hours). The mean relative permeation into the receptor fluid occurring within half of the sampling period (t0.5) was calculated from individual replicate data according to recommendations of the EFSA Guidance Document (2017).

Conclusion

In conclusion, following topical application of [14C]-Cymoxanil, applied as a dry residue prepared from the Test Preparation (0.1155 g/L) to human skin in vitro, the absorbed dose of [14C]-Cymoxanil was 3.05% (0.029 µg equiv./cm2) of the applied dose.

The 8 h dislodgeable dose was 95.1%, demonstrating high decontamination of the skin. The total dislodgeable dose was 95.2%.

The dermal delivery and mass balance of [14C]-Cymoxanil were 3.20% (0.031 µg equiv./cm2) and 98.6% (0.88 µg equiv./cm2) of the applied dose, respectively. The final dermal absorption value, as de-

fined per EFSA Guidance (2017), was 3.20% ± 2.94%

(Maas W. 2020)

A 2.11 Other/Special Studies (KCP 7.4)

In the following studies on the active substance zoxamide are provided, which were also submitted in July 2020 to the RMS for zoxamide (Latvia) and concerned MSs for an interzonal evaluation.

A 2.11.1 Study 1

Since RH-24549 appears >10% in a nature of hydrolysis study with zoxamide (study no. RB66JN) requested by EFSA (2017), an AMES test has been performed and is provided in the following.

Comments of zRMS PL:	Confirmatory-like study which is under evaluation by the RMS for Zoxamide in an interzonal procedure, thus it was not evaluated in this assessment. RH-24549 was not mutagenic in reverse mutation assay, neither in the presence nor in the absence of metabolic activation.

Reference:	**KCP 7.4**
Report	Schreib, G. 2019: Reverse mutation assay using bacteria *(Salmonella typhimurium and Escherichia coli)* with RH-24549 Gowan Crop Protection Ltd., UK Eurofins BioPharma GmbH, Germany, Report No. STUGC19AA1580-1, GLP, Not published
Guideline(s):	OECD 471 (1997) EEC B.13/14 (2008) OPPTS 870.5100 (1998)
Deviations:	No
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test material (Lot/Batch No.)	RH-24549 (FCC12097)
Purity:	99.95 % (w/w)
Solvent:	
Test organisms	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 *Escherichia coli strain* WP2 uvrA Properly maintained and checked for appropriate genetic markers (rfa mutation, R factor).
Controls	
Negative:	Medium with aqua destillate

Solvent:	dimethylsulfoxide (DMSO)
Positive:	*Without metabolic activation* (-S9) NaN_3; sodium azide Concentration: 10 µg/plate Test strain: *S. typhimurium*: TA100; TA1535 4-NOPD; 4-nitro-o-phenylene-diamine Concetration: 10 µg/plate for TA98, 40 µg/plate for TA1537 Test strain: *S. typhimurium*: TA98; TA1537 MMS; methylmethanesulfonate Concentration: 1µL/plate Test strain: *E.coli* WP2 uvrA
	With metabolic activation (+S9) 2-AA; 2-aminoanthracene Concentrations: 2.5 µg/plate for TA98; TA100; TA1535; TA1537 and 10 µg/plate for *E.coli* WP2 uvrA Test strains: *S. typhimurium*: TA98; TA100; TA1535; TA1537; *E.coli* WP2 uvrA
Activation	S9 from Eurofins Munich and Trinova Biochem GmbH Gießen, derived from male Wistar rats. Protein concentrations in the S9 preparation of Eurofins Munich were 38.0 and 35.7 mg/mL (lot: 270418 and 210918, respectively), in the S9 preparation of Trinova 35.6 mg/mL (lot: 4123). Protein concentrations were adjusted to 30 mg/mL. Tested for sterility and metabolic activation ability (Eurofins) or alkoxyresorufin-0-dealkylase activities, presence of adventitious agents and promutagen activation (Trinova).
S9 Mix composition:	according to Ames et al. (1973)
Test concentrations	**Plate incorporation assay:** 3.16, 10.0, 31.6, 100, 316, 1000, 2500 and 5000 µg/plate **Pre-incubation assay:** 3.16, 10.0, 31.6, 100, 316, 1000, 2500 and 5000 µg/plate
Post exposure observation period	incubated at 37 °C for at least 48 h in the dark
Remarks	None

The test item RH-24549 was investigated for its potential to induce gene mutations according to the plate incorporation test (experiment I) and the pre-incubation test (experiment II) using *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and tester strain *E. coli* WP2 uvrA.

In a reverse gene mutation assay four strains of *Salmonella typhimurium* and one strain of *E. coli* were exposed to RH-24549 using dimethylsulfoxide (DMSO) as solvent.

The test item was dissolved in DMSO and diluted prior to treatment. The solvent was compatible with the survival of the bacteria and the S9 activity.

Based on the results of a pre-experiment, two independent experiments were performed at the following concentrations:

Experiment I: 3.16, 10.0, 31.6, 100, 316, 1000, 2500 and 5000 µg/plate,

Experiment II: 3.16, 10.0, 31.6, 100, 316, 1000, 2500 and 5000 µg/plate.

Each assay was conducted with and without metabolic activation. The test item concentrations including the controls were tested in triplicate. Similar procedures and test concentrations were used in the pre- and the main experiment.

For the plate incorporation method the following materials were mixed in a test tube and poured over the surface of a minimal agar plate: 100 µL Test solution at each dose level, solvent or negative control or reference mutagen solution (positive control), 500 µL S9 mix (for testing with metabolic activation) or S9 mix substitution buffer (for testing without metabolic activation), 100 µL bacteria suspension, pre-culture of the strain), 2000 µL overlay agar. For the pre-incubation method 100 µL of the test item-preparation was pre-incubated with the tester strains (100 µL) and sterile buffer or the metabolic activation system (500 µL) for 60 min at 37 °C prior to adding the overlay agar (2000 µL) and pouring onto the surface of a minimal agar plate. For each strain and dose level, including the controls, three plates were used. After solidification the plates were inverted and incubated at 37 °C for at least 48 h in the dark.

The colonies were counted using a ProtoCOL counter (Meintrup DWS Laborgeräte GmbH). In case of precipitation of the test item, the revertant colonies were counted by hand. In addition, tester strains with a low spontaneous mutation frequency like TA1535 and TA1537 were counted manually.

Cytotoxicity was detected by a clearing or rather diminution of the background lawn or a reduction in the number of revertants down to a mutation factor of approximately ≤ 0.5 in relation to the solvent control.

The Mutation Factor was calculated by dividing the mean value of the revertant counts by the mean values of the solvent control (the exact and not the rounded values are used for calculation). A test item is considered as mutagenic if a clear and dose-related increase in the number of revertants occurs and/or a biologically relevant positive response for at least one of the dose groups occurs in at least one tester strain with or without metabolic activation. A biologically relevant increase was described as follows: If in tester strains TA98, TA100 and E. coli WP2 uvrA the number of reversions is at least twice as high and if in tester strains TA1535 and TA1537 the number of reversions is at least three times higher as compared to the reversion rate of the solvent control.

According to OECD guidelines, the biological relevance of the results is the criterion for the interpretation of results, a statistical evaluation of the results was therefore not regarded necessary. A test item producing neither a dose related increase in the number of revertants nor a reproducible biologically relevant positive response at any of the dose groups was considered to be non-mutagenic.

Results and discussions

No precipitation of the test item was observed in any tester strain used in experiment I and II (with and without metabolic activation). Toxic effects of the test item were noted in most tester strains evaluated in experiment I and II. In experiment I toxic effects of the test item were observed in tester strain TA98 at a concentration of 5000 µg/plate (without metabolic activation). In tester strain TA100 toxic effects of the test item were noted at a concentration of 5000 µg/plate (with and without metabolic activation). In tester strain *E. coli* WP2 uvrA toxic effects of the test item were observed at concentrations of 2500 µg/plate and higher (without metabolic activation). The reduction in the number of revertants down to a mutation factor of ≤ 0.5 found in tester strain TA1535 at a concentration of 100 µg/plate (with metabolic activation) was regarded as not biologically relevant due to lack of a dose-response relationship. In experiment II toxic effects of the test item were noted in tester strains TA98, TA100, TA1537 and *E. coli* WP2 uvrA at a concentration of 5000 µg/plate (with and without metabolic activation). In tester strain TA1535 toxic effects of the test item were noted at concentrations of 2500 µg/plate and higher (without metabolic activation) and at a concentration of 5000 µg/plate (with metabolic activation). No biologically relevant increases in revertant colony numbers of any of the five tester strains were observed following treatment with RH-24549 at any concentration level, neither in the presence nor absence of metabolic activation in experiment I and II.

All validity criteria were met :

- for each strain the bacteria demonstrate their typical responses to ampicillin (TA98, TA100)
- the negative controls with and without S9 mix were within the following ranges (mean values of the spontaneous reversion frequency are within the historical control data range (2016 -2018):

Strain	- S9		+ S9	
	Min	Max	min	Max
TA98	11	58	15	58
TA100	55	155	60	155
TA1535	4	41	3	38
TA1537	3	35	3	34
E. coli WP2 uvrA	32	66	30	79

- corresponding background growth on both negative control and test plates was observed
- the positive controls showed a distinct enhancement of revertant rates over the control plate
- at least five different concentrations of each tester strain were analysable.

Conclusion

In a reverse gene mutation assay four strains of *Salmonella typhimurium* and one strain of *E. coli* were exposed to RH-24549 using dimethylsulfoxide (DMSO) as solvent. Under the experimental conditions, the test item did not cause gene mutations by base pair changes or frameshifts in the genome of the tester strains used. Thus, RH-24549 was not mutagenic, neither in the presence nor in the absence of metabolic activation. The sensitivity of both the plate incorporation and pre-incubation procedures to detect mutagenesis was adequately demonstrated by the results obtained with the positive controls. The study is valid.

(Schreib G. 2019)

A 2.11.2 Study 2

Since RH-129151 appears >10% in a nature of hydrolysis study with zoxamide (study no. RB66JN) requested by EFSA (2017), and can appear in processed commodities, an *in-vitro* genotox package has been performed and is provided in the following.

Comments of PL zRMS:	Confirmatory-like study which is under evaluation by the RMS for Zoxamide in an interzonal procedure, thus it was not evaluated in this assessment. RH-129151 was not mutagenic, neither in the presence nor in the absence of metabolic activation

Reference:	**KCP 7.4**
Report	Schreib, G. 2019: Reverse mutation assay using bacteria *(Salmonella typhimurium* and *Escherichia coli)* with RH-129151 Gowan Crop Protection Ltd., UK Eurofins BioPharma GmbH, Germany, Report No. 188626, GLP, Not published
Guideline(s):	OECD 471 (1997) EEC B.13/14 (2008) OPPTS 870.5100 (1998)

Deviations:	In the study protocol the document *OECD Principles of Good Laboratory Practice (as revised in 1997); OECD Environmental Health and Safety Publications; Series on Principles of Good Laboratory Practice and Compliance Monitoring - Number 1. Environment Directorate, Organisation for Economic Co-operation and Development, Paris 1998* was mentioned, but not that it is consistent with: United States Environmental Protection Agency, FIFRA, Title 40 CFR Part 160, Federal Register, 01 July 2015. This deviation does not change the integrity of the study.
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test material (Lot/Batch No.)	RH-129151 (632031-P1050-36)
Purity:	98.91 % (w/w)
Test organisms	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 *Escherichia coli strain* WP2 uvrA Properly maintained and checked for appropriate genetic markers (rfa mutation, R factor).
Controls	
Negative:	Medium with aqua destillate
Solvent:	dimethylsulfoxide (DMSO)
Positive:	<u>*Without metabolic activation* (-S9)</u> NaN_3; sodium azide Concentration: 10 µg/plate Test strain: *S. typhimurium*: TA100; TA1535 4-NOPD; 4-nitro-o-phenylene-diamine Concetration: 10 µg/plate for TA98, 40 µg/plate for TA1537 Test strain: *S. typhimurium*: TA98; TA1537 MMS; methylmethanesulfonate Concentration: 1µL/plate Test strain: *E.coli* WP2 uvrA
	<u>*With metabolic activation* (+S9)</u> 2-AA; 2-aminoanthracene Concentrations: 2.5 µg/plate for TA98; TA100; TA1535; TA1537 and 10 µg/plate for *E.coli* WP2 uvrA Test strains: *S. typhimurium*: TA98; TA100; TA1535; TA1537; *E.coli* WP2 uvrA
Activation	S9 from Eurofins Munich, derived from male Wistar rats. Protein concentrations in the S9 preparation (lot: 210918) was 35.7 mg/mL and was adjusted to 30 mg/mL. Tested for sterility and metabolic activation ability.
S9 Mix composition:	according to Ames et al. (1973)

Test concentrations	**Plate incorporation assay:** 3.16, 10.0, 31.6, 100, 316, 1000, 2500 and 5000 µg/plate **Pre-incubation assay:** 3.16, 10.0, 31.6, 100, 316, 1000 and 2500 µg/plate
Post exposure observation period	incubated at 37 °C for at least 48 h in the dark
Remarks	None

The test item RH-129151 was investigated for its potential to induce gene mutations according to the plate incorporation test (experiment I) and the pre-incubation test (experiment II) using *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and tester strain *E. coli* WP2 uvrA.

In a reverse gene mutation assay four strains of *Salmonella typhimurium* and one strain of *E. coli* were exposed to RH-129151 using dimethylsulfoxide (DMSO) as solvent.

The test item was dissolved in DMSO, processed by ultrasound for 10 min at 37 °C, and diluted prior to treatment. The solvent was compatible with the survival of the bacteria and the S9 activity.

Based on the results of a pre-experiment, two independent experiments were performed at the following concentrations:

Experiment I: 3.16, 10.0, 31.6, 100, 316, 1000, 2500 and 5000 µg/plate,

Experiment II: 3.16, 10.0, 31.6, 100, 316, 1000 and 2500 µg/plate.

Each assay was conducted with and without metabolic activation. The test item concentrations including the controls were tested in triplicate. Similar procedures were used in the pre- and the main experiment.

For the plate incorporation method, the following materials were mixed in a test tube and poured over the surface of a minimal agar plate: 100 µL Test solution at each dose level, solvent or negative control or reference mutagen solution (positive control), 500 µL S9 mix (for testing with metabolic activation) or S9 mix substitution buffer (for testing without metabolic activation), 100 µL bacteria suspension, pre-culture of the strain), 2000 µL overlay agar. For the pre-incubation method 100 µL of the test item-preparation was pre-incubated with the tester strains (100 µL) and sterile buffer or the metabolic activation system (500 µL) for 60 min at 37 °C prior to adding the overlay agar (2000 µL) and pouring onto the surface of a minimal agar plate. For each strain and dose level, including the controls, three plates were used. After solidification the plates were inverted and incubated at 37 °C for at least 48 h in the dark.

The colonies were counted using a ProtoCOL counter (Meintrup DWS Laborgeräte GmbH). In case of precipitation of the test item, the revertant colonies were counted by hand. In addition, tester strains with a low spontaneous mutation frequency like TA1535 and TA1537 were counted manually.

Cytotoxicity was detected by a clearing or rather diminution of the background lawn or a reduction in the number of revertants down to a mutation factor of approximately ≤ 0.5 in relation to the solvent control.

The Mutation Factor was calculated by dividing the mean value of the revertant counts by the mean values of the solvent control (the exact and not the rounded values are used for calculation). A test item is considered as mutagenic if a clear and dose-related increase in the number of revertants occurs and/or a biologically relevant positive response for at least one of the dose groups occurs in at least one tester strain with or without metabolic activation. A biologically relevant increase was described as follows: If in tester strains TA98, TA100 and E. coli WP2 uvrA the number of reversions is at least twice as high and if in tester strains TA1535 and TA1537 the number of reversions is at least three times higher as compared to the reversion rate of the solvent control.

According to OECD guidelines, the biological relevance of the results is the criterion for the interpretation of results, a statistical evaluation of the results was therefore not regarded necessary. A test item producing

neither a dose related increase in the number of revertants nor a reproducible biologically relevant positive response at any of the dose groups was considered to be non-mutagenic.

Results and discussions

Precipitation of the test item was observed in all tester strains used in experiment I and II (with and without metabolic activation) at a concentration of 1000 µg/plate and higher. The observed precipitation did not interfere with the scoring; thus, it did not impact the results. No toxic effects of the test item were noted in any of the five tester strains used up to the highest dose group evaluated with and without metabolic activation in experiment I and II. The reduction in the number of revertants down to a mutation factor of ≤ 0.5 found in experiment I in tester strain TA 1535 at a concentration of 2500 µg/plate (with metabolic activation) was regarded as not biologically relevant due to lack of a dose-response relationship. No biologically relevant increases in revertant colony numbers of any of the five tester strains were observed following treatment with RH-129151 at any concentration level, neither in the presence nor absence of metabolic activation in experiment I and II.

All validity criteria were met :

- for each strain the bacteria demonstrate their typical responses to ampicillin (TA98, TA100)
- the negative controls with and without S9 mix were within the following ranges (mean values of the spontaneous reversion frequency are within the historical control data range (2016 -2018):

Strain	**- S9**		**+ S9**	
	Min	Max	Min	Max
TA98	11	58	15	58
TA100	55	155	60	155
TA1535	4	41	3	38
TA1537	3	35	3	34
E. coli WP2 uvrA	32	66	30	79

- corresponding background growth on both negative control and test plates was observed
- the positive controls showed a distinct enhancement of revertant rates over the control plate
- at least five different concentrations of each tester strain were analysable.

Conclusion

In a reverse gene mutation assay four strains of *Salmonella typhimurium* and one strain of *E. coli* were exposed to RH-129151 using dimethylsulfoxide (DMSO) as solvent. Under the experimental conditions, the test item did not cause gene mutations by base pair changes or frameshifts in the genome of the tester strains used. Thus, RH-129151 was not mutagenic, neither in the presence nor in the absence of metabolic activation. The sensitivity of both the plate incorporation and pre-incubation procedures to detect mutagenesis was adequately demonstrated by the results obtained with the positive controls. The study is valid.

(Schreib G. 2019)

A 2.11.3 Study 3

Comments of PL as zRMS:	Confirmatory-like study which is under evaluation by the RMS for Zoxamide in an interzonal procedure, thus it was not evaluated in this assessment. RH-129151 is considered to be non-mutagenic in the HPRT locus using V79 cells of the Chinese Hamster.

Reference:	**KCP 7.4**
Report	Voges, Y., 2020: *In vitro* Mammalian Cell Gene Mutation Test (HPRT-Locus) in Chinese Hamster V79 Cells with RH-129151 Gowan Crop Protection Ltd., UK Eurofins BioPharma, Germany, Report No.188628, GLP, Not Published
Guideline(s):	OECD 476 (2016) EEC B.17 (2008) OPPTS 870.5300 (1998)
Deviations:	No
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test material (Lot/Batch No.)	RH-129151 (632031-P1050-36)
Purity:	98.91%
Test cells	V79 cells *in vitro* Eurofins BioPharma Product Testing Munich GmbH culture, properly maintained and purified.
Medium:	Culture medium: MEM medium supplemented with 10 % fetal bovine serum (FBS) 100 U/100 µg/mL penicillin/streptomycin 2 mM L-glutamine 25 mM HEPES 2.5 µg/mL amphotericin B Treatment medium: MEM medium supplemented with 0 % fetal bovine serum (FBS) 100 U/100 µg/mL penicillin/streptomycin 2 mM L-glutamine 25 mM HEPES 2.5 µg/mL amphotericin B Selective medium: MEM medium supplemented with 10 % fetal bovine serum (FBS) 100 U/100 µg/mL penicillin/streptomycin 2 mM L-glutamine 25 mM HEPES 2.5 µg/mL amphotericin B 11 µg/mL 6-thioguanine (TG)
Controls	
Negative:	Medium

Solvent:	THF (0.5 %, v/v)
Positive:	*Without metabolic activation* EMS; ethylmethanesulfonate Dissolved in medium (MEM) Concentration: 300 µg/mL *With metabolic activation* DMBA; 7,12-dimethylbenz(a)anthracene Dissolved in DMSO, dimethylsulfoxide (1% in medium) EMS; ethylmethanesulfonate Concentration: 1.5 µg/mL
Activation	Mammalian microsomal S9 mix from Eurofins Munich, Germany, derived from male Wistar rats. Protein concentration in Eurofins lot 210918 was 35.7 mg/mL. Checked for bilogical activity and sterility.
S 9 mix:	S9 cofactor solution with final protein concentration of 0.75 mg/mL, 8 mM MgC_{l2}, 33 mM KCl, 5 mM Glucose-6-phosphate and 5 mM NADP in 100 mM sodium-phosphate-buffer pH 7.4
Test concentrations	without metabolic activation: 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 7.0, 8.0 and 9.0 µg/mL with metabolic activation: 10, 25, 50, 75, 100, 125, 150, 175, 200 and 250 µg/mL repetition of the experiment with metabolic activation: 25, 50, 75, 100, 125, 140, 150, 160, 170, 180, 200 and 250 µg/mL
Post exposure observation period	4 h
Remarks	None

The test item RH-129151 was assessed for its potential to induce gene mutations at the HPRT locus using V79 cells of the Chinese hamster. The main experiments were carried out without and with metabolic activation. The experiments with metabolic activation were performed by including liver microsomes and NADP for efficient detection of a wide variety of carcinogens requiring metabolic activation. The test item was dissolved in THF. The solvent was compatible with the survival of the cells and the S9 activity.

Based on the results of a pre-experiment, tests with and without metabolic activation were performed as a 4 h short-term exposure assay. The test item was investigated at the following concentrations:

without metabolic activation: 3.5, 4.0, 4.5, 5.5, 6.0 and 8.0 µg/mL and

with metabolic activation: 25, 50, 75, 150 and 200 µg/mL.

Repetition of the experiment with metabolic activation due to unclear results in the first test: 150, 160, 170, 180, 200 and 250 µg/mL.

Main test:

Approx. 5 x 10^6 cells per treatment group were seeded in complete culture medium in a 175 cm^2 culture flask. Approx. 24 h after seeding, the cells were exposed to designated concentrations of the test item either in the presence or absence of metabolic activation. After 4 h the cultures were checked for precipitation and the treatment medium containing the test item (MEM without FBS) was removed. The cells were washed twice with PBS, trypsinised and counted with a cell counter.

For **expression**, one 175 cm² flask was seeded with at least 2 x 10^6 cells per treatment group in complete culture medium (MEM supplemented with 10% FBS). Cells were subcultured within the following (7 – 9) days after treatment in complete culture medium in a sufficient number of cells (at least 2 x 106 cells per treatment group).

For **survival**, two 25 cm² flasks were seeded with approx. 200 cells in complete culture medium for each treatment group. After incubation for an appropriate time (6 – 7 days) colonies were fixed with methanol, stained with Giemsa and counted.

For **mutant frequency**, at the end of the expression period (after 7 to 9 days) about 4 x 10^5 cells for each treatment group were seeded in 5 cell culture petri dishes (diameter 90 mm) with selective medium containing 11 µg/mL 6-thioguanine for further incubation. After incubation for 9 – 11 days colonies were fixed with methanol, stained with Giemsa and counted.

For **cloning efficiency**, at the end of the expression period (after 7 to 9 days), two 25 cm² flasks were seeded with approx. 200 cells in complete culture medium for each treatment group. After incubation for 6 – 8 days colonies were fixed with methanol, stained with Giemsa and counted.

The non-parametric Mann-Whitney test was applied to the mutation data to prove the concentration groups for any significant difference in mutant frequency compared to the solvent controls. Mutant frequencies of the solvent controls were used as reference.

A test chemical was considered to be clearly negative if, in all experimental conditions examined none of the test concentrations exhibits a statistically significant increase compared with the concurrent negative control, there is no concentration-related increase when evaluated with an appropriate trend-test, and all results are inside the distribution of the historical negative control data. A test chemical is considered to be clearly positive if, in any of the experimental conditions examined at least one of the test concentrations exhibits a statistically significant increase compared with the concurrent negative control, the increase is concentration-related when evaluated with an appropriate trend test, and any of the results are outside the distribution of the historical negative control data. Moreover, if there is by chance a low spontaneous mutant frequency in the corresponding negative and solvent controls a concentration-related increase of the mutations within their range has to be discussed. According to the OECD guideline, the biological relevance is considered first for the interpretation of results.

Results and discussions

The pH-value detected with the test item was within the physiological range (pH 7.0 ± 0.4).

Precipitation of the test item was noted in the experiments with metabolic activation at concentrations of 200 µg/mL and higher.

Toxicity: A biologically relevant growth inhibition (reduction of relative survival below 70%) was observed after the treatment with the test item in experiment with and without metabolic activation. In the experiment without metabolic activation the relative survival was 15% for the highest concentration (8.0 µg/mL) evaluated. Due to the occurrence of precipitation the highest biologically relevant concentration evaluated with metabolic activation was 200 µg/mL with a relative survival of 33% for the first conducted experiment. For the repetition experiment with metabolic activation the relative survival was 85% at concentration 250 µg/mL. This discrepancy is presumable due to the occurrence of precipitate.

Mutagenicity: In the main experiment without and with metabolic activation and the repetition experiment all validity criteria were met. The mean mutant values of the negative and solvent controls fall within the historical data range of the test facility and the cloning efficiencies of the negative and solvent controls are >50%. The positive controls, DMBA (1.5 µg/mL) and EMS (300 µg/mL) showed statistically significant increases in mutant frequency, thereby demonstrating both the sensitivity and validity of the test systems.

In the experiment **without metabolic activation** the mutant values of the negative, solvent controls and most mutant values found for all applied concentrations of the test item were within the historical control data of the test facility Eurofins Munich (about 8.7 – 42.5 mutants per 106 cells). The positive control EMS induced a distinct increase in mutant frequency with 224.2 mutants/106 cells. The mutant frequencies induced by the test item did not show a biologically relevant increase. None of the observed mutant frequencies was statistically significantly increased over those of the solvent controls. Additionally, no significant concentration-related increase was determined in the χ^2 test for trend. The highest mutant frequency was observed at a concentration of 4.0 µg/mL (11.8 mutants per 106 cells) with a relative survival of 70%.

In the experiment **with metabolic activation** the mutant values of the negative, solvent controls and most mutant values found for all, but one applied concentration of the test item were within the historical control data of the test facility Eurofins Munich (about 9.2 – 45.3 mutants per 106 cells). The positive control DMBA induced a distinct increase in mutant frequency with 397.8 mutants/106 cells. A statistical analysis displayed that one of the mutant frequencies (at concentration 150 µg/mL with 57.8 mutants/106 cells) was clearly significantly increased over those of the solvent controls and outside the historical control data, but there was no significant concentration-related increase determined in the χ^2 test for trend. Due to the not clear results in the experiment with metabolic activation and after consultation with the sponsor an independent repetition experiment was performed with closer concentration spacing within the relevant range.

During the **repetition of the main experiment with metabolic activation** the mutant values of the negative, solvent controls and most mutant values of the test item concentrations found were within the historical control data of the test facility Eurofins Munich (about 9.2 – 45.3 mutants per 10^6 cells). The positive control DMBA induced a distinct increase in mutant frequency with 211.9 mutants/10^6 cells. A statistical analysis displayed that one of the mutant frequencies was significantly increased over those of the solvent controls (at concentration 200 µg/mL with 48.1 mutants/10^6 cells, but no statistically significantly concentration-related increase was determined in the χ^2 test for trend. The observed slight increase can be attributed to biological variability and was regarded as not biologically relevant. Additionally, since precipitate was noted at concentrations of 200 µg/mL and higher, this increased mutant frequency has to be evaluated with caution, as artefactual effects may result from the precipitate.

The test was regarded acceptable :

- Negative and/or solvent controls fall within the performing laboratories 95th control limits of the historical control data range: 8.7 – 42.5 mutants/10^6 cells (without metabolic activation) and 9.2 – 45.3 mutants/10^6 cells (with metabolic activation) (January 2015 – December 2018).
- The absolute cloning efficiency ([number of positive cultures x 100] / total number of seeded cultures) of the negative and /or solvent controls was > 50%.
- The positive controls (EMS and DMBA) induced a statistically significant increase compared to the concurrent negative control and are compatible with the laboratory historical data base.
- Two experimental conditions (e.g. with and without metabolic activation) were tested unless one results in a positive response.

Conclusion

In conclusion, in the described in vitro cell gene mutagenicity test under the experimental conditions reported, RH-129151 is considered to be non-mutagenic in the HPRT locus using V79 cells of the Chinese Hamster.
Reference mutagens were tested in parallel to the test item and demonstrated the sensitivity of the test system. The study is valid.

(Voges Y. 2020)

A 2.11.4 Study 4

Comments of zRMS PL:	Confirmatory-like study which is under evaluation by the RMS for Zoxamide in an interzonal procedure, thus it was not evaluated in this assessment. RH-141452 was not mutagenic in *in vitro* mammalian cell gene mutation test (HPRT-Locus) in Chinese Hamster V79 cells, neither in the presence nor in the absence of metabolic activation.

Reference:	**KCP 7.4**
Report	Voges, Y., 2020: *In vitro* mammalian cell gene mutation test (HPRT-Locus) in Chinese Hamster V79 cells with RH-141452 Gowan Crop Protection Ltd., UK Eurofins BioPharma, Germany, Report No. 188620, GLP, Not Published
Guideline(s):	OECD 476 (2016) EEC B.17 (2008) OPPTS 870.5300 (1998)
Deviations:	Additional test item material was required for testing (subsequent delivery). Final arrival of the Test Item: 26 November 2018 / 01 August 2019. Final batch no.: 632031-P1050-44 and 55954-24-06. These deviations did not influence the quality or integrity of the present study.
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test material (Lot/Batch No.)	RH-141452 (632031-P1050-44 and 55954-24-06)
Purity:	96.27%
Test cells	V79 cells *in vitro* Eurofins BioPharma Product Testing Munich GmbH culture, properly maintained and purified.
Medium:	Culture medium: MEM medium supplemented with 10 % fetal bovine serum (FBS) 100 U/100 µg/mL penicillin/streptomycin 2 mM L-glutamine 25 mM HEPES 2.5 µg/mL amphotericin B Treatment medium: MEM medium supplemented with 0 % fetal bovine serum (FBS) 100 U/100 µg/mL penicillin/streptomycin 2 mM L-glutamine 25 mM HEPES 2.5 µg/mL amphotericin B

	Selective medium: MEM medium supplemented with 10 % fetal bovine serum (FBS) 100 U/100 µg/mL penicillin/streptomycin 2 mM L-glutamine 25 mM HEPES 2.5 µg/mL amphotericin B 11 µg/mL 6-thioguanine (TG)
Controls	
Negative:	Medium
Solvent:	DMSO (1 %, v/v)
Positive:	*Without metabolic activation* EMS; ethylmethanesulfonate Dissolved in medium (MEM) Concentration: 300 µg/mL *With metabolic activation* DMBA; 7,12-dimethylbenz(a)anthracene Dissolved in DMSO, dimethylsulfoxide (1% in medium) EMS; ethylmethanesulfonate Concentration: 1.0 µg/mL
Activation	Mammalian microsomal S9 mix from Eurofins Munich, Germany, derived from male Wistar rats. Protein concentration in Eurofins lot 220219 was 35.7 mg/mL. Checked for bilogical activity and sterility.
S 9 mix:	S9 cofactor solution with final protein concentration of 0.75 mg/mL, 8 mM MgC_{l2}, 33 mM KCl, 5 mM Glucose-6-phosphate and 5 mM NADP in 100 mM sodium-phosphate-buffer pH 7.4
Test concentrations	without metabolic activation: 50, 100, 150, 200, 225, 250, 300, 400, 500, 1000, 1500 and 2000 µg/mL with metabolic activation: 100, 150, 175, 200, 210, 215 and 220 µg/mL
Post exposure observation period	4 h
Remarks	None

The test item RH-141452 was assessed for its potential to induce gene mutations at the HPRT locus using V79 cells of the Chinese hamster. The main experiments were carried out without and with metabolic activation. The experiments with metabolic activation were performed by including liver microsomes and NADP for efficient detection of a wide variety of carcinogens requiring metabolic activation. The test item was dissolved in DMSO. The solvent (final concentration of 1 %, v/v) was compatible with the survival of the cells and the S9 activity.

Based on the results of a pre-experiment, tests with and without metabolic activation were performed as a 4 h short-term exposure assay. The test item was investigated at the following concentrations:

without metabolic activation: 50, 100, 150, 200, 225, 250, 300, 400, 500, 1000, 1500 and 2000 µg/mL

and

with metabolic activation: 100, 150, 175, 200, 210, 215 and 220 µg/mL

Main test:

Approx. 5 x 10^6 cells per treatment group were seeded in complete culture medium in a 175 cm^2 culture flask. Approx. 24 h after seeding, the cells were exposed to designated concentrations of the test item either in the presence or absence of metabolic activation. After 4 h the cultures were checked for precipitation and the treatment medium containing the test item (MEM without FBS) was removed. The cells were washed twice with PBS, trypsinised and counted with a cell counter.

For **expression**, one 175 cm² flask was seeded with at least 2 x 10^6 cells per treatment group in complete culture medium (MEM supplemented with 10% FBS). Cells were subcultured within the following (7 – 9) days after treatment in complete culture medium in a sufficient number of cells (at least 2 x 106 cells per treatment group).

For **survival**, two 25 cm² flasks were seeded with approx. 200 cells in complete culture medium for each treatment group. After incubation for an appropriate time (6 – 7 days) colonies were fixed with methanol, stained with Giemsa and counted.

For **mutant frequency**, at the end of the expression period (after 7 to 9 days) about 4 x 10^5 cells for each treatment group were seeded in 5 cell culture petri dishes (diameter 90 mm) with selective medium containing 11 µg/mL 6-thioguanine for further incubation. After incubation for 9 – 11 days colonies were fixed with methanol, stained with Giemsa and counted.

For **cloning efficiency**, at the end of the expression period (after 7 to 9 days), two 25 cm² flasks were seeded with approx. 200 cells in complete culture medium for each treatment group. After incubation for 6 – 8 days colonies were fixed with methanol, stained with Giemsa and counted.

The non-parametric Mann-Whitney test was applied to the mutation data to prove the concentration groups for any significant difference in mutant frequency compared to the solvent controls. Mutant frequencies of the solvent controls were used as reference.

A test chemical was considered to be clearly negative if, in all experimental conditions examined none of the test concentrations exhibits a statistically significant increase compared with the concurrent negative control, there is no concentration-related increase when evaluated with an appropriate trend-test, and all results are inside the distribution of the historical negative control data. A test chemical is considered to be clearly positive if, in any of the experimental conditions examined at least one of the test concentrations exhibits a statistically significant increase compared with the concurrent negative control, the increase is concentration-related when evaluated with an appropriate trend test, and any of the results are outside the distribution of the historical negative control data. Moreover, if there is by chance a low spontaneous mutant frequency in the corresponding negative and solvent controls a concentration-related increase of the mutations within their range has to be discussed. According to the OECD guideline, the biological relevance is considered first for the interpretation of results.

Results and discussions

The pH-value detected with the test item was within the physiological range (pH 7.0 ± 0.4).

No precipitation of the test item was noted in the main experiments.

Toxicity: A biologically relevant growth inhibition (reduction of relative survival below 70%) was observed after treatment with the test item in experiment with and without metabolic activation. In the experiment without metabolic activation the relative survival was 19% for the highest concentration (1500 µg/mL) evaluated. The highest biologically relevant concentration evaluated with metabolic activation was 210 µg/mL with a relative survival of 13%.

Mutagenicity: In the main experiment metabolic activation all validity criteria were met. Most of the mutant values of the negative and solvent controls fall within the historical data range of the test facility and the cloning efficiencies of the negative and solvent controls are > 50%. In the experiment without metabolic activation, the individual mutant value of one of the negative controls was slightly increased and outside the historic control range. Since no technical failure occurred, the data was considered acceptable for addition to the laboratory historical database. The positive controls, DMBA (1.0 µg/mL) and EMS (300 µg/mL) showed statistically significant increases in mutant frequency, thereby demonstrating both the sensitivity and validity of the test systems.

In the experiment **without metabolic activation** the mean mutant values of the negative, solvent controls and all mutant values found for all applied concentrations of the test item were within the historical control data of the test facility Eurofins Munich (about 8.7 - 42.5 mutants per 10^6 cells). The positive control EMS induced a distinct increase in mutant frequency with 281.5 mutants/10^6 cells. Additionally, the individual mutant value of one of the negative controls was slightly increased (54.4 mutants/10^6 cells) and outside the historic control range. However, the mean value of this control was within the historical data range of the test facility and since no technical failure occurred, the data was considered acceptable for addition to the laboratory historical database. The mutant frequencies induced by the test item did not show a biologically relevant increase. None of the observed mutant frequencies was statistically significantly increased over those of the solvent controls and no significant concentration-related increase was determined in the χ^2 test for trend. The highest mutant frequency was observed at a concentration of 1000 µg/mL (40.9 mutants per 10^6 cells) with a relative survival of 45%.

In the experiment **with metabolic activation** the mean mutant values of the negative, solvent controls and all mutant values found for all applied concentrations of the test item were within the historical control data of the test facility Eurofins Munich (about 9.2 - 45.3 mutants per 10^6 cells). The positive control DMBA induced a distinct increase in mutant frequency with 597.3 mutants/10^6 cells. In the experiment with metabolic activation, the individual mutant value of one of the negative controls was slightly decreased (7.0 mutants/10^6 cells) and outside the historic control range. However, the mean value of this control (9.7 mutants/106 cells) was within the historical data range of the test facility and therefore considered acceptable. The mutant frequencies induced by the test item did not show a biologically relevant increase. None of the observed mutant frequencies was statistically significantly increased over those of the negative controls and no significant concentration-related increase was determined in the χ^2 test for trend. The highest mutant frequency was observed at a concentration of 150 µg/mL (14.7 mutants per 10^6 cells) with a relative survival of 62%.

The test was regarded acceptable :

- Negative and/or solvent controls fall within the performing laboratories 95th control limits of the historical control data range: 8.7 - 42.5 mutants/10^6 cells (without metabolic activation) and 9.2 - 45.3 mutants/10^6 cells (with metabolic activation) (January 2015 - December 2018).
- The absolute cloning efficiency ([number of positive cultures x 100] / total number of seeded cultures) of the negative and /or solvent controls was > 50%.
- The positive controls (EMS and DMBA) induced a statistically significant increase compared to the concurrent negative control and are compatible with the laboratory historical data base.
- Two experimental conditions (e.g. with and without metabolic activation) were tested unless one results in a positive response.

Conclusion

In conclusion, in the described in vitro cell gene mutagenicity test under the experimental conditions reported, RH-141452 is considered to be non-mutagenic in the HPRT locus using V79 cells of the Chinese Hamster.

Reference mutagens were tested in parallel to the test item and demonstrated the sensitivity of the test system. The study is valid.

(Voges Y. 2020)

A 2.11.5 Study 5

Comments of zRMS PL:	Confirmatory-like study which is under evaluation by the RMS for Zoxamide in an interzonal procedure, thus it was not evaluated in this assessment. RH-150721 was not mutagenic, neither in the presence nor in the absence of metabolic activation in the in vitro mammalian cell gene mutation assay (Thymidine Kinase locus) in mouse lymphoma L5178Y cells .

Reference:	**KCP 7.4/05**
Report	Schreib, G., 2017: *In vitro* mammalian cell gene mutation assay (Thymidine Kinase Locus/TK$^{+/-}$) in mouse lymphoma L5178Y cells with RH-150721 Gowan Crop Protection Ltd., UK Eurofins BioPharma, Germany, Report No. 171360, GLP, Not Published
Guideline(s):	OECD 490 (2016) EEC B.17 (2008)
Deviations:	No
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test material (Lot/Batch No.)	RH-150721 (79942-4-16)
Purity:	98.07 % (w/w)
Test cells	mouse lymphoma cell line L5178Y, culture of Eurofins, Munich, Germany
Medium:	Culture medium: RPMI 1640 complete medium with 10 % horse serum (HS) 100 U/100 µg/mL penicillin/streptomycin 1 mM sodium pyruvate 2 mM L-glutamine 25 mM HEPES 2.5 µg/mL amphotericin B Treatment medium: RPMI 1640 complete medium with 5 % horse serum (HS) for short-term exposure, 7.5 % for long-term exposure 100 U/100 µg/mL penicillin/streptomycin 2 mM L-glutamine 25 mM HEPES

	2.5 µg/mL amphotericin B Selective medium: RPMI 1640 complete medium with 20 % horse serum (HS) 100 U/100 µg/mL penicillin/streptomycin 1 mM sodium pyruvate 2 mM L-glutamine 25 mM HEPES 2.5 µg/mL amphotericin B 5 µg/mL TFT
Controls	
Negative:	Medium
Solvent:	RPMI cell culture medium was used as solvent (RPMI + 5% HS)
Positive:	*Without metabolic activation* EMS; ethylmethanesulfonate Dissolved in medium Concentration: 200 and 300 µg/mL MMS; methylmethanesulfonate Dissolved in 0.9% NaCI Concentration: 8 µg/mL and 10 µg/mL *With metabolic activation* B(A)P; benz(a)pyrene Dissolved in DMSO, dimethylsulfoxide (1% in RPMI medium) Concentration: 1.5 and 2.5 µg/mL
Activation	Liver microsome preparations (S9 mix) prepared by Eurofins Munich, Germany, derived from male Wistar rats. Protein concentration in Eurofins lot 030217 was 32.4 mg/mL. Checked for bilogical activity and sterility.
S 9 mix:	S9 cofactor solution with final protein concentration of 0.75 mg/mL, 8 mM MgC_{l2}, 33 mM KCl, 5 mM Glucose-6-phosphate and 5 mM NADP in 100 mM sodium-phosphate-buffer pH 7.4
Test concentrations	Exposure (concentration(s), no. of applications) Experiment I without metabolic activation: 10, 20, 50, 82, 89, 112, 115 and 118µg/mL with metabolic activation: 100, 150, 200, 203, 207 and 208µg/mL Experiment II without metabolic activation: 20, 30, 35, 40, 45, 54, 60 and 64µg/mL
Post exposure observation period	4, 24, 48 h
Remarks	None

The test item RH-150721 was assessed for its potential to induce mutations at the mouse lymphoma thymidine kinase locus using the cell line L5178Y.

Concentrations used in the main experiments were selected based on data from the pre-experiments. In experiment I 118 µg/mL (without metabolic activation) and 208 µg/mL (with metabolic activation) were chosen as the highest concentrations. In experiment II 64 µg/mL (without metabolic activation) was selected as the highest concentration. Experiment I was performed without and with metabolic activation over 4 h (short-term exposure assay), experiment II without metabolic activation over 24 h (long-term exposure assay). The experiments with metabolic activation were started by including liver microsome preparations (S9 mix). The test item was dissolved in RPMI cell culture medium was used as solvent (RPMI + 5% HS).

For a short-term exposure experiment 1 x 10^7 cells were suspended in 11 ml RPMI medium with 5% horse serum (25 cm2 flasks) and exposed to designated concentrations of the test item either in the presence or absence of metabolic activation in the mutation experiment. After 4 h the test item was removed by centrifugation (200 x g, 10 min) and the cells were washed twice with PBS. Subsequently, the cells were suspended in 30 ml_ complete culture medium and incubated for an expression and growth period of 2 days in total at 37 °C in 5% C02/95% humidified air. The cell density was determined each day and adjusted to 3 x 10^5 cells/mL in a total culture volume of 20 mL. For a long-term exposure experiment 5 x 10^6 cells were suspended in 25 mL RPMI medium with 7.5% horse serum (75 cm2 flasks) and exposed to designated concentrations of the test item in the absence of metabolic activation. After 24 h the test item was removed by centrifugation (200 x g, 10 min) and the cells were washed twice with PBS. Subsequently, 3 x 10^5 cells/mL were suspended in 14 mL complete culture medium and incubated for an expression and growth period of 2 days at 37 °C in 5% $C0_2$/95% humidified air. The cell density was determined each day and adjusted to 3 x 10^5 cells/mL in a total culture volume of 14 mL. After the expression period the cloning efficiency (CE) of the cells was determined by seeding a statistical number of 1.6 cells/well in two 96-well plates. The cells were incubated for at least 6 days at 37 °C in a humidified atmosphere with 5% C02. Analysis of the results was based on the number of cultures with cell growth (positive wells) and those without cell growth (negative wells) compared to the total number of cultures seeded. Additionally, cultures were seeded in selective medium. Cells from each experimental group were seeded in four 96-well plates at a density of approximately 2000 cells/well in 200 µL selective medium with TFT. The plates were scored after an incubation period of about 12 days at 37 °C in 5% $C0_2$/95% humidified air. The mutant frequency was calculated by dividing the number of TFT resistant colonies by the number of cells plated for selection, corrected for the plating efficiency of cells from the same culture grown in the absence of TFT. For the microwell method used here the Poisson distribution was used to calculate the plating efficiencies for cells cloned without and with TFT selection. Based on the null hypothesis of the Poisson distribution, the probable number of clones/well (P) is equal to - ln(negative wells/total wells) and the plating efficiency (PE) equals P/(number of cells plated per well). Mutant frequency then was calculated as MF = (PE(cultures in selective medium)/PE(cultures in non-selective medium)).

Suspension growth (SG) of the cell cultures reflects the number of times the cell number increases from the starting cell density. When carrying out a short-term treatment (4 h) a 2-day growth period was considered, when carrying out a long-term treatment the treatment a period of 24 h followed by a 2 days growth period. The relative total growth (RTG) was calculated as the product of the relative suspension growth (RSG; calculated by comparing the SG of the dose groups with the SG of the control) and the relative cloning efficiency (ROE) for each culture: RTG = RSG x RCE /100. The mutant frequencies obtained from the experiments were compared with the Global Evaluation Factor (GEF) from ten laboratories. It is defined as the mean of the negative/vehicle mutant frequency plus one standard deviation. Applying this definition to the collected data, the GEF was 126 for the microwell method.

The non-parametric Mann-Whitney test was applied to the mutation data to prove the dose groups for any significant difference in mutant frequency compared to the negative/solvent controls. Mutant frequencies of the solvent/negative controls were used as reference.

The test item is considered mutagenic if the induced mutant frequency meets or exceeds the Global Evaluation factor (GEF) of 126 mutants per 106 cells and a dose-dependent increase in mutant frequency is detected. Besides, combined with a positive effect in the mutant frequency, an increased occurrence of small colonies (>40% of total colonies) is an indication for potential clastogenic effects and/or chromosomal aberrations. According to the OECD guideline, the biological relevance is considered first for the interpretation of results. Statistical methods might be used as an aid in evaluation of the test result. A test item is considered to be negative if the induced mutant frequency is below the GEF and the trend of the test is negative.

Results and discussions

The pH-value detected with the test item and the osmolality were within the physiological range.

Precipitation of the test item was noted in the pre-experiment I without and with metabolic activation at the following concentrations: 500, 1000 and 2000 pg/mL. These concentrations were not further used in the experiments since they resulted in extremely low levels of RSG. No precipitation of the test item was noted in the main experiment I, pre-experiment II or main experiment II (without and with metabolic activation).

Toxicity: Growth inhibition was observed in experiment I and II without and with metabolic activation. In experiment I without metabolic activation the relative total growth (RTG) was 12.1% for the highest concentration (118 pg/mL) evaluated. The highest concentration evaluated with metabolic activation was 208 pg/mL with a RTG of 11.0%. In experiment II without metabolic activation the relative total growth (RTG) was 10.7% for the highest concentration (64 pg/mL) evaluated.

Mutagenicity:

The mutant frequencies obtained from all experiments were compared to the Global Evaluation Factor (GEF) of 126. Criterion for mutagenicity is the extension of the GEF by the induced mutant frequency as well as a dose-dependent increase in mutant frequency. The positive controls EMS (300 and 200 pg/mL), MMS (8 and 10 pg/mL) and B[a]P (2.5 pg/mL) showed distinct effects in mutation frequency, thus proving the ability of the test system to detect potential mutagenic effects.

In the **experiment I without metabolic activation** all validity criteria were met. The negative controls showed mutant frequencies within the acceptance range of 50-170 mutants/10^6 cells, according to the IWGT criteria. The mutant frequencies of the negative controls were 60.3 and 73.1 mutants/10^6 cells, the positive controls EMS and MMS induced a distinct increase in mutant frequency with 686.9 and 838.3 mutants/10^6 cells. The mutant frequencies induced by the test item did not show any biologically relevant increase. The GEF of 126 was not exceeded in any of the dose groups showing induced mutant frequencies between 4.0 and 28.4 mutants/10^6 cells. None of the observed mutant frequencies was statistically significantly increased over those of the negative controls.

In the **experiment I with metabolic activation** all validity criteria were met. The negative controls showed mutant frequencies within the acceptance range of 50-170 mutants/10^6 cells, according to the IWGT criteria. The mutant frequencies of the negative controls were 97.7 and 70.4 mutants/10^6 cells, the positive control B[a]P induced a distinct increase in mutant frequency with 881.0 mutants/10^6 cells. The mutant frequencies induced by the test item did not show any biologically relevant increase. The GEF of 126 was not exceeded in any of the dose groups showing induced mutant frequencies between -9.8 and 59.2 mutants/10^6 cells. Statistical analysis displayed that one of the mutant frequencies was significantly increased over those of the negative controls; however, the GEF was not exceeded. Therefore, this effect was considered as not biologically relevant.

In **experiment II without metabolic activation** all validity criteria were met. The negative controls showed mutant frequencies within the acceptance range of 50-170 mutants/106 cells, according to the IWGT criteria. The mutant frequencies of the negative controls were 57.5 and 56.7 mutants/10^6 cells, the positive controls EMS and MMS induced a distinct increase in mutant frequency with 2179.4 and 541.0 mutants/10^6 cells. The mutant frequencies induced by the test item did not show any biologically relevant increase. The GEF of 126 was not exceeded in any of the dose groups showing induced mutant frequencies between 5.5 and 63.4 mutants/10^6 cells. A statistical analysis displayed that several of the mutant frequencies were significantly increased over those of the negative controls, however the GEF was not exceeded. Therefore, this effect was considered as not biologically relevant.

Historical data for all mutant frequencies for negative and positive controls of experiment I and II were within the historical range of the test facility Eurofins Munich.

Clastogenicity:

The positive controls MMS (8 and 10 µg/mL) and B[*a*]P (2.5 µg/mL) induced a significant increase in mutant frequency and a biologically significant increase of small colonies (>40%), thus proving the ability of the test system to indicate potential clastogenic effects.

In **experiment I without metabolic activation** the percentage of small colonies in the negative controls was found to be 16.0% and 23.2%. The percentage of small colonies of the positive control MMS was found to be 41.1%. In the highest dose groups 17.2% (112 µg/mL), 23.6% (115 µg/mL) and 15.4% (118g/mL) of small colonies were found. As none of the values exceeded 40%, all dose groups were considered as not clastogenic. **With metabolic activation** the percentage of small colonies in the negative controls was found to be 11.1% and 15.2%. The percentage of small colonies of the positive control B[*a*]P was found to be 41.5%. In the highest dose groups 21.8% (203 µg/mL), 15.3% (207 µg/mL) and 22.2% (208 µg/mL) of small colonies were found. As none of the values exceeded 40%, all dose groups were considered as not clastogenic.

In **experiment II without metabolic activation** the percentage of small colonies in the negative controls was found to be 19.0% and 13.2%. The percentage of small colonies of the positive control MMS was found to be 47.8%. In the highest dose groups 18.3% (54 µg/mL), 21.1% (60 µg/mL) and 21.7% (64µg/mL) of small colonies were found. As none of the values exceeded 40%, all dose groups were considered as not clastogenic.

The test was regarded acceptable :

- At least three out of four 96-well plates from the TFT resistance-testing portion of the experiment are scorable.
- The cloning efficiency of the negative and/or solvent controls is in the range 65% -120%.
- The spontaneous mutant frequency in the negative and/or solvent controls is in the range 50-170 mutants per 10^6 cells
- The cell number of the negative/solvent controls should undergo 8-32-fold increase during a 2-day growth period (short-term treatment) or 32-180-fold increase during a 3-day growth period (long-term treatment)
- The clastogenic positive controls (MMS and B[a]P) have to produce an induced mutant frequency (total mutant frequency minus concurrent negative control mutant frequency) of at least 300 mutants per 10^6 cells with at least 40% of the colonies being small colonies or with an induced small colony mutant frequency of at least 150 mutants per 10^6 cells The RTG must be greater than 10%.

Conclusion

The test item RH-150721 is considered to be non-mutagenic in the *in vitro* mammalian cell gene mutation assay (Thymidine Kinase locus) in mouse lymphoma L5178Y cells under the experimental conditions

reported. Reference mutagens were tested in parallel to the test item and demonstrated the sensitivity of the test system. The study is valid.

(Schreib G. 2017)

A 2.11.6 Study 6

Comments of zRMS PL :	Confirmatory-like study which is under evaluation by the RMS for Zoxamide in an interzonal procedure, thus it was not evaluated in this assessment. RH-129151was not causing chromosomal aberrations, neither in the presence nor in the absence of metabolic activation in the in vitro mammalian micronucleus assay in Chinese Hamster V79 Cells.

Reference:	**KCP 7.4**
Report	Donath, C., 2020: *In vitro* mammalian micronucleus assay in Chinese Hamster V79 Cells with RH-129151 Gowan Crop Protection Ltd., UK Eurofins BioPharma, Germany, Report No. 188627, GLP, Not published
Guideline(s):	OECD 487 (2016) EEC B.49 (2017)
Deviations:	No
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test material (Lot/Batch No.)	RH-129151 (632031-P1050-36)
Purity:	98.91%
Test cells	Chinese Hamster V79 (ATCC, CCL-93) cells *in vitro* Eurofins Munich, Germany culture Three or four day-old stock cultures (in exponential growth), more than 50% confluent, were rinsed with Ca-Mg-free PBS solution, trypsinised with a 0.05% trypsin solution in Ca-Mg-free PBS at 37°C for 5 min. By adding complete culture medium the detachment was stopped and a single cell suspension was prepared.
Medium:	Culture medium: MEM medium supplemented with 10 % fetal bovine serum (FBS) 100 U/100 µg/mL penicillin/streptomycin 2 mM L-glutamine 25 mM HEPES 2.5 µg/mL amphotericin B Treatment medium (short-term exposure): Complete culture medium with

	0 % fetal bovine serum (FBS) After treatment medium (long-term exposure): Complete culture medium with 10 % fetal bovine serum (FBS) 1.5 µg/mL cytochalasin B
Controls	
Negative:	Culture medium
Solvent:	THF (0.5%, v/v)
Positive:	**Clastogenic Controls** *Without metabolic activation* MMS, mmethylmethanesulfonate Concentration: 25 µg/mL *With metabolic activation* CPA (cyclophosphamide) dissolved in MEM Concentration: 2.5 µg/mL **Aneugenic Controls** *Without metabolic activation* Colchicine dissolved in MEM Concentration: 0.16-2.0 µg/mL
Activation	S9 liver microsomal fraction from Eurofins Munich and Trinova Biochem GmbH Giessen, Germany, derived from male Wistar rats. Checked for bilogical activity and sterility.
S 9 mix:	S9 cofactor solution with final protein concentration of 0.75 mg/mL, 8 mM MgC_{l2}, 33 mM KCl, 5 mM Glucose-6-phosphate and 5 mM NADP in 100 mM sodium-phosphate-buffer pH 7.4
Test concentrations	**Experiment I** without metabolic activation: 0.50, 1.0, 1.50, 1.75, 2.0, 2.1, 2.2, 2.3, 2.4 and 2.5 µg/mL **with** metabolic activation: 5, 10, 25, 30, 35, 40, 45, 50 and 75 µg/mL **Experiment II** without metabolic activation: 1.0, 2.5, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 12.5 and 15.0 µg/mL **For the microscopic analyses of micronuclei frequencies** Experiment I with short-term exposure (4 h): without metabolic activation: 2.2, 2.3 and 2.5 µg/mL with metabolic activation: 10, 25 and 30 µg/mL with metabolic activation (repetition): 40, 45 and 50 µg/mL Experiment II with long-term exposure (24 h): without metabolic activation: 1.0, 2.5, 5.0 and 7.0 µg/mL
Post exposure observation period	4 - 72 h
Remarks	None

The *in vitro* micronucleus assay has been performed to assess the possible potential of RH-129151 to induce clastogenic and aneugenic activity in cells that have undergone cell division during or after exposure.

The test item was dissolved in THF and diluted in cell culture medium to reach a final concentration of 0.5% v/v THF in the samples. The solvent was compatible with the survival of the cells and the S9 activity.

Based on the results of a pre-test, experiments with and without metabolic activation were performed in duplicate cultures:

Experiment I

without metabolic activation: 0.50, 1.0, 1.50, 1.75, 2.0, 2.1, 2.2, 2.3, 2.4 and 2.5 µg/mL

with metabolic activation: 5, 10, 25, 30, 35, 40, 45, 50 and 75 µg/mL

Experiment II without metabolic activation: 1.0, 2.5, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 12.5 and 15.0 µg/mL

The following study design was performed:

	Without S9		**With S9**	
	Experiment I	**Experiment II**	**Experiment I**	**Experiment I repitition**
Exposure period	4 h	24 h	4 h	24 h
Cytochalasin B exposure	20 h	23 h	20 h	20 h
Preparation interval	24 h	24 h	24 h	24 h
Total culture period*	72 h	72 h	72 h	72 h

* Exposure started 48 h after culture initiation

Experiment I: Exponentially growing V79 cells were seeded into 25 cm^2 cell culture flasks (two flasks per test group). Approx. 50 000 cells were seeded per cell culture flask, containing 5 mL complete culture medium (minimum essential medium supplemented with 10% FBS). After an attachment period of approx. 48 h, the complete culture medium was removed and subsequently the test item was added to the treatment medium in appropriate concentrations. The cells were incubated with the test item for 4 h in presence or absence of metabolic activation. At the end of the incubation, the treatment medium was removed and the cells were washed twice with PBS. Subsequently, the cells were incubated in complete culture medium + 1.5 µg/mL cytochalasin B for 20 h at 37 °C.

Experiment II: If negative or equivocal results are obtained, they should be confirmed using continuous treatment (long-term treatment) without metabolic activation. Approx. 50 000 exponentially growing V79 cells were seeded in 25 cm^2 cell culture flasks in absence of metabolic activation. After an attachment period of approx. 48 h the test item was added in complete culture medium. 1 h later 1.5 µg/mL cytochalasin B were added and the cells were incubated for 23 h at 37 °C. At the end of the treatment the cell culture medium was removed and the cells were prepared for microscopic analysis.

At the end of the cultivation, the complete culture medium was removed. Subsequently, cells were trypsinated and resuspended in about 9 ml complete culture medium. The cultures were transferred into tubes and incubated with hypotonic solution (0.4% KCl) for some minutes at room temperature. Prior to this an aliquot of each culture was removed to determine the cell count by a cell counter (ALSystems). After the treatment with the hypotonic solution the cells were fixed with methanol + glacial acetic acid (3+1). The cells were resuspended gently and the suspension was dropped onto clean glass slides. Consecutively, the cells were dried on a heating plate. Finally, the cells were stained with acridine orange solution.

For each experimental point, at least 2000 binucleated cells per concentration (1000 binucleated cells per slide) were analysed for micronuclei according to the criteria of Fenech (2000), i.e. clearly surrounded by a

nuclear membrane, having an area of less than one-third of that of the main nucleus, being located within the cytoplasm of the cell and not linked to the main nucleus via nucleoplasmic bridges. Mononucleated and multinucleated cells and cells with more than six micronuclei were not considered.

A cytokinesis block proliferation index (CBPI) and % cytostasis were calculated.

A test item was considered clearly positive if, in any of the experimental conditions examined:

- at least one of the test concentrations exhibits a statistically significant increase compared with the concurrent negative/solvent control,
- the increase is concentration-related in at least one experimental condition when evaluated with an appropriate trend test and
- any of the results are outside the distribution of the historical negative/solvent control data (e.g. poisson-based 95% control limits).

Results and discussions

The pH-value detected with the test item was within the physiological range (pH 7.0 ± 0.4). No precipitate of the test item was noted.

Cytotoxicity: In experiment I without metabolic activation no increase of the cytostasis above 30% was noted up to 2.2 µg/mL. At 2.3 µg/mL a cytostasis of 42% and at 2.5 µg/mL a cytostasis of 54% was noted. In experiment I with metabolic activation no increase of the cytostasis above 30% was noted up to 25 µg/mL. At 30 µg/mL a cytostasis of 50% was observed. In experiment I with metabolic activation (repetition) no increase of the cytostasis above 30% was noted up to 40 µg/mL. At 45 µg/mL a cytostasis of 44% and at 50 µg/mL a cytostasis of 53% was observed. In experiment II without metabolic activation no increase of the cytostasis above 30% was noted up to 2.5 µg/mL. At 5.0 µg/mL a cytostasis of 45% and at 7.0 µg/mL a cytostasis of 51% was observed.

Clastogenicity / Aneugenicity: In experiment I without metabolic activation the micronucleated cell frequency of the negative control (1.05%) was within the historical control limits of the negative control (0.37% – 1.37%). The micronucleated cell frequency of the solvent control (1.55%) was slightly above the historical control limits of the solvent control (0.47% – 1.48%). Since exceedance of the upper historical control limit was marginal and the data were considered acceptable for addition to the laboratory historical database, the experiment was considered valid. The mean values of micronucleated cells found after treatment with the test item were 1.00% (2.2 µg/mL), 0.65% (2.3 µg/mL) and 1.35% (2.5 µg/mL). The numbers of micronucleated cells were within the historical control limits of the solvent control and did not show a biologically relevant increase compared to the concurrent solvent control.
In experiment I with metabolic activation the micronucleated cell frequency of the negative control (1.60%) was within the historical control limits of the negative control (0.42% – 1.64%) and the micronucleated cell frequency of the solvent control (1.60%) was within the historical control limits of the solvent control (0.35% – 1.75%). The mean values of micronucleated cells found after treatment with the test item were 1.25% (10 µg/mL), 1.40% (25 µg/mL) and 1.85% (30 µg/mL). The numbers of micronucleated cells for the concentrations of 10 and 25 µg/mL were within the historical control limits of the solvent control and did not show a biologically relevant increase compared to the concurrent solvent control. The concentration of 30 µg/mL showed a number of micronucleated cells that was slightly above the upper historical control limit of the solvent control. Since this effect was not statistically significant and taking into account the micronuclei frequency observed for the solvent control (1.60%), this increase was regarded as not biologically relevant.

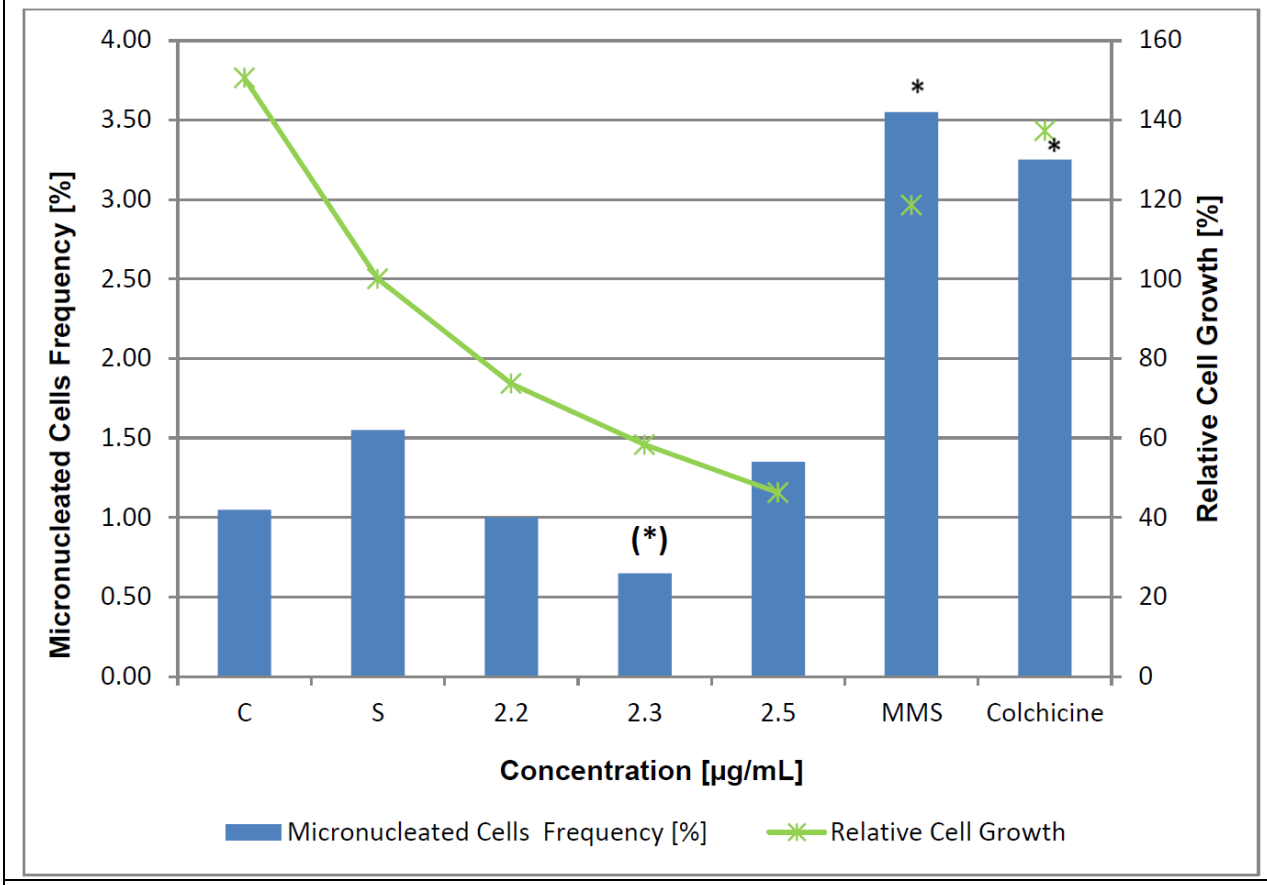
This experimental condition with metabolic activation and short-term treatment was repeated to confirm that the effect seen has no biological relevance. In the repetition experiment the micronucleated cell frequency of the negative control (0.45%) was within the historical control limits of the negative control (0.42% – 1.64%)

and the micronucleated cell frequency of the solvent control (0.75%) was within the historical control limits of the solvent control (0.35% – 1.75%). The mean values of micronucleated cells found after treatment with the test item were 0.70% (40 µg/mL), 0.55% (45 µg/mL) and 0.70% (50 µg/mL). The numbers of micronucleated cells were within the historical control limits of the solvent control and did not show a biologically relevant increase compared to the concurrent solvent control. Thus, the results of the repetition experiment confirmed that the test item did not induce increases of the micronuclei frequency.
In experiment II without metabolic activation the micronucleated cell frequency of the negative control (0.80%) was within the historical control limits of the negative control (0.37% – 1.37%) and the micronucleated cell frequency of the solvent control (0.95%) was within the historical control limits of the solvent control (0.47% – 1.48%). The mean values of micronucleated cells found after treatment with the test item were 0.75% (1.0 µg/mL), 1.25% (2.5 µg/mL), 0.80% (5.0 µg/mL) and 1.10% (7.0 µg/mL). The numbers of micronucleated cells were within the historical control limits of the solvent control and did not show a biologically relevant increase compared to the concurrent solvent control.

No statistically significant enhancement ($p<0.05$) of cells with micronuclei was noted in the dose groups of the test item evaluated in experiment I and II with and without metabolic activation.

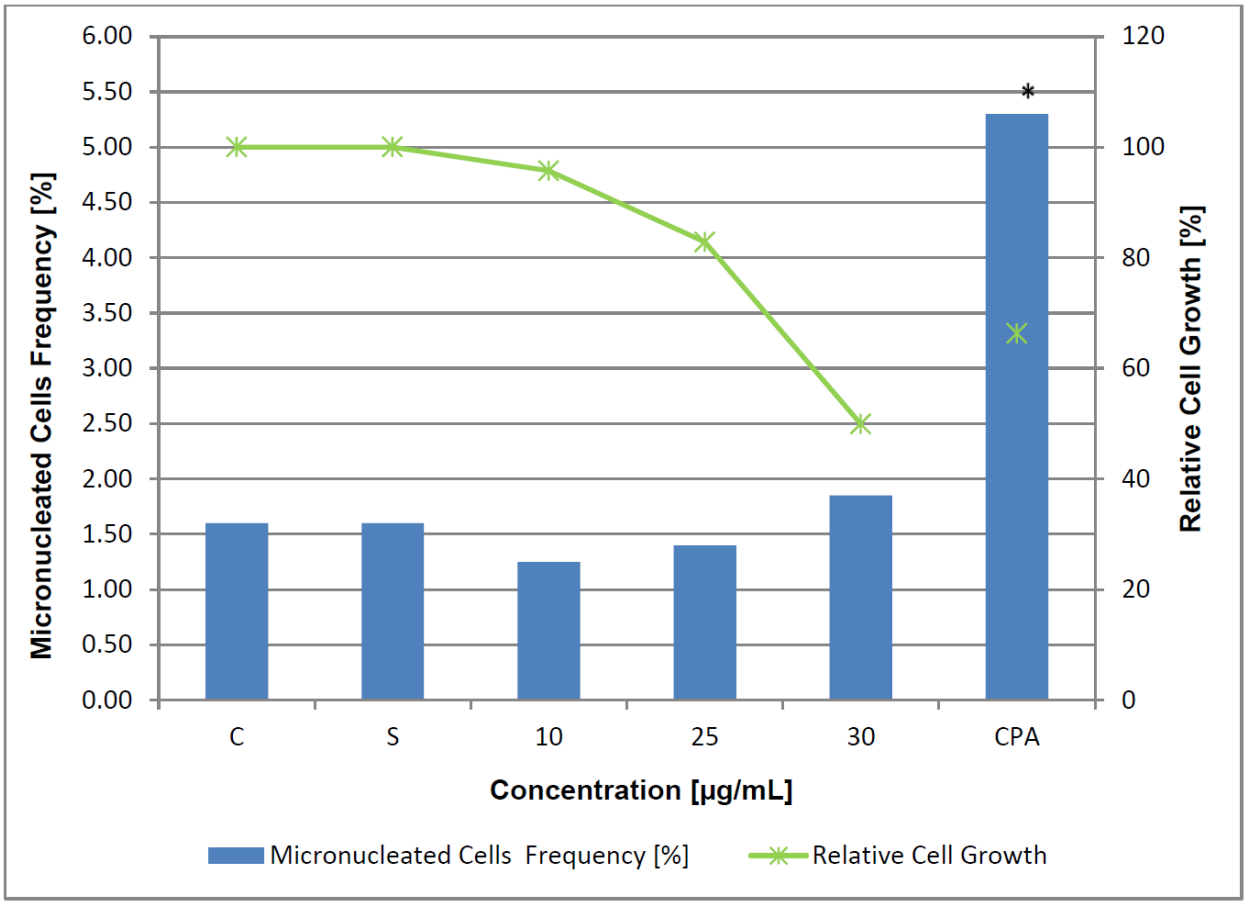
No statistically significant increase in the frequency of micronucleated cells under the experimental conditions of the study was observed in experiment I and II without metabolic activation. In experiment I with metabolic activation a concentration-related increase in the frequency of micronucleated cells was noted. Since the corresponding numbers of micronucleated cells lay below or just slightly and not statistically significantly above the value of the corresponding solvent control and were within the historical control limits of the solvent control or just slightly above the upper limit, this concentration-related increase was regarded as not biologically relevant. Moreover, in the repetition experiment I performed with metabolic activation no statistically significant increase in the frequency of micronucleated cells under this experimental condition was observed.

MMS (25 µg/mL) and CPA (2.5 µg/mL) were used as clastogenic controls and colchicine as aneugenic control (0.16 and 2.0 µg/mL). They induced distinct and statistically significant increases of the micronucleus frequency. This demonstrates the validity of the assay.



The CBPI was determined in 500 cells per culture of each test group.
The relative values of the CBPI are related to the solvent control.
Micronucleated Cell Frequency was determined in 2000 cells (1000 cells per slide), except for the solvent control (3000 cells) and the concentration of 2.5 µg/mL (4000 cells).
C: Negative control (culture medium)
S: Solvent control (THF 0.5% v/v in culture medium)
MMS: Methylmethanesulfonate, positive control (without metabolic activation) [25 µg/mL]
Colchicine: Positive control (without metabolic activation) [2.0 µg/mL]
CBPI: Cytokinesis Block Proliferation Index, CBPI = $((c_1 \times 1) + (c_2 \times 2) + (c_x \times 3))/n$
Relative Cell Growth: 100 x ((CBPI Test conc – 1) / (CBPI control -1))
c1: mononucleate cells
c2: binucleate cells
cx: multinucleate cells
n: total number of cells
*: statistically significant increase of micronucleated cells
(*): statistically significant decrease of micronucleated cells

Figure A 1: Micronucleus frequency and growth rate in the main experiment without metabolic activation



The CBPI was determined in 500 cells per culture of each test group.
The relative values of the CBPI are related to the solvent control.
Micronucleated cell frequency was determined in 2000 cells (1000 cells per slide), except for solvent control and at a concentration of 120 µg/mL (2000 cells per slide), except for the concentration of 30 µg/mL (3000 cells).
C: Negative control (culture medium)
S: Solvent control (THF 0.5% v/v in culture medium)
CPA: Cyclophosphamide, positive control (with metabolic activation) [2.5 µg/mL]
CBPI: Cytokinesis Block Proliferation Index, CBPI = $((c_1 \times 1) + (c_2 \times 2) + (c_x \times 3))/n$
Relative Cell growth: 100 x ((CBPI Test conc – 1) / (CBPI control -1))
c1: mononucleate cells
c2: binucleate cells
cx: multinucleate cells
n: total number of cells
*: statistically significant increase of micronucleated cells

Figure A 2: Micronucleus frequency and growth rate in the main experiment with metabolic activation

The test was regarded acceptable:

- The concurrent negative/solvent control was acceptable for addition to the laboratory historical negative/solvent control database.
- Concurrent positive controls induced responses that were compatible with those generated in the laboratory's historical positive control data base and produced a statistically significant increase compared to the concurrent negative/solvent control.
- Cell proliferation criteria in the negative/solvent control according to OECD 487 have been fulfilled (all experimental conditions were tested unless one resulted in positive results, an adequate number of cells and concentrations was analysable, criteria for the selection of top concentration were fulfilled).
- All experimental conditions were tested unless one resulted in positive results.

- Adequate number of cells and concentrations were analysable.
- Criteria for the selection of top concentration were fulfilled.

Conclusion

Under the experimental conditions of an *in vitro* mammalian micronucleus assay with Chinese Hamster V79 cells the test item did not induce structural and/or numerical chromosomal damage. Therefore, RH-129151 is considered to be non-mutagenic with respect to clastogenicity and/or aneugenicity.
Positive controls demonstrated the sensitivity of the test system.
The study is valid.

(Donath C. 2020)

A 2.11.7 Study 7

Comments of zRMS PL:	Confirmatory-like study which is under evaluation by the RMS for Zoxamide in an interzonal procedure, thus it was not evaluated in this assessment. RH-141452 was not causing structural and/or numerical chromosomal aberrations, neither in the presence nor in the absence of metabolic activation in the *in vitro* mammalian micronucleus assay in Chinese Hamster V79 Cells.

Reference:	**KCP 7.4/07**
Report	Donath, C., 2019: *In vitro* mammalian micronucleus assay in Chinese Hamster V79 Cells with RH-141452 Gowan Crop Protection Ltd., UK Eurofins BioPharma GmbH, Germany, Report No. 188616, GLP, Not published
Guideline(s):	OECD 487 (2016) EEC B.49 (2017)
Deviations:	No
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test material (Lot/Batch No.)	RH-141452 (632031-P1050-44)
Purity:	96.27 % (w/w)
Test cells	Chinese Hamster V79 (ATCC, CCL-93) cells *in vitro* Eurofins Munich, Germany culture Three or four day-old stock cultures (in exponential growth), more than 50% confluent, were rinsed with Ca-Mg-free PBS solution, trypsinised with a 0.05% trypsin solution in Ca-Mg-free PBS at 37°C for 5 min. By adding complete culture medium the detachment was stopped and a single cell suspension was prepared.
Medium:	Culture medium:

	MEM medium supplemented with 10 % fetal bovine serum (FBS) 100 U/100 µg/mL penicillin/streptomycin 2 mM L-glutamine 25 mM HEPES 2.5 µg/mL amphotericin B Treatment medium (short-term exposure): Complete culture medium with 0 % fetal bovine serum (FBS) After treatment medium (long-term exposure): Complete culture medium with 10 % fetal bovine serum (FBS) 1.5 µg/mL cytochalasin B
Controls	
Negative:	Culture medium
Solvent:	DMSO (1 %, v/v)
Positive:	**Clastogenic Controls** *Without metabolic activation* MMS (mmethylmethanesulfonate) disolved MEM Concentration: 25 µg/mL *With metabolic activation* CPA (cyclophosphamide) dissolved in MEM Concentration: 2.5 µg/mL **Aneugenic Controls** *Without metabolic activation* Colchicine dissolved in MEM Concentration: 0.08-2.0 µg/mL
Activation	S9 liver microsomal fraction from Eurofins Munich, Germany, derived from male Wistar rats. Lot 210918, protein concentration 35.7 mg/mL, checked for biological activity and sterility.
S 9 mix:	S9 cofactor solution with final protein concentration of 0.75 mg/mL, 8 mM $MgCl_2$, 33 mM KCl, 5 mM Glucose-6-phosphate and 5 mM NADP in 100 mM sodium-phosphate-buffer pH 7.4
Test concentrations	**Experiment I** without metabolic activation: 10, 20, 30, 40, 50, 60, 65, 70 µg/mL with metabolic activation: 25, 50, 100, 120, 130, 140, 150, 160, 175 µg/mL **Experiment II** without metabolic activation: 20, 30, 40, 50, 60, 65, 70, 75, 80, 85, 90 µg/mL **For the microscopic analyses of micronuclei frequencies** Experiment I with short-term exposure (4 h): without metabolic activation: 40, 50, 60, 65 µg/mL with metabolic activation: 120, 140, 150 µg/mL

	Experiment II with long-term exposure (24 h): without metabolic activation: 20, 50, 60 µg/mL
Post exposure observation period	4-72 h
Remarks	None

The *in vitro* micronucleus assay has been performed to assess the possible potential of RH-141452 to induce clastogenic and aneugenic activity in cells that have undergone cell division during or after exposure.

The test item was dissolved in DMSO and diluted in cell culture medium to reach a final concentration of 1 % (v/v) DMSO in the samples. The solvent was compatible with the survival of the cells and the S9 activity.

Based on the results of a pre-test, experiments with and without metabolic activation were performed in duplicate cultures:

Experiment I

without metabolic activation: 10, 20, 30, 40, 50, 60, 65, 70 µg/mL

with metabolic activation: 25, 50, 100, 120, 130, 140, 150, 160, 175 µg/mL

Experiment II without metabolic activation: 20, 30, 40, 50, 60, 65, 70, 75, 80, 85, 90 µg/mL

The following study design was performed:

	Without S9		**With S9**
	Experiment I	**Experiment II**	**Experiment I**
Exposure period	4 h	24 h	4 h
Cytochalasin B exposure	20 h	23 h	20 h
Preparation interval	24 h	24 h	24 h
Total culture period*	72 h	72 h	72 h

* Exposure started 48 h after culture initiation

Experiment I: Exponentially growing V79 cells were seeded into 25 cm^2 cell culture flasks (two flasks per test group). Approx. 50 000 cells were seeded per cell culture flask, containing 5 mL complete culture medium (minimum essential medium supplemented with 10% FBS). After an attachment period of approx. 48 h, the complete culture medium was removed and subsequently the test item was added to the treatment medium in appropriate concentrations. The cells were incubated with the test item for 4 h in presence or absence of metabolic activation. At the end of the incubation, the treatment medium was removed and the cells were washed twice with PBS. Subsequently, the cells were incubated in complete culture medium + 1.5 µg/mL cytochalasin B for 20 h at 37 °C.

Experiment II: If negative or equivocal results are obtained, they should be confirmed using continuous treatment (long-term treatment) without metabolic activation. Approx. 50 000 exponentially growing V79 cells were seeded in 25 cm^2 cell culture flasks in absence of metabolic activation. After an attachment period of approx. 48 h the test item was added in complete culture medium. 1 h later 1.5 µg/mL cytochalasin B were added and the cells were incubated for 23 h at 37 °C. At the end of the treatment the cell culture medium was removed and the cells were prepared for microscopic analysis.

At the end of the cultivation, the complete culture medium was removed. Subsequently, cells were trypsinated and resuspended in about 9 ml complete culture medium. The cultures were transferred into tubes and incubated with hypotonic solution (0.4% KCl) for some minutes at room temperature. Prior to this an aliquot of each culture was removed to determine the cell count by a cell counter (ALSystems). After the

treatment with the hypotonic solution the cells were fixed with methanol + glacial acetic acid (3+1). The cells were resuspended gently and the suspension was dropped onto clean glass slides. Consecutively, the cells were dried on a heating plate. Finally, the cells were stained with acridine orange solution.

For each experimental point, at least 2000 binucleated cells per concentration (1000 binucleated cells per slide) were analysed for micronuclei according to the criteria of Fenech (2000), i.e. clearly surrounded by a nuclear membrane, having an area of less than one-third of that of the main nucleus, being located within the cytoplasm of the cell and not linked to the main nucleus via nucleoplasmic bridges. Mononucleated and multinucleated cells and cells with more than six micronuclei were not considered.

A cytokinesis block proliferation index (CBPI) and % cytostasis were calculated.

A test item was considered clearly positive if, in any of the experimental conditions examined:

- at least one of the test concentrations exhibits a statistically significant increase compared with the concurrent negative/solvent control,
- the increase is concentration-related in at least one experimental condition when evaluated with an appropriate trend test and
- any of the results are outside the distribution of the historical negative/solvent control data (e.g. poisson-based 95% control limits).

Results and discussions

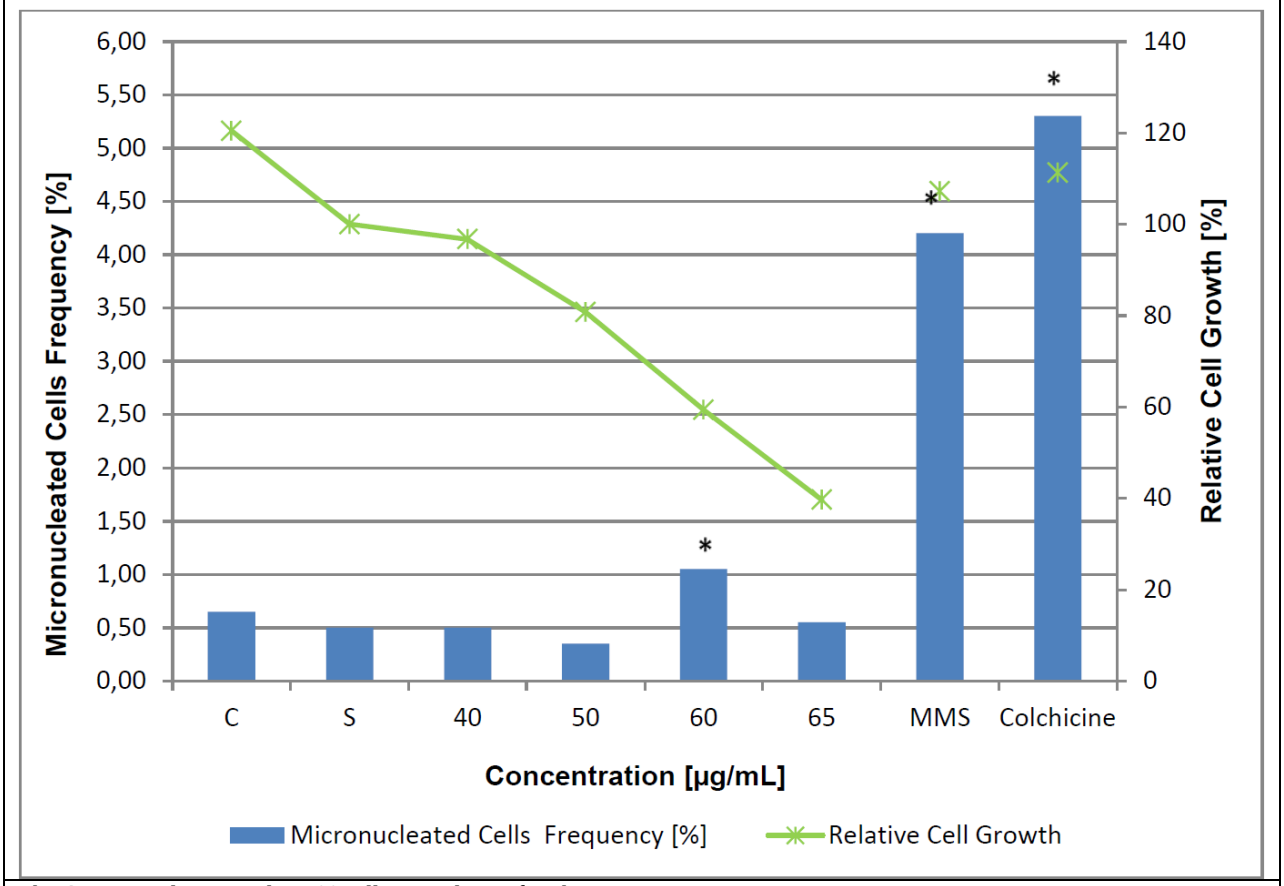
The pH-value detected with the test item was within the physiological range (pH 7.0 ± 0.4). No precipitate of the test item was noted.

Cytotoxicity: In experiment I without metabolic activation no increase of the cytostasis above 30% was noted up to 50 µg/mL. At 60 µg/mL a cytostasis of 41% and at 65 µg/mL a cytostasis of 60% was noted. In experiment I with metabolic activation no increase of the cytostasis above 30% was noted up to 120 µg/mL. At 140 µg/mL a cytostasis of 42% and at 150 µg/mL a cytostasis of 57% was observed. In experiment II without metabolic activation no increase of the cytostasis above 30% was noted up to 20 µg/mL. At 50 µg/mL a cytostasis of 47% and at 60 µg/mL a cytostasis of 53% was observed.

Clastogenicity / aneugenicity: In experiment I without metabolic activation the micronucleated cell frequency of the negative control (0.65%) was within the historical control limits of the negative control (0.37% – 1.37%) and the micronucleated cell frequency of the solvent control (0.50%) was within the historical control limits of the solvent control (0.47% – 1.48%). The mean values of micronucleated cells found after treatment with the test item were 0.50% (40 µg/mL), 0.35% (50 µg/mL), 1.05% (60 µg/mL) and 0.55% (65 µg/mL). The numbers of micronucleated cells were within or below the historical control limits of the solvent control and did not show a biologically relevant increase compared to the concurrent solvent control. The concentration of 60 µg/mL showed a statistically significant increase of micronuclei compared to the concurrent solvent control. However, the number of micronucleated cells was within the historical control limits of the solvent control. Therefore, this increase was regarded as not biologically relevant.
In experiment I with metabolic activation the micronucleated cell frequency of the negative control (0.55%) was within the historical control limits of the negative control (0.42% – 1.64%) and the micronucleated cell frequency of the solvent control (0.80%) was within the historical control limits of the solvent control (0.35% – 1.75%). The mean values of micronucleated cells found after treatment with the test item were 1.25% (120 µg/mL), 0.95% (140 µg/mL) and 0.85% (150 µg/mL). The numbers of micronucleated cells were within the historical control limits of the solvent control and did not show a biologically relevant increase compared to the concurrent solvent control. The concentration of 120 µg/mL showed a statistically significant increase of micronuclei compared to the concurrent solvent control. However, the number of micronucleated cells was within the historical control limits of the solvent control. Therefore, this increase was regarded as not biologically relevant.

In experiment II without metabolic activation the micronucleated cell frequency of the negative control (1.35%) was within the historical control limits of the negative control (0.37% – 1.37%) and the micronucleated cell frequency of the solvent control (1.00%) was within the historical control limits of the solvent control (0.47% – 1.48%). The mean values of micronucleated cells found after treatment with the test item were 1.55% (20 µg/mL), 1.30% (50 µg/mL) and 1.18% (60 µg/mL). The numbers of micronucleated cells for the concentrations of 50 and 60 µg/mL were within the historical control limits of the solvent control and did not show a biologically relevant increase compared to the concurrent solvent control. The micronuclei frequency for the concentration of 20 µg/mL was slightly above the upper historical control limit of the solvent control. However, since the exceedance was marginal and the two higher concentrations evaluated showed numbers of micronucleated cells within the historical control limits, this increase was considered as not biologically relevant.
In experiment I with and without metabolic activation a statistically significant increase of cells with micronuclei was noted at a concentration of 120 µg/mL and 60 µg/mL, respectively. However, the frequency of micronucleated cells was within the historical control limits of the solvent control, did not follow a dose-response relationship and were not reproducible in experiment II. No statistically significant enhancement ($p<0.05$) of cells with micronuclei was noted in the concentration groups of the test item evaluated in experiment II without metabolic activation. Therefore, the increases in experiment I were regarded as not biologically relevant.

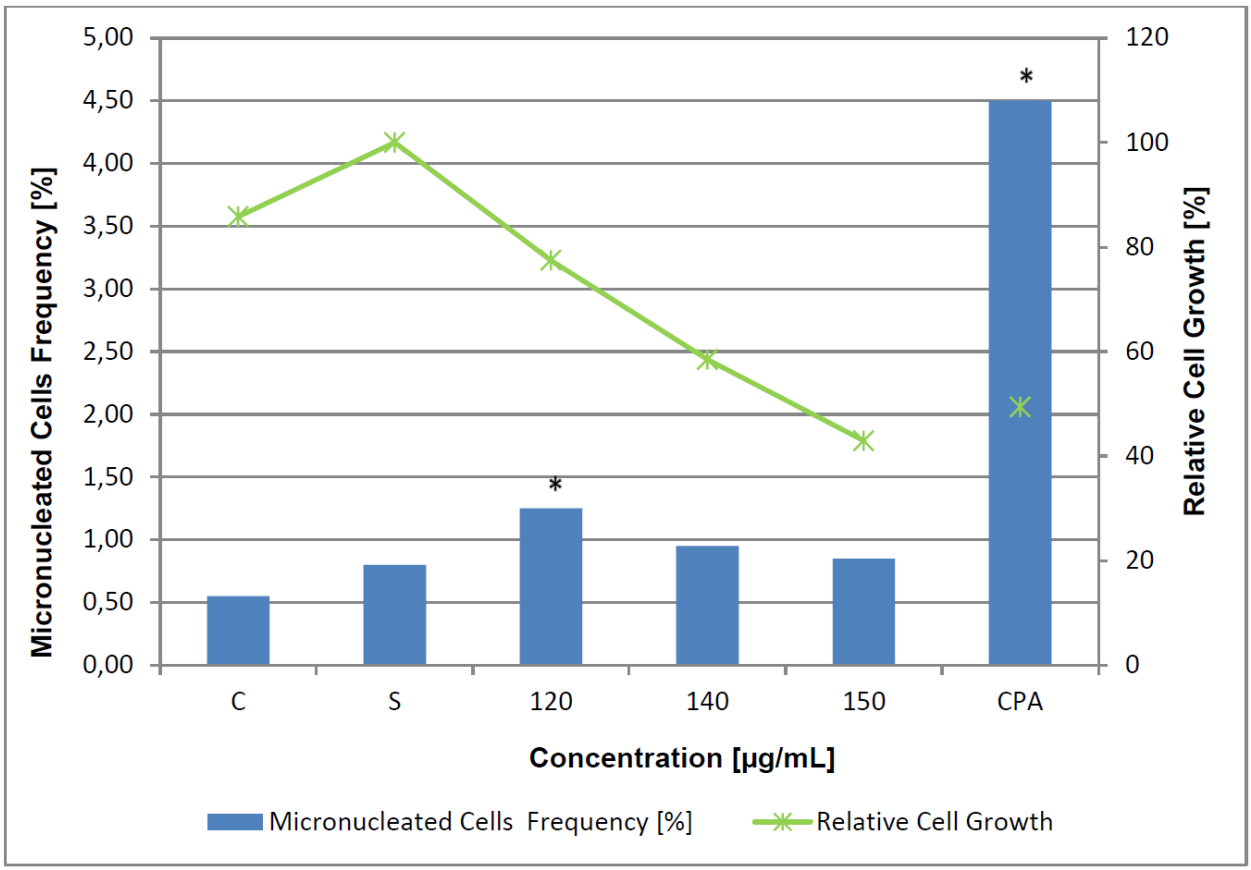
No statistically significant increase in the frequency of micronucleated cells under the experimental conditions of the study was observed in experiment I and II. MMS (25 µg/mL) and CPA (2.5 µg/mL) were used as clastogenic controls and colchicine as aneugenic control (0.08 and 2.0 µg/mL). They induced distinct and statistically significant increases of the micronucleus frequency. This demonstrates the validity of the assay.



The CBPI was determined in 500 cells per culture of each test group.
The relative values of the CBPI are related to the solvent control.
Micronucleated cell frequency was determined in 2000 cells (1000 cells per slide).

C: Negative control (culture medium)
S: Solvent control (DMSO 1% v/v in culture medium)
MMS: Methylmethanesulfonate, positive control (without metabolic activation) [25 µg/mL]
Colchicine: Positive control (without metabolic activation) [2.0 µg/mL]
CBPI: Cytokinesis Block Proliferation Index, CBPI = ((c_1 x 1) + (c_2 x 2) + (c_x x 3))/n
Relative cell growth: 100 x ((CBPI Test conc – 1) / (CBPI control -1))
c_1: mononucleate cells
c_2: binucleate cells
c_x: multinucleate cells
n: total number of cells
*: statistically significant increase of micronucleated cells

Figure A 3: Micronucleus frequency and growth rate in the main experiment without metabolic activation



The CBPI was determined in 500 cells per culture of each test group.
The relative values of the CBPI are related to the solvent control.
Micronucleated cell frequency was determined in 2000 cells (1000 cells per slide), except for solvent control and at a concentration of 120 µg/mL (2000 cells per slide).
C: Negative control (culture medium)
S: Solvent control (DMSO 1% v/v in culture medium)
CPA: Cyclophosphamide, positive control (with metabolic activation) [2.5 µg/mL]
CBPI: Cytokinesis Block Proliferation Index, CBPI = ((c_1 x 1) + (c_2 x 2) + (c_x x 3))/n
Relative cell growth: 100 x ((CBPI test conc – 1) / (CBPI control -1))
c_1: mononucleate cells
c_2: binucleate cells
c_x: multinucleate cells
n: total number of cells
*: statistically significant increase of micronucleated cells

Figure A 4: Micronucleus frequency and growth rate in the main experiment with metabolic activation

The test was regarded acceptable:

- The concurrent negative/solvent control was acceptable for addition to the laboratory historical negative/solvent control database.
- Concurrent positive controls induced responses that were compatible with those generated in the laboratory's historical positive control data base and produced a statistically significant increase compared to the concurrent negative/solvent control.
- Cell proliferation criteria in the negative/solvent control according to OECD 487 have been fulfilled (all experimental conditions were tested unless one resulted in positive results, an adequate number of cells and concentrations was analysable, criteria for the selection of top concentration were fulfilled).
- All experimental conditions were tested unless one resulted in positive results.
- Adequate number of cells and concentrations were analysable.
- Criteria for the selection of top concentration were fulfilled.

Conclusion

Under the experimental conditions of an *in vitro* mammalian micronucleus assay with Chinese Hamster V79 cells the test item did not induce structural and/or numerical chromosomal damage. Therefore, RH-141452 is considered to be non-mutagenic with respect to clastogenicity and/or aneugenicity.
Positive controls demonstrated the sensitivity of the test system.
The study is valid.

(Donath C. 2019)

A 2.11.8 Study 8

Comments of zRMS PL :	Confirmatory-like study which is under evaluation by the RMS for Zoxamide in an interzonal procedure, thus it was not evaluated in this assessment. RH-141452 was not causing structural and/or numerical chromosomal aberrations, neither in the presence nor in the absence of metabolic activation in the *in vitro* mammalian micronucleus assay in Chinese Hamster V79 Cells.

Reference:	**KCP 7.4**
Report	Donath, C., 2017: *In vitro* mammalian micronucleus assay in Chinese hamster V79 Cells with RH-150721 Gowan Crop Protection Ltd., UK Eurofins BioPharma, Germany, Report No. 171361, GLP, Not published
Guideline(s):	OECD 487 (2016)
Deviations:	MMS (methylmethanesulfonate) at a final concentration of 25 µg/mL instead of EMS (ethylmethanesulfonate) at a final concentration of 400- 1200 µg/mL was used in the study. This typing error has been recognised in time and did not influence the quality or integrity of the present study.
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test material (Lot/Batch No.)	RH-150721 (79942-4-16)
Purity:	98.07 % (w/w)
Test cells	Chinese Hamster V79 (ATCC, CCL-93) cells *in vitro* Eurofins Munich, Germany culture Three or four day-old stock cultures (in exponential growth), more than 50% confluent, were rinsed with Ca-Mg-free PBS solution, trypsinised with a 0.2% trypsin solution in Ca-Mg-free PBS at 37°C for 5 min. By adding complete culture medium the detachment was stopped and a single cell suspension was prepared.
Medium:	Culture medium: MEM medium supplemented with 10 % fetal bovine serum (FBS) 100 U/100 µg/mL penicillin/streptomycin 2 mM L-glutamine 25 mM HEPES 2.5 µg/mL amphotericin B Treatment medium (short-term exposure): Complete culture medium with 0 % fetal bovine serum (FBS) After treatment medium (long-term exposure): Complete culture medium with 10 % fetal bovine serum (FBS) 1.5 µg/mL cytochalasin B
Controls	
Negative:	Culture medium
Solvent:	DMSO (1 %, v/v)
Positive:	**Clastogenic Controls** *Without metabolic activation* MMS (mmethylmethanesulfonate) disolved MEM Concentration: 25 µg/mL *With metabolic activation* CPA (cyclophosphamide) dissolved in MEM Concentration: 2.5 µg/mL **Aneugenic Controls** *Without metabolic activation* Colchicine dissolved in MEM Concentration: 0.16-2.0 µg/mL
Activation	S9 liver microsomal fraction from Eurofins Munich, Germany, derived from male Wistar rats. Lot 0300217, protein concentration 32.4 mg/mL, checked for biological activity and sterility.
S 9 mix:	S9 cofactor solution with final protein concentration of 0.75 mg/mL, 8 mM $MgCl_2$, 33 mM KCl, 5 mM Glucose-6-phosphate and 5 mM NADP in 100

	mM sodium-phosphate-buffer pH 7.4
Test concentrations	**Experiment I (short-term exposure, 4 h)** without metabolic activation: 10, 25, 50, 60, 70, 80, 90, 100, 110 and 120 µg/mL with metabolic activation: 25, 50, 100, 125, 150, 175, 200, 225, 250, 275 and 300 µg/mL **Experiment II (long-term exposure, 24 h)** without metabolic activation: 0.25, 0.5, 1.0, 2.5, 5, 10, 20, 30, 40, 50, 60, 70 and 80 µg/mL **For the microscopic analyses of micronuclei frequencies** Experiment I with short-term exposure (4 h): without metabolic activation: 50, 70, 80 µg/mL with metabolic activation: 100, 125, 150, 175 µg/mL Experiment II with long-term exposure (24 h): without metabolic activation: 30, 50, 60 µg/mL
Post exposure observation period	4- 72 h
Remarks	None

The *in vitro* micronucleus assay has been performed to assess the possible potential of RH-150721 to induce clastogenic and aneugenic activity in cells that have undergone cell division during or after exposure.

The test item was dissolved in DMSO and diluted in cell culture medium to reach a final concentration of 1 % (v/v) DMSO in the samples. The solvent was compatible with the survival of the cells and the S9 activity.

Based on the results of a pre-test, experiments with and without metabolic activation were performed in duplicate cultures:

Experiment I (short-term exposure, 4 h)

without metabolic activation: 10, 25, 50, 60, 70, 80, 90, 100, 110 and 120 µg/mL

with metabolic activation: 25, 50, 100, 125, 150, 175, 200, 225, 250, 275 and 300 µg/mL

Experiment II (long-term exposure, 24 h) without metabolic activation: 0.25, 0.5, 1.0, 2.5, 5, 10, 20, 30, 40, 50, 60, 70 and 80 µg/mL

The following study design was performed:

	Without S9		**With S9**
	Experiment I	**Experiment II**	**Experiment I**
Exposure period	4 h	24 h	4 h
Cytochalasin B exposure	20 h	23 h	20 h
Preparation interval	24 h	24 h	24 h
Total culture period*	72 h	72 h	72 h

* Exposure started 48 h after culture initiation

Experiment I: Exponentially growing V79 cells were seeded into 25 cm^2 cell culture flasks (two flasks per test group). Approx. 50 000 cells were seeded per cell culture flask, containing 5 mL complete culture medium (minimum essential medium supplemented with 10% FBS). After an attachment period of approx. 48

h, the complete culture medium was removed and subsequently the test item was added to the treatment medium in appropriate concentrations. The cells were incubated with the test item for 4 h in presence or absence of metabolic activation. At the end of the incubation, the treatment medium was removed and the cells were washed twice with PBS. Subsequently, the cells were incubated in complete culture medium + 1.5 µg/mL cytochalasin B for 20 h at 37 °C.

Experiment II: If negative or equivocal results are obtained, they should be confirmed using continuous treatment (long-term treatment) without metabolic activation. Approx. 50 000 exponentially growing V79 cells were seeded in 25 cm^2 cell culture flasks in absence of metabolic activation. After an attachment period of approx. 48 h the test item was added in complete culture medium. 1 h later 1.5 µg/mL cytochalasin B were added and the cells were incubated for 23 h at 37 °C. At the end of the treatment the cell culture medium was removed and the cells were prepared for microscopic analysis.

At the end of the cultivation, the complete culture medium was removed. Subsequently, cells were trypsinated and resuspended in about 9 ml complete culture medium. The cultures were transferred into tubes and incubated with hypotonic solution (0.4% KCl) for some minutes at room temperature. Prior to this an aliquot of each culture was removed to determine the cell count by a cell counter (ALSystems). After the treatment with the hypotonic solution the cells were fixed with methanol + glacial acetic acid (3+1). The cells were resuspended gently and the suspension was dropped onto clean glass slides. Consecutively, the cells were dried on a heating plate. Finally, the cells were stained with acridine orange solution.

For each experimental point, at least 2000 binucleated cells per concentration (1000 binucleated cells per slide) were analysed for micronuclei according to the criteria of Fenech (2000), i.e. clearly surrounded by a nuclear membrane, having an area of less than one-third of that of the main nucleus, being located within the cytoplasm of the cell and not linked to the main nucleus via nucleoplasmic bridges. Mononucleated and multinucleated cells and cells with more than six micronuclei were not considered.

A cytokinesis block proliferation index (CBPI) and % cytostasis were calculated.

A test item was considered clearly positive if, in any of the experimental conditions examined:

- at least one of the test concentrations exhibits a statistically significant increase compared with the concurrent negative/solvent control,
- the increase is concentration-related in at least one experimental condition when evaluated with an appropriate trend test and
- any of the results are outside the distribution of the historical negative/solvent control data (e.g. poisson-based 95% control limits).

Results and discussions

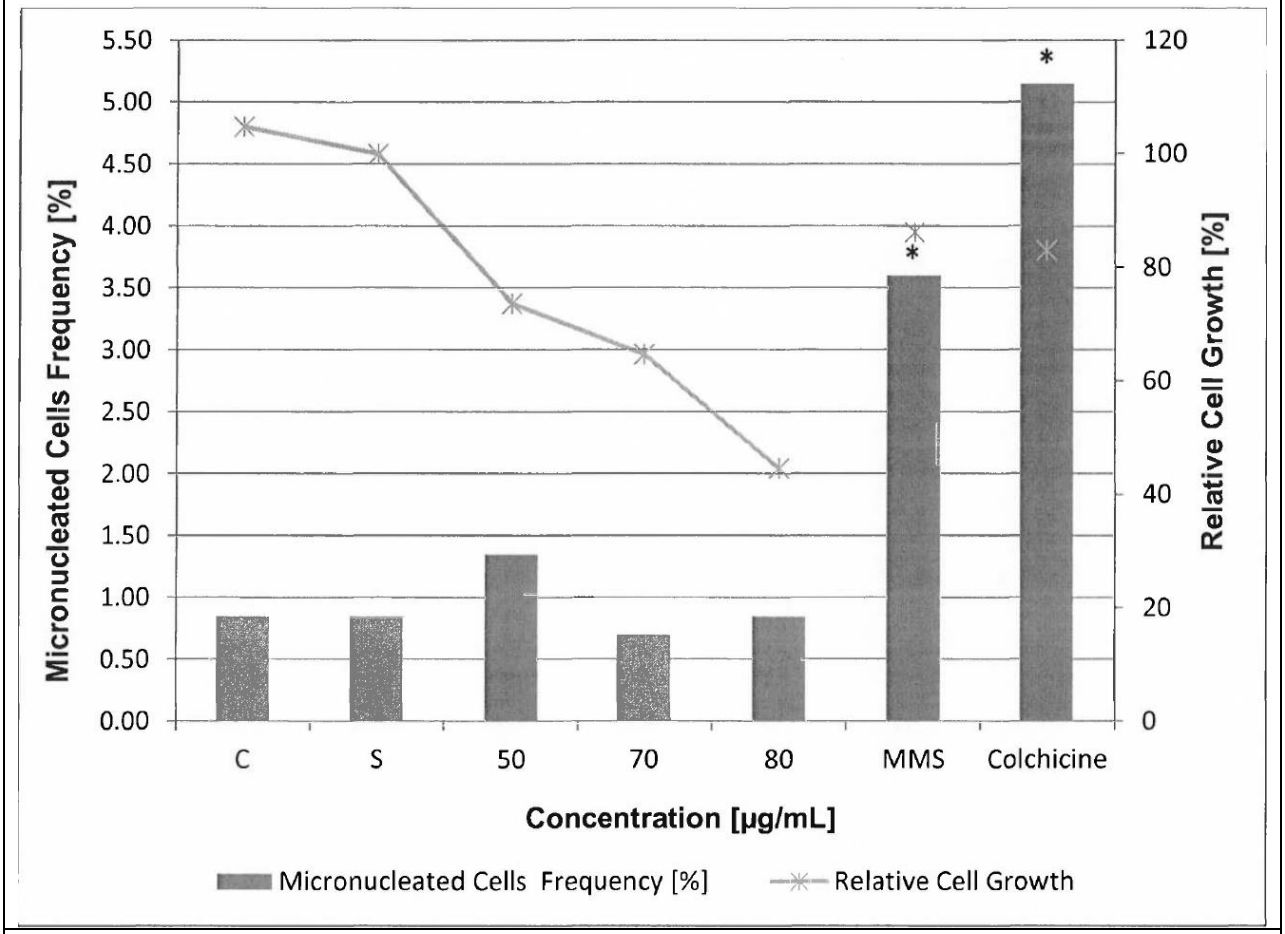
The pH-value detected with the test item was within the physiological range (pH 7.0 ± 0.4). No precipitate of the test item was noted.

Cytotoxicity: In experiment I with metabolic activation no increase of the cytostasis above 30% was noted. In experiment I without metabolic activation no increase of the cytostasis above 30% was noted up to 50 pg/mL. At 70 pg/mL a cytostasis of 35% and at 80 pg/mL a cytostasis of 55% was noted. In experiment II without metabolic activation no increase of the cytostasis above 30% was noted up to 30 pg/mL At 50 pg/mL a cytostasis of 31% and at 60 pg/mL a cytostasis of 53% was noted.

Clastogenicity / aneugenicity: In experiment I without metabolic activation the micronucleated cell frequency of the negative control (0.85%) and the solvent control (0.85%) were within the historical control limits of the negative control (0.39% - 1.40%) and the solvent control (0.45% - 1.55%), respectively. The mean values of micronucleated cells found after treatment with the test item were 1.35% (50 pg/mL), 0.70% (70 pg/mL) and 0.85% (80 pg/mL). The numbers of micronucleated cells were within the historical control

limits of the solvent control and did not show any biologically relevant increase compared to the concurrent solvent control.
In experiment I with metabolic activation the micronucleated cell frequency of the negative control (0.90%) and the solvent control (1.23%) were within the historical control limits of the negative control (0.37% - 1.68%) and the solvent control (0.23% - 1.88%), respectively. The mean values of micronucleated cells found after treatment with the test item were 1.35% (100 pg/mL), 0.90% (125 pg/mL), 1.30% (150 pg/mL) and 1.30% (175 pg/mL). The numbers of micronucleated cells were within the historical control limits of the solvent control and did not show any biologically relevant increase compared to the concurrent solvent control.
In experiment II without metabolic activation the micronucleated cell frequency of the negative control (0.95%) and the solvent control (1.05%) were within the historical control limits of the negative control (0.39% - 1.40%) and the solvent control (0.45% - 1.55%), respectively. The mean values of micronucleated cells found after treatment with the test item were 1.50% (30 pg/mL), 0.45% (50 pg/mL) and 0.73% (60 pg/ml). The numbers of micronucleated cells were within historical control limits of the solvent control and did not show any biologically relevant increase compared to the concurrent solvent control.

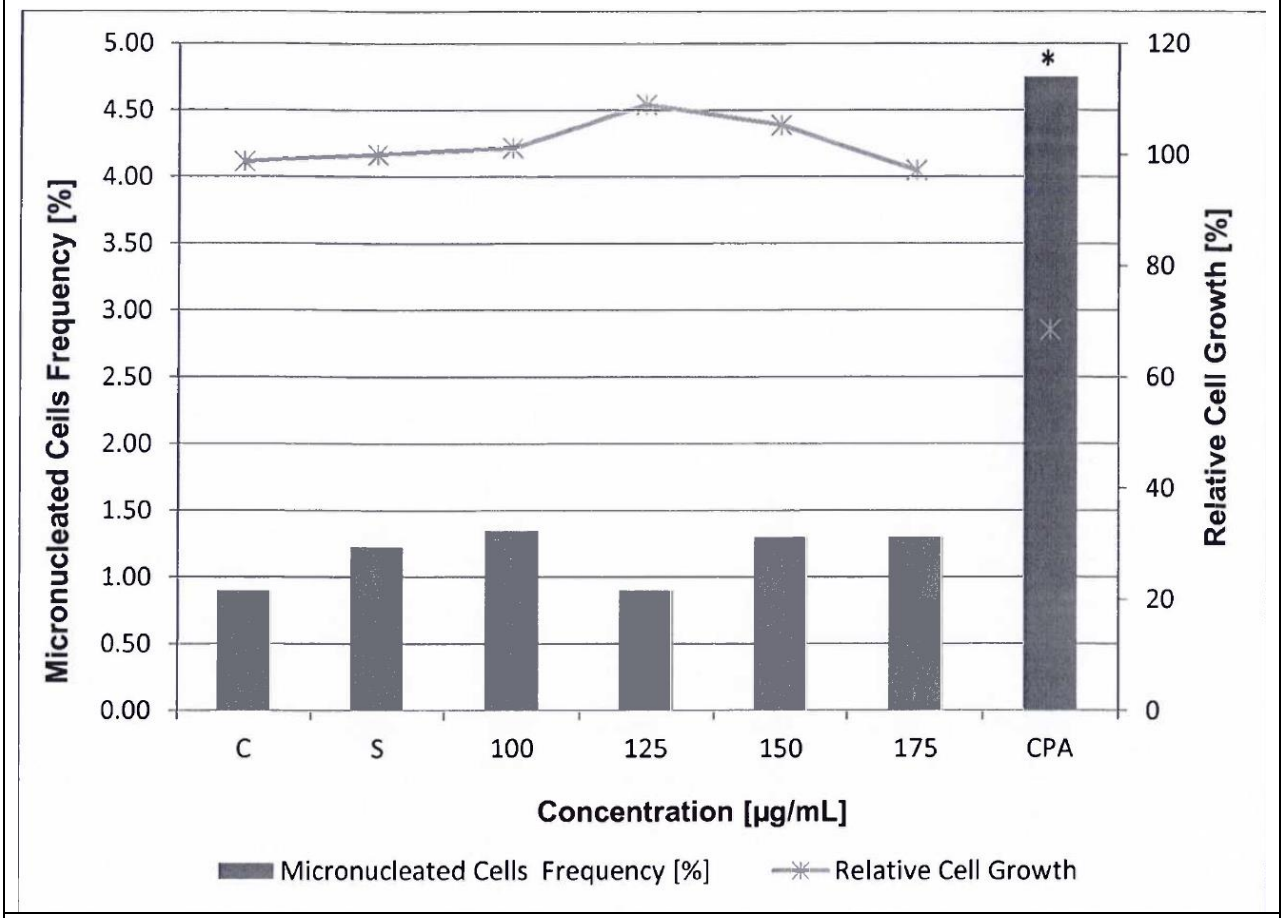
No statistically significant enhancement ($p<0.05$) of cells with micronuclei was noted in the dose groups of the test item evaluated in experiment I and II. A Chi^2 test demonstrated no statistically significant increase in the frequency of micronucleated cells in experiment I and II. MMS (25 pg/mL) and CPA (2.5 pg/mL) were used as clastogenic controls and colchicine as aneugenic control (0.16 and 2.0 pg/mL). They induced distinct and statistically significant increases of the micronucleus frequency, demonstrating the validity of the assay.



The CBPI was determined in 500 cells per culture of each test group.
The relative values of the CBPI are related to the solvent control.
Micronucleated Cell frequency was determined in 2000 cells (1000 cells per slide).

C: Negative control (culture medium)
S: Solvent control (DMSO 1% v/v in culture medium)
MMS: Methylmethanesulfonate, positive control (without metabolic activation) [25 µg/mL]
Colchicine: Positive Control (without metabolic activation) [2.0 µg/mL]
CBPI: Cytokinesis Block Proliferation Index, CBPI = ((ci x 1) + (c2 x 2) + (cx x 3))/n
Relative cell growth: 100 x ((CBPI rest conc - 1) / (CBPI control -1))
ci: mononucleate cells
C2~. binucleate cells
cx: multinucleate cells
n: total number of cells
*: statistically significant increase of micronucleated cells

Figure A 5: Micronucleus frequency and growth rate in the main experiment without metabolic activation



The CBPI was determined in 500 cells per culture of each test group.
The relative values of the CBPI are related to the solvent control.
Micronucleated Cell Frequency was determined in 2000 cells (1000 cells per slide), except for solvent control (2000 cells per culture).
C: Negative control (culture medium)
S: Solvent control (DMSO 1% v/v in culture medium)
CPA: Cyclophosphamide, positive control (with metabolic activation) [2.5 µg/mL]
CBPI: Cytokinesis Block Proliferation Index, CBPI = ((c1 x 1) + (c2 x 2) + (cx x 3))/n
Relative Cell Growth: 100 x ((CBPI Test conc – 1) / (CBPI control -1))
c_1: mononucleate cells
c_2: binucleate cells
c_x: multinucleate cells
n: total number of cells
*: statistically significant increase of micronucleated cells

Figure A 6: Micronucleus frequency and growth rate in the main experiment with metabolic activation

The test was regarded acceptable :

- The concurrent negative/solvent control was acceptable for addition to the laboratory historical negative/solvent control database.
- Concurrent positive controls induced responses that were compatible with those generated in the laboratory's historical positive control data base and produced a statistically significant increase compared to the concurrent negative/solvent control.
- Cell proliferation criteria in the negative/solvent control according to OECD 487 have been fulfilled (all experimental conditions were tested unless one resulted in positive results, an adequate number of cells and concentrations was analysable, criteria for the selection of top concentration were fulfilled).
- All experimental conditions were tested unless one resulted in positive results.
- Adequate number of cells and concentrations were analysable.
- Criteria for the selection of top concentration were fulfilled.

Conclusion

Under the experimental conditions of an *in vitro* mammalian micronucleus assay with Chinese Hamster V79 cells the test item did not induce structural and/or numerical chromosomal damage. Therefore, RH-150721 is considered to be non-mutagenic with respect to clastogenicity and/or aneugenicity.
Positive controls demonstrated the sensitivity of the test system.
The study is valid.

(Donath C. 2017)

A 2.11.9 Study 9

Comments of zRMS PL :	Confirmatory-like study which is under evaluation by the RMS for Zoxamide in an interzonal procedure, thus it was not evaluated in this assessment. Evidence of systemic plasma exposure to RH-141455 was observed (AUClast: 34.5 µg h/mL) in 2 days oral dietary pharmacokinetic study in Sprague Dawley Rats, the maximum concentration was observed at 53.5 hours

Reference:	**KCP 7.4/09**
Report	xxx, 2019: RH-141455: 2 days oral dietary pharmacokinetic study in Sprague Dawley Rats Gowan Crop Protection Ltd., UK xxx, Report No. U-19044, No GLP, Not published
Guideline(s):	None (investigative study)
Deviations:	No
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test material (Lot/Batch No.)	RH-141455 (HHGCP017-00-1)
Purity:	99.6 % (w/w)

Vehicle:	rodent powder diet
Test organisms	Albino Rat, Sprague Dawley purchased from Envigo, The Netherlands
Age:	7-10 weeks
Body weight:	Body weights did not differ by more than 20% in the group at study initiation.
Acclimation period:	1 day
Water:	Tap water, *ad libitum*
Diet:	rodent powder diet *ad libitum*
Housing:	1 animal per cage in polycarbonate rat cages with bedding materials
Test design	
Total number of animals:	4 (males)
Environmental conditions	
Temperature:	21.6 to 22.6°C
Humidity:	Relative humidity ranged from 51 to 66%
Air changes:	17 fresh air changes per hour
Photoperiod:	12-hour light/dark cycles
Test conentrations	15000 ppm in diet
Exposure time	2 days
Remarks	Designed as a pre-test to assess plasma exposures and pharmacokinetic profiles of RH- 141455 following oral dietary administration.

The study was designed to assess plasma exposures and pharmacokinetic profiles of RH- 141455 following oral dietary administration at 15000 ppm for two days in male Sprague Dawley rats. A total of 4 male rats were used. RH-141455 was formulated in powdered diet at concentration of 15000 ppm and fed from day 1 through day 3. The observations included daily morbidity/mortality check, clinical signs, body weights, feed consumption, bioanalytical & TK parameters (days 2 & 3), and gross pathology. A total of 10 rat plasma samples were analysed. Blood was collected via retro-orbital plexus puncture under mild isoflurane anaesthesia at time points from 4 pm on day 2 till 4 pm on day 3. Plasma was separated by centrifuging the whole blood sample and stored at < -60°C until bioanalysis. The bioanalysis was performed by LC-MS/MS method. After extraction and centrifugation, the supernatant was transferred into auto sampler vials and volumes of 10 µL were injected on a HPLC column with LC-MS/MS detection. The samples were analysed using a calibration curve range from 0.064 µg/mL to 50.370 µg/mL. Pharmacokinetics parameters were evaluated using validated Phoenix® WinNonlin® (version 6.3) software.

Results and discussions

All rats in the study survived until scheduled sacrifice and no clinical signs were observed. Body weights were slightly increased on day 3 as compared to day 1. There were no test item related effects on body weight. The average feed consumption amounted to approximately 26 g/rat/day. There were no test item-related gross changes in any of the organs examined. Blood collection times were considered from the time of feed input. RH-141455 was detectable in rat plasma after dietary exposure at concentrations of 0.3 to 1.5 µg/ml (AUC_{last}: 34.5 µg h/mL). The T_{max} of RH-141455 was observed on day 3 with a peak plasma concentration (C_{max}) of 1.4 µg/mL.

Table A 8: Pharmacokinetic parameters of RH-141455

Group No.	Dose	Sex	T_{max} (h)	C_{max} (µg/mL)	AUC_{last} (µg h/mL)	T_{last} (h)	C_{last} (ug/mL)
Group 1	15000 ppm	Male	53.5	1.4	34.5	53.5	1.4

Conclusion

The plasma pharmacokinetics profile of RH-141455 was determined when formulated powdered diet was fed to male Sprague Dawley rats at 15000 ppm for two days. No test item related effects were seen. Evidence of systemic plasma exposure to RH-141455 was observed ($AUCl_{ast}$: 34.5 µg h/mL), the maximum concentration was observed at 53.5 hours (on day 3).

(xxx 2019)

A 2.11.10 Study 10

Comments of zRMS PL :	Confirmatory-like study which is under evaluation by the RMS for Zoxamide in an interzonal procedure, thus it was not evaluated in this assessment. The No-Observed-Adverse-Effect Level (NOAEL) of RH-141455 in 14-day oral dietary dose range finding study in Sprague Dawley rats was found to be 15000 ppm, which is equivalent to 1123 and 1069 mg/kg body weight/day for the males and females, respectively.

Reference:	**KCP 7.4**
Report	xxx, 2020: RH-141455: 14-day oral dietary dose range finding study in Sprague Dawley rats Gowan Crop Protection Ltd., UK xxx, Report No. U-19071, No GLP, Not published
Guideline(s):	OECD 407 (2008) EEC B.7 (2008) OPPTS 870.3050 (2000) (except for duration of dosing)
Deviations:	The blood collection for toxicokinetics on day 2 (6 P.M) & day 3 (6 A.M) and on day 14 (6 A.M) was delayed between 1- 18 minutes from the scheduled time (± 10 minutes) across the groups. However, this deviation is regarded to have no impact on the integrity of the study.
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test material (Lot/Batch No.)	RH-141455 (HHGCP017-00-1)
Purity:	99.6 % (w/w)
Vehicle:	Teklad Global 18% Protein Rodent Diet (meal)

	manufactured by Envigo
Test organisms	Albino Rat, Sprague Dawley purchased from Envigo, Italy
Age:	6-7 weeks
Body weight:	Male:199.25 – 228.38 g Female: 145.82 – 176.08 g
Acclimation period:	3 days
Water:	Tap water, *ad libitum*
Diet:	Teklad Global 18% Protein Rodent Diet (meal) *ad libitum* (except overnight fasting prior to clinical pathology blood collection) manufactured by Envigo
Housing:	2 per sex per cage in polycarbonate rat cages with bedding materials
Test design	
No. of groups:	4
No. of animals/group:	6 rats/sex/group
Number of animals:	48 (24 Males + 24 Females)
Extra animals:	6 (3 Males + 3 Females)
Total number of animals:	54 (27 Males + 27 Females)
Environmental conditions	
Temperature:	21.0 to 24.1℃
Humidity:	Relative humidity ranged from 45 to 68%
Air changes:	16 fresh air changes per hour
Photoperiod:	12-hour light/dark cycles
Test conentrations	1000, 5000 and 15000 ppm in diet
Exposure time	14 days
Remarks	Designed as range-finding study for the 90 days oral toxicity study.

In a dose range finding study, RH-141455 was administered to rats for 14 days at levels of 1000, 5000 and 15000 ppm for male and female to groups of Sprague Dawley rats (6 rats/sex/group). The dose in ppm were equivalent to 76, 368 and 1123 mg/kg body weight/day in males and 86, 457 and 1069 mg/kg body weight/day in females, respectively. The control group was fed with basal diet for same duration of study.

Table A 9: Study design

Test group	Concentration in diet (ppm)	Dose (mg/kg/day)		Animals assigned	
		Male	Female	Male	Female
1	0	0	0	6	6
2	1000	76	86	6	6
3	5000	368	457	6	6
4	15000	1123	1069	6	6

There were 6 animals of each sex per group. Males and females were randomised into 4 dose groups using Pristima, based on body weight to control bias. RH-141455 was administered in the diet for 14 days to Sprague Dawley rats – at 0, 1000, 5000 and 15000 ppm. Husbandry conditions were in accordance with the recommendations of Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC) and Committee for the Purpose of Control and Supervision of Experiments on Animals, Government of India.

The required amount of test material was weighed and mixed with the diet using electric mixer. Diet were prepared approximately weekly. Prepared diets were stored at room temperature before use. The concentration of the test substance in the feed was analytically verified weekly by reverse phase HPLC-DAD. The method has been validated according to SANCO/3029/99 rev. 4 (2000); see analytical method validation report no. U-19069 for the estimation of RH-141455 in rat feed.

The animals were observed twice daily for morbidity and mortality. All rats were observed for clinical signs at least once daily. Detailed clinical examinations were done once on day 1 of dosing and weekly thereafter.

Body weights were recorded on days 1, 2, 4, 8, 11 and 14.

Food consumption and compound intake was recorded on days 1, 2, 4, 8, 11. Food left over was measured on days 4, 8, 11 and 14. Test substance intake was calculated.

Ophthalmoscopic examination was not reported.

Blood samples were collected from three rats per sex per time point as per the sampling schedule. Blood was collected via retro-orbital plexus puncture under mild isoflurane anaesthesia. The sampling intervals were as follows: 6 P.M (Day 2 and 13) and 6 A.M, 9 A.M, 12 P.M and 6 P.M on Day 3 and 14. Toxicokinetics evaluation was performed by validated Phoenix® WinNonlin® software (Pharsight Corporation, USA).

Blood plasma samples prepared with K_2EDTA as anti-coagulant and analysed by LC-MS/MS for RH-141455 and an internal standard (tolbutamide). A total of 186 (96 for day 1 and 90 from day 14) samples were analysed. The method has been validated according to SANCO/3029/99 rev. 4 (2000) for a concentration range of 0.064 µg/mL to 50.007 µg/mL.

Blood was collected from all animals at the terminal sacrifice (day 15) for haematology, coagulation and clinical chemistry evaluations. The animals were fasted overnight prior to terminal blood collection. Blood was collected via retro-orbital plexus puncture of right eye under mild isoflurane anaesthesia. The following haematology, coagulation and clinical chemistry parameters were measured: haematology – red blood cell count, haemoglobin concentration, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, platelet count, reticulocyte count, total white blood cell count, leukocyte differential (neutrophils, lymphocytes, monocytes, eosinophils and basophils); coagulation – active partial thromboplastin time, prothrombin time and fibrinogen; clinical chemistry – albumin/globulin ratio, alanine aminotransferase, albumin, alkaline phosphatase, aspartate aminotransferase, blood urea nitrogen, calcium, chloride, creatinine, creatinine kinase, gamma-glutamyl trans-peptidase, globulin, glucose, high-density lipoprotein, inorganic phosphorus, low-density lipoprotein, potassium, sodium, total bilirubin, total cholesterol, total protein and triglycerides.

Urine was collected from all animals at the end of dosing phase (day 15). During urine collection, animals were fasted overnight in metabolic cages. The following parameters were measured: volume, colour, appearance, glucose, bilirubin, ketone, blood, pH, protein, urobilinogen, nitrite, leukocyte and specific gravity.

Gross necropsy was conducted on all animals at the end of dosing phase (day 15). The following organs were collected, weighed, assessed histopathologically after fixation in 10% neutral buffered formalin and

staining with haematoxylin and eosin: adrenals, brain, epididymites, heart, kidneys, liver, ovaries, pituitary, prostate, seminal vesicles and coagulating glands, spleen, testes, thymus, thyroid with parathyroid and uterus with cervix. The other organs or tissues such as aorta, biceps femoris muscles, cecum, colon, diaphragm, duodenum, oesophagus, eyes, femur bone with joint, gastrocnemius muscle, gross changes, harderian glands, ileum with Peyer's patches, jejunum, lungs, lymph nodes (mandibular and mesenteric), mammary gland, optic nerve, oviducts, pancreas, Quadriceps muscle, rectum, salivary glands (mandibular, sublingual and parotid), sciatic nerve, skin (inguinal region), soleus muscle, spinal cord, sternum with marrow, stomach, tongue, trachea, urinary bladder and vagina, were also examined histopathologically. Histopathology was performed on all tissues of animals from control and high dose groups. Peer review was performed by another in-house pathologist.

For comparative statistics, data were evaluated using Levene's Test for homogeneity of variance, with significance at 5% level. Data determined to be homogeneous was evaluated for analysis of variance (ANOVA). When the ANOVA verified significance at 5% level, pairwise comparisons of each treated group with the control group was made using a Dunnett Test, to identify statistical differences (at 5% level). When the data were found to be non-homogeneous, ANOVA was done using suitable transformation. When the homogeneity tests were significant even after transformation, data were evaluated using a Kruskal-Wallis Test for group factor significance. When significance (at 5% level) existed between groups, Dunn's pairwise comparison was performed.

Results and discussions

The dose formulation analysis results of Day 1 were within the acceptable limits i.e., 99.60 to 100.94 % (< ±20 % of nominal concentrations). The control samples analysed did not show presence of test item.

There were no treatment-related clinical signs of toxicity observed in animals at any dose.

No mortalities occurred in the test animals at any dose level.

No test substance-related changes were noticed in body weight and body weight gain parameters in any of the dose groups when compared to control group.

There were no test substance-related changes in feed consumption in any of the dose groups when compared to control group. Statistically significant increase in feed consumption on Days 4-8 (27%) was noticed in low dose females (1000 ppm) when compared to control group. Above mentioned changes in feed consumption were considered as an incidental, as the magnitude of changes were minimal and occurred during dosing phase. The test substance intake achieved over the entire treatment period (Day 1 - 14) for 1000, 5000 and 15000 ppm concentration levels in diet was equivalent to 76, 368 and 1123 mg/kg body weight/day in males and 86, 457 and 1069 mg/kg body weight/day in females, respectively.

There were no test substance-related changes in haematology and coagulation parameters in the study. Minimal but statistically significant differences in the haematology parameters (RBC, HGB, HCT, WBC and lymphocytes) in the low dose (1000 ppm) females compared to the concurrent controls were considered incidental because of the lack of dose-response relationship.

There were no test substance-related changes in clinical chemistry parameters in the study.

There were no test substance-related changes in urinalysis parameters in the study.

There were no test substance-related changes in organ weight changes in the study. All changes in organ weight parameters were considered incidental and not test substance-related because the magnitude of changes was comparable to concurrent controls and/or lacked a dose-response relationship. Variations in the uterine with cervical weights among the treated groups compared the concurrent controls were likely related to the changes during normal reproductive cycle.

There were no test substance-related gross changes in the study. Uterine dilation in two controls and one mid dose (5000 ppm) females correlated with the microscopic finding of luminal dilation of the uterine horns consistent with changes associated with normal physiological reproductive cycle. There were no test substance-related microscopic findings in the study. All microscopic changes were considered incidental or spontaneous and not related to the test substance, because they were randomly distributed across the test substance and control groups and were generally observed in the rats of this age and strain (McInnes, 2012[5]).

Following oral feeding of RH-141455 in rats, T_{max} was observed at 42 hours in males and 42 to 54 hours in females for day 2. For day 13, T_{max} was observed at 294 to 306 hours in males and 294 to 309 hours in females. Plasma concentrations of RH-141455 were observed at all time points in treated groups. T_{last} was observed at 54 hours for day 2 and 318 hours for day 13. Increase in AUC_{24h} and C_{max} were less than dose proportional over 1000 to 15000 ppm dose range in males and females in day 2 and day 13. No sex related difference was observed in RH-141455 concentrations. Repeat dose feeding of RH-141455 in diet showed evidence of systemic plasma exposure to RH-141455 and increase in exposure was less than dose proportional at 1000, 5000 and 15000 ppm in Sprague Dawley rats. RH-141455 exposure found comparable between males and females. No accumulation of RH-141455 was observed in male and female rats on day 13 following daily feeding for 2 weeks.

Table A 10: The toxicokinetic parameters of RH-141455

Day Nominal	Sex	Dose in ppm (mg/kg/day)	T_{max} in h (time from start of exposure)	C_{max} (ug/mL)	AUC_{24h} (h*ug/mL)	T_{last} in h (time from start of exposure)	C_{last} (ug/mL)
Day 2	Male	1000 (76)	12 (42)	0.700	13.33	24 (54)	0.493
		5000 (368)	12 (42)	1.480	28.70	24 (54)	1.047
		15000 (1123)	12 (42)	3.064	68.84	24 (54)	1.948
Day 2	Female	1000 (86)	15 (45)	0.596	12.79	24 (54)	0.445
		5000 (442)	24 (54)	1.287	28.30	24 (54)	1.287
		15000 (1069)	12 (42)	2.650	52.68	24 (54)	2.218
Day 13	Male	1000 (76)	0 (294)	1.152	16.23	24 (318)	0.505
		5000 (368)	0 (294)	0.986	21.80	24 (318)	0.977
		15000 (1123)	12 (306)	2.378	51.38	24 (318)	2.177
Day 13	Female	1000 (86)	15 (309)	0.598	11.34	24 (318)	0.416
		5000 (442)	0 (294)	1.096	21.81	24 (318)	0.911
		15000 (1069)	0 (294)	2.606	50.62	24 (318)	2.083

Conclusion

In a dose range finding study, RH-141455 was administered to rats for 14 days at levels of 1000, 5000 and 15000 ppm for male and female to groups of Sprague Dawley rats (6 rats/sex/group). The dose in ppm were equivalent to 76, 368 and 1123 mg/kg body weight/day in males and 86, 457 and 1069 mg/kg body weight/day in females, respectively. The control group was fed with basal diet for same duration of study.

There was no morbidity or mortality or adverse clinical sign up to 15000 ppm in both sexes. There were

[5] McInnes, E. F. (2012). Background lesions in laboratory animal, A color atlas. Chapter 2 Wistar and Sprague-Dawley rats (pp. 17-36). Saunders Elsevier

no test material-related changes in mean body weight and food consumption in both sexes compared with control group animals. There was no test item related change in hematology, coagulation, clinical chemistry and urinalysis parameter. No test item related gross findings, organ weight changes or histopathology findings were observed in the study. The No-Observed-Adverse-Effect Level (NOAEL) of RH-141455 was found to be 15000 ppm, which is equivalent to 1123 and 1069 mg/kg body weight/day for the males and females, respectively.

(xxx 2020)

A 2.11.11 Study 11

Comments of zRMS PL:	Confirmatory-like study which is under evaluation by the RMS for Zoxamide in an interzonal procedure, thus it was not evaluated in this assessment. The No-Observed-Adverse-Effect Level (NOAEL) of RH-141455 in 90-day oral dietary dose range finding study in Sprague Dawley rats was found to be 16000 ppm, which is equivalent to 924 and 1119 mg/kg body weight/day for the males and females, respectively

Reference:	**KCP 7.4/11**
Report	xxx 2020: RH-141455: 90-day oral dietary toxicity study with toxicokinetics and 28-day recovery period in Sprague Dawley rats Gowan Crop Protection Ltd., UK xxx, Report No. U-19102, GLP, Not published
Guideline(s):	OECD 408 (2018) OPPTS 870.3100 (1998) ICH Harmonized Tripartite Guideline S3A (Toxicokinetics, 1994) Notification No. 12-Nousan-8147, MAFF, Japan (2000)
Deviations:	No
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test material (Lot/Batch No.)	RH-141455 (HHGCP017-00-1)
Purity:	99.6 % (w/w)
Vehicle:	Teklad Global 18% Protein Rodent Diet (meal) manufactured by Envigo
Test organisms	Albino Rat, Sprague Dawley purchased from Envigo, Netherlands
Age:	6-7 weeks
Body weight:	Males:193.75 to 222.07 g Females:148.36 to 170.32 g The weight variation of the animals at randomisation was within ± 20% of the mean weight for each sex.
Acclimation period:	5 days
Water:	Tap water, *ad libitum*

Diet:	Teklad Global 18% Protein Rodent Diet (meal) manufactured by Envigo
Housing:	one or two per sex per cage in polycarbonate rat cages with bedding materials
Test design	
No. of groups:	6 (2 main groups + 2 recovery groups + 2 TK groups)
No. of animals/group:	10 rats/sex/group in main groups 6 rats/sex/group in recovery groups 6 rats/sex/group in test item TK group and 3 rats/sex/group in control TK group
Number of animals:	82 (41 Males + 41 Females)
Extra animals:	20 (10 Males + 10 Females)
Total number of animals:	102 (51 Males + 51 Females)
Environmental conditions	
Temperature:	20.1 – 24.3°C
Humidity:	Relative humidity ranged from 47 – 69%
Air changes:	22 fresh air changes per hour
Photoperiod:	12-hour light/dark cycles
Test conentrations	16000 mg/kg in diet
Exposure time	90 days
Remarks	None.

This study was designed to assess the potential sub-chronic toxicity and toxicokinetics (TK) profile of RH-141455 when fed in formulated diet to Sprague Dawley rats for 90 days. A total of 41 males and 41 females Sprague Dawley rats were randomly assigned to 2 main groups (10 rats/sex/group), 2 recovery groups (6 rats/sex/group) and 2 TK groups (3/6 rats/sex/group for control and test-substance related, respectively). The dose of 16000 ppm was aimed to reach the guideline recommended limit dose of 1000 mg/kg body weight/day. The control group was fed with basal diet for same duration of study.

Table A 11: Study design

Main Groups:

Test group	Concentration in diet (ppm)	Dose (mg/kg/day)		Animals assigned	
		Male	Female	Male	Female
1	0	0	0	10	10
2	16000	924	1119	10	10

Recovery Groups:

Test group	Concentration in diet (ppm)	Dose (mg/kg/day)		Animals assigned	
		Male	Female	Male	Female
1	0	0	0	6	6
2	16000	930	1090	6	6

TK Groups:

Test group	Concentration in diet (ppm)	Dose (mg/kg/day)		Animals assigned	
		Male	Female	Male	Female
1	0	0	0	3	3

2	16000	912	1102	6	6

Males and females were randomized into 2 main groups (10 rats/sex/group), 2 recovery groups (6 rats/sex/group) and 2 TK groups (3 rats/sex/group for control and 6 rats/sex/group for RH-141455) using Pristima, based on body weight to control bias. RH-141455 was incorporated in the diet and fed at concentration level of 16000 ppm for 90 days to Sprague Dawley rats. Care of animals compiled with the recommendations of Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC) and Committee for the Purpose of Control and Supervision of Experiments on Animals, Government of India. The principles of OECD Series on Testing and Assessment Document No. 19: Environment Directorate, Guidance Document on The Recognition, Assessment, and Use of Clinical Signs as Humane Endpoints for Experimental Animals Used in Safety Evaluation were followed.

The required amount of test material was weighed and mixed with the diet using electric mixer. Diet formulations were prepared under monochromatic light and used within the stability period (stable up to 8 days) of the test substance in the diet. The concentration of the test substance in the feed were analysed on days 1, 42 and 84 of dosing period by reverse phase high performance liquid chromatography with UV detection. The method has been validated according to SANCO/3029/99 rev. 4 (2000) under Syngene study no. U-19069. The results confirmed the test item concentrations in the rat diet at 80 - 120 % with a relative standard deviation (% RSD) ≤ 20 %.

The animals were observed for morbidity and mortality twice daily during study period and once on the day of terminal and recovery sacrifice. All rats were observed for clinical signs once daily during dosing period and recovery period. Detailed clinical examinations were done prior to initiation of treatment and weekly once thereafter all main and recovery group animals until scheduled necropsy. Detailed clinical examinations were not performed for the toxicokinetics groups.

Body weight were recorded for all animals on first day of treatment (Day 1) and weekly thereafter. Terminal (fasting) body weights were measured on the days of scheduled necropsy (Day 91 for main group and Day 119 for recovery group).

Food input and food left over was measured for all animals at once weekly interval from day of start of treatment until scheduled sacrifice.

Ophthalmological examination was performed using an ophthalmoscope once for all animals during acclimatization period and at the last week of dosing phase (day 87). Mydriasis was induced before examination of eyes using a mydriatic agent.

Following examinations were conducted on day 86 of the dosing phase in the control and test substance group. Neurological evaluation for individual animals, as applicable, were recorded in the form of scores as per test facility SOP.

The following neuromuscular observations were performed: 1. Grip strength, 2. Hind limb foot spray.

Rectal temperature was recorded in Fahrenheit (°F) using digital thermometer.

The following motor activity parameters were observed: 1. Distance travelled, 2. Horizontal counts, 3. Ambulatory counts, and 4. Vertical counts.

Blood samples were collected from three rats per sex per time point from the TK groups as per the sampling schedule. Blood was collected via retro-orbital plexus puncture under mild isoflurane anaesthesia. The sampling intervals for test substance treated group were as follows: 5 P.M and 9 P.M (day 2 and 86), 7 A.M, 10 A.M, 2 P.M and 5 P.M on Day 3 and 87. The sampling intervals for control group were as follows: 5 P.M (day 2 and 86), 7 A.M and 2 P.M and 5 P.M, on day 3 and 87. The bioanalysis was performed by LC-MS/MS method using protein precipitation as sample extraction technique. The supernatant - after extraction and centrifugation - was transferred into auto sampler vials. Volumes of 10 µL of

the supernatant were injected onto a HPLC column for chromatographic separation, followed by mass spectrometer detection. The samples were analysed with a validated method (Syngene Study No. U-19104) at a LOQ of 0.100 µg/mL. Toxicokinetic evaluation was performed by validated Phoenix® WinNonlin® software (Pharsight Corporation, USA).

Blood was collected from all animals of main groups at the dosing phase (day 91) and for recovery groups at the end of recovery phase (day 29) for haematology, coagulation and clinical chemistry evaluations. The animals were fasted overnight prior to terminal blood collection. Blood was collected via retro-orbital plexus puncture of right eye under mild isoflurane anaesthesia. The following haematology, coagulation and clinical chemistry parameters were measured: haematology – red blood cell count, haemoglobin concentration, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, platelet count, reticulocyte count, total white blood cell count, leukocyte differential (neutrophils, lymphocytes, monocytes, eosinophils and basophils); coagulation – active partial thromboplastin time, prothrombin time and fibrinogen; clinical chemistry – albumin/globulin ratio, alanine aminotransferase, albumin, alkaline phosphatase, aspartate aminotransferase, blood urea nitrogen, calcium, chloride, creatinine, creatinine kinase, gamma-glutamyl trans-peptidase, globulin, glucose, high-density lipoprotein, inorganic phosphorus, low-density lipoprotein, potassium, sodium, total bilirubin, total cholesterol, total protein, triglycerides and T3, T4 and TSH.

Urine was collected from all animals at the end of dosing period (day 91 of dosing phase) and at the end of recovery period (day 29 of recovery phase). During urine collection, animals were fasted overnight in metabolic cages. The following parameters were measured: volume, colour, appearance, glucose, bilirubin, ketone, blood, pH, protein, urobilinogen, nitrite, leukocyte and specific gravity.

Gross necropsy was conducted on all animals at the end of dosing phase for main groups (day 91; terminal sacrifice) and at the end of recovery phase of recovery group (Day 29; recovery phase). The following organs were collected, weighed, assessed histopathologically after fixation in 10% neutral buffered formalin and staining with haematoxylin and eosin: adrenals, brain, epididymites, heart, kidneys, liver, ovaries, pituitary, prostate, seminal vesicles and coagulating glands, spleen, testes, thymus, thyroid with parathyroid and uterus with cervix. The other organs or tissues such as aorta, biceps femoris muscles, cecum, colon, diaphragm, duodenum, oesophagus, eyes, femur bone with joint, gastrocnemius muscle, gross changes, harderian glands, ileum with Peyer's patches, jejunum, lacrimal gland, lungs, lymph nodes (mandibular and mesenteric), mammary gland, optic nerve, oviducts, pancreas, Quadriceps muscle, rectum, salivary glands (mandibular, sublingual and parotid), sciatic nerve, skin (inguinal region), soleus muscle, spinal cord, sternum with marrow, stomach, tongue, trachea, urinary bladder and vagina, were also examined histopathologically. Histopathology was performed on all tissues of animals from control and test substance treated groups on main group. Histopathology was not extended to recovery groups, because there were no target organs in the test substance treated main group. Peer review was performed by another in-house pathologist.

Body weight and body weight gain, food consumption, grip strength, hind limb foot spray, body temperature, organ weights, organ weight ratios and laboratory parameters (haematology, coagulation and clinical chemistry) were analysed using Student's t-test.

Results and discussions

No mortality was observed during the study.

Soft stool was observed in males from day 56 of dosing phase of 16000 ppm main, recovery and TK groups. Soft stool was observed in females from day 70 of dosing phase of 16000 ppm main group, and also observed from day 62 of dosing phase of 16000 ppm recovery and TK groups. However, these clinical signs were recovered in recovery groups from day 2 of recovery phase in males and females. Soft

stool was considered as test substance related. This clinical sign was not associated adverse changes in body weights and other parameters, hence, not considered as adverse change.

No test substance-related changes were noticed in overall body weight and body weight gain parameters in test substance treated group when compared to control group.

Table A 12: Cumulative body weight gain

main group males

Dose (ppm)	Day 1-8 n=10	Day 29-36 n=10	Day 57-64 n=10	Day 1-90 n=10
0	40.08[1] (10.75)[2]	22.96 (4.30)	13.45 (4.50)	237.75 (35.40)
16000	45.19 (3.92)	20.97 (3.52)	13.36 (6.51)	213.80 (20.74)

[1]: Weight in gains; [2]: Standard deviation

main group females

Dose (ppm)	Day 1-8 n=10	Day 29-36 n=10	Day 57-64 n=10	Day 1-90 n=10
0	22.27[1] (4.61)[2]	7.65 (2.86)	4.91 (2.70)	97.87 (5.29)
16000	27.41+ (2.68)	12.31 (10.78)	3.35 (3.83)	101.28 (4.27)

[1]: Weight in gains; [2]: Standard deviation, +: Significantly higher than the control group at 5% level.

There were no test substance-related changes in feed consumption in females, when compared to control. Minimal reduction (5%) on overall food consumption was observed in 16000 ppm main group males as compared to control group. Statistically significant decrease in feed consumption on days 8-15 (14%), days 36-43 (10%), days 43-50 (7%) was noticed in 16000 ppm main group males when compared to control group. Statistically significant increase in feed consumption on day 15-22 (16%) and on recovery day 1-8 (5%) were observed in 16000 ppm recovery female group. Minimal reduction on overall food consumption in males was considered to as test substance related. Above mentioned changes in feed consumption in females were considered as an incidental, as the magnitude of changes were minimal and occurred randomly during dosing phase. The test substance intake achieved over the entire treatment period (day 1-90) for 16000 ppm concentration levels in diet was equivalent to 924 mg/kg body weight/day in males and 1119 mg/kg body weight/day in females, respectively. In 16000 ppm recovery group, the test substance intake achieved over the entire treatment period (day 1-90) was equivalent to 930 mg/kg body weight/day in males and 1090 mg/kg body weight/day in females, respectively. In 16000 ppm TK group, the test substance intake achieved over the entire treatment period (day 1-85) was equivalent to 912 mg/kg body weight/day in males and 1102 mg/kg body weight/day in females, respectively.

No eye abnormalities were observed in ophthalmological examination performed toward the end of treatment period in males and females.

No test substance-related changes were observed in functional observation battery (FOB) parameters for main group animals as compared to control group.

No test substance-related changes were observed in grip strength and landing foot splay parameters as compared to control group.

No test substance-related changes were observed in rectal temperature as compared to control group.

No test substance-related changes were observed in motor activity parameters as compared to control group.

Following oral feeding of RH-141455 in rats, T_{max} was observed on both day 2 and 86 at 7 A.M in males and females, i.e. 45/2061 hours from first exposure of animals. Plasma concentrations of RH-141455 were noted up to last time points in treated groups. T_{last} was observed at 55 hours for day 2 and 2071 hours for day 86. No concentrations in control group were observed. No sex related difference was observed in RH-141455 concentrations on day 2. Male to female AUC_{24h} and C_{max} ratio on day 86 was 0.81 and 0.84, respectively. Day 86/day 2 AUC_{24h} ratios were 0.48 in males and 0.65 in females at 16000 ppm. Repeat dose feeding of RH-141455 at 16000 ppm in diet for 90 days showed evidence of systemic plasma exposure to RH-141455 in Sprague Dawley rats.

A statistically significant but minimal decrease in mean blood neutrophil counts (38%) was seen in females administered RH-141455 at terminal sacrifice, which was recovered at the end of recovery period. This change in neutrophils was considered toxicologically not significant because of low magnitude, lack of microscopic correlate and occurrence in only one sex.

A statistically significant minimal increase in serum creatine in males (12%) and females (12%) and statistically significant minimal increase in BUN in females (19%) were observed at the terminal sacrifice in 16000 ppm. The mean creatinine values were within the historical control data range. At the end of recovery period, changes in creatinine and BUN were not evident. These small differences in BUN and creatinine values were considered likely relevant to in-life observation of soft stools during the dosing phase (possibly due to a minimal water loss via soft stools).These changes were considered toxicologically not relevant because of very lower magnitude of change, absence of renal pathology, and no evidence of such changes at the endo of recovery period. A statistically significant minimal increase in total bilirubin (14%) was noted in males at the terminal sacrifice in 16000 ppm. The bilirubin values were out of historical control data. In recovery males, although mean bilirubin values were slightly higher (31%) at the end of recovery period compared to concurrent controls, the bilirubin values were within the range of historical control data. This change was considered toxicologically not relevant because of its lower magnitude, lack of correlative microscopic findings, absence of concurrent increases in ALT and GGT (markers for hepatobiliary injury) and occurrence in only one sex. Marginal decrease (11%) in mean values of T4 was noted in males and females of 16000 ppm, when compared to the concurrent control group at the terminal sacrifice. This change in T4 was considered toxicologically not significant because of concurrent increase in TSH and its complete recovery at the end of recovery period. These changes were recovered at the end of recovery period.

At terminal sacrifice, slight decrease in mean urine volume was noted in 16000 ppm group. In addition, a marginal increase in mean urine specific gravity was noted in males of 16000 ppm group (control group mean: 1.0188; 16000 ppm group mean: 1.0328). These changes were recovered at the end of recovery period. These small changes in urine volume and specific gravity were considered likely related to in-life observation of soft stools but were considered toxicologically not significant because of their lower magnitude, lack of correlative microscopic findings, and complete recovery at the end of recovery period.

All the differences in organ weight parameters at the end and recovery sacrifices were considered incidental, as the magnitude of differences were marginal, lacked microscopic correlate and/or occurred only in one of three parameters (absolute weight, relative to body weight or relative to brain weight).

RH-141455-related cecal enlargement (with contents) was noted in males (8/10) and females (3/10) of 16000 ppm group at the terminal sacrifice. This finding had no microscopic correlate and recovered the end of recovery period, therefore, considered as non-adverse finding. Other gross findings at the end of terminal sacrifice such as a yellow focus in the liver of one male in 16000 ppm, or uterine dilation in female control group was considered an incidental finding or normal physiological uterine change. There were no test substance-related microscopic findings in the study. All microscopic changes were considered incidental or spontaneous and not related to the test substance, because they were randomly distrib-

uted across the test substance and control groups and/or were generally observed in the rats of this age and strain.

Conclusion

This study was designed to assess the potential sub-chronic toxicity and toxicokinetics (TK) profile of RH-141455 when fed in formulated diet to Sprague Dawley rats for 90 days. A total of 41 males and 41 females Sprague Dawley rats were randomly assigned to 2 main groups (10 rats/sex/group), 2 recovery groups (6 rats/sex/group) and 2 TK groups (3/6 rats/sex/group for control and test-substance related, respectively). The dose of 16000 ppm was aimed to reach the guideline recommended limit dose of 1000 mg/kg body weight/day. The control group was fed with basal diet for same duration of study.

There was no morbidity or mortality or ophthalmic changes at 16000 ppm in both sexes. Soft stool was observed in males from day 56 of dosing phase and day 70 of dosing phase in females of 16000 ppm. However, these clinical signs recovered in recovery groups. RH-141455 administration did not result in any effects on the functional observation battery, grip strength, landing foot splay, rectal temperature and motor activity.

There were no test material-related changes in overall mean body weight and body weight gain at 16000 ppm in both sexes when compared to control group animals. Minimal reduction (5%) on overall food consumption and food conversion efficiency was observed test substance treated main group males as compared to control group.

There was no relevant test material-related change in hematology and coagulation parameters. Minimal increase in serum creatine in both sexes and blood urea nitrogen (BUN) in females were not considered adverse, as creatinine values were within the historical control data range. At the end of recovery period, changes in creatinine and BUN were not evident and these small differences in BUN and creatinine value were considered likely related to in-life observation of soft stools during the dosing phase (possibly due to a minimal water loss via soft stools). These changes were considered toxicologically not relevant because of very lower magnitude of changes, absence of renal pathology, and no evidence of such changes at the end of recovery period. A statistically significant marginal increase in total bilirubin was considered toxicologically not relevant because its lower magnitude, lack of correlative microscopic findings, absence of concurrent increase in ALT and GGT (markers for hepatobiliary injury) and occurrence in only one sex. At terminal sacrifice, cecal enlargement (with contents) was noticed in males (8/10) and females (3/10) administered RH-141455 group. This finding had no microscopic correlate and recovered at the recovery period, therefore, considered as non-adverse findings. No test substance-related microscopic findings noted in the study.

NOAEL of RH-141455 was 16000 ppm, which is equivalent to 924 and 1119 mg/kg body weight/day for the males and females, respectively. This NOAEL was based on absence of adverse effects at 16000 ppm in male and female rats.

(xxx 2020)

A 2.11.12 Study 12

Comments of zRMS PL :	Confirmatory-like study which is under evaluation by the RMS for Zoxamide in an interzonal procedure, thus it was not evaluated in this assessment. Evidence of systemic plasma exposure to RH-150721 and downstream metabolite RH-141455 was not observed in 2 days oral dietary pharmacokinetic study in Sprague Dawley Rats fed a diet containing RH-150721 at concentration of 15000 ppm from day 1 through day 3. The resulting concentrations were below the quantification limits for both RH-150721 and RH-141455. Pharmacokinetic parameters could not be calculated.

Reference:	**KCP 7.4**
Report	xxx, 2020: RH-150721: 2-day oral dietary pharmacokinetic study in Sprague Dawley Rats Gowan Crop Protection Ltd., UK xxx, Report No. U-19134, No GLP, Not published
Guideline(s):	OECD 408 (2018)
Deviations:	The draft report was later than expected by the sponsor. This deviation is not regarded to have an influence on the integrity of the study. 12 March 2020
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test material (Lot/Batch No.)	RH-150721 (HHGCP004-00-3)
Purity:	99.9%
Reference material:	RH-141455 (HHGCP014-00-1)
Purity:	99.6%
Vehicle:	Teklad certified irradiated global 18% protein rodent diet (meal)
Test organisms	Albino rat, Sprague Dawley purchased from Envigo, The Netherlands
Age:	10-11 weeks
Body weight:	Body weights did not differ by more than 20% in the group at study initiation.
Acclimation period:	1 day
Water:	Tap water, *ad libitum*
Diet:	Teklad certified irradiated global 18% protein rodent diet (meal) diet *ad libitum*
Housing:	1 animal per cage in polycarbonate rat cages with bedding materials
Test design	
Total number of animals:	4 (males)
Environmental conditions	

Temperature:	20.3 to 24.1°C
Humidity:	Relative humidity ranged from 54-64 %
Air changes:	22 fresh air changes per hour
Photoperiod:	12-hour light/dark cycles
Test conentrations	15000 ppm in diet
Exposure time	2 days
Remarks	None

The study was designed to assess plasma exposures and pharmacokinetic profiles of RH-150721 and its downstream metabolite RH-141455 following oral dietary administration at 15000 ppm for two days in male Sprague Dawley rats. A total of 4 male Sprague Dawley rats were used for pharmacokinetic evaluation. RH-150721 was formulated in powdered diet at concentration of 15000 ppm and fed from day 1 through day 3. The observations included daily morbidity/ mortality check, clinical signs, body weights, feed consumption, bioanalytical & PK parameters (days 2 & 3), and gross pathology. Blood samples were collected at 4 PM, 10 PM (day 2), 7 AM, 12 PM and 4 PM (day 3) via retro-orbital plexus puncture under mild isoflurane anesthesia, plasma was separated and a method for the determination of RH-150721 and RH-141455 via LC-MS/MS, using an internal standard (tolbutamide) was developed. Pharmacokinetic parameters were evaluated.

Results and discussions

No mortality/morbidity was observed during in-life phase of the study. No clinical signs were observed during in-life phase of the study. A test item related decrease in day 3 body weights (8%) compared to day 1 was noticed. At terminal sacrifice on day 3, there were no test item-related gross changes in any of the organs examined.

A total of 10 rat plasma samples were analysed with a validated LCMS/MS method using an internal standard (tolbutamide) at a calibration range of 0.057 µg/mL to 44.583 µg/mL. The resulting concentration data were below the quantification limits for both RH-150721 and RH-141455. Hence, pharmacokinetic (PK) evaluation using Phoenix® WinNonlin® software was not performed.

Conclusion

The study was designed to assess plasma exposures and pharmacokinetic profiles of RH-150721 (and downstream metabolite RH-141455) following oral dietary administration of RH-150721 at 15000 ppm for two days in male Sprague Dawley rats. The resulting concentrations were below the quantification limits for both RH-150721 and RH-141455. Pharmacokinetic parameters could not be calculated.

(xxx 2020)

A 2.11.13 Study 13

Comments of zRMS PL :	Confirmatory-like study which is under evaluation by the RMS for Zoxamide in an interzonal procedure, thus it was not evaluated in this assessment. The No-Observed-Adverse-Effect Level (NOAEL) of RH-150721 in 14-day oral dietary dose range finding study in Sprague Dawley rats was found to be is 5000 ppm, which is equivalent to appr. 334 mg/kg bw/day in males and 382 mg/kg bw/day in females

Reference:	**KCP 7.4**
Report	xxx, 2020: RH-150721: 14-day oral dietary dose range finding study in Sprague Dawley Rats Gowan Crop Protection Ltd., UK xxx, Report No. U-19189, GLP, Not Published
Guideline(s):	OECD 407 (2008)
Deviations:	The draft report was provided to the sponsor with a delay of 1 month. On day 14 (7 A.M), blood sampling has been delayed between 4-27 minutes from the scheduled time (± 25 minutes) across all the groups. These findings will not change the findings and the integrity of the study.
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test material (Lot/Batch No.)	RH-150721 (HHGCP004-00-3)
Purity:	99.9 % (w/w)
Vehicle:	Teklad Global 18% Protein Rodent Diet (meal) manufactured by Envigo
Stability:	Test material was stable for at least 8 days at room temperature.
Test organisms	Albino rat, Sprague Dawley purchased from Envigo, Netherlands
Age:	7-8 weeks
Body weight:	Males: 245.26- 274.99 g Females: 187.88 – 205.94 g The weight variation of the animals at randomisation was within ± 20% of the mean weight for each sex.
Acclimation period:	4 days
Water:	Tap water, *ad libitum* (except overnight fasting prior to clinical pathology blood collection)
Diet:	Teklad Global 18% Protein Rodent Diet (meal) manufactured by Envigo
Housing:	2 per sex per cage in polycarbonate rat cages with bedding materials
Test design	
No. of groups:	4 groups
No. of animals/group:	6 rats/sex/group
Number of animals:	48 (24 Males + 24 Females)
Extra animals:	12 (6 Males + 6 Females)
Total number of animals:	60 (30 Males + 30 Females)
Environmental conditions	
Temperature:	20.1 – 23.8°C

Humidity:	Relative humidity ranged from 51 - 67%
Air changes:	20 fresh air changes per hour
Photoperiod:	12-hour light/dark cycles
Test conentrations	1000, 5000 and 16000 ppm in diet
Exposure time	14 days
Remarks	None

In a dose range finding study, RH-150721 was administered to rats for 14 days at levels of 1000, 5000 and 16000 ppm for male and female to groups of Sprague Dawley rats (6 rats/sex/group). The high dose group was administered with 16000 ppm for 7 days. The dose was reduced to 8000 ppm from day 8 due to unpalatability. The concentrations of 1000 and 5000 ppm in the diet were equivalent to 66 and 334 mg/kg body weight/day in males and 77 and 382 mg/kg body weight/day in females, respectively. Test substance intake achieved over the treatment period (day 1-7) for 1600 ppm was equivalent to 911 mg/kg body weight/day in males and 1051 mg/kg body weight/day in females, respectively. Test substance intake achieved over the treatment period (day 8-14) for 8000 ppm was equivalent to 618 mg/kg body weight/day in males and 664 mg/kg body weight/day in females, respectively. The control group was fed with basal diet for same duration of study.

There were 6 animals of each sex per group. Males and females were randomised into 4 dose groups using Pristima, based on body weight to control bias. RH-150721 was administered in the diet for 14 days to Sprague Dawley rats – 0, 1000, 5000 and 16000/8000 ppm. Husbandry conditions were in accordance with the recommendations of Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC) and Committee for the Purpose of Control and Supervision of Experiments on Animals, Government of India. The principles of OECD Series on Testing and Assessment Document No. 19: Environment Directorate, Guidance Document on The Recognition, Assessment, and Use of Clinical Signs as Humane Endpoints for Experimental Animals Used in Safety Evaluation were followed.

Table A 13: Study design

Test group	Concentration in diet (ppm)	Dose (mg/kg/day)		Animals assigned	
		Male	Female	Male	Female
1	0	0	0	6	6
2	1000	66	77	6	6
3	5000	334	382	6	6
4	15000 (Day 1-7)	911	1051	6	6
	8000 (Day 8-14)*	618	664		

* Due to significant reduction in body weight and food consumption, the high dose was reduced from 16000 ppm to 8000 ppm in diet from Day 8 of the dosing phase.

The required amount of test material was weighed and mixed with the diet using electric mixer. Diet were prepared approximately weekly. Prepared diets were stored at room temperature before use. The concentration of the test substance in the feed was analytically verified with a reverse phase analytical method using UV-DAD detection. The method has been validated according to SANCO 3099 rev. 4 under study no. U-19162. The active ingredient content in the prepared dose formulations were analysed 99.96-102.7 % of nominal, and there within the acceptance limits of 80 to 120 % with % RSDs ≤ 20 %.

The animals were observed twice daily for morbidity and mortality. All rats were observed for clinical signs at least once daily. Detailed clinical examinations were done once on Day 1 of dosing and weekly thereafter.

Body weight were recorded on days 1, 2, 3, 7, 11 and 14.

Food input was recorded on Days 1, 3, 4, 7, 8, 9, 11, 13 and food leftover was measured on days 3, 5, 7, 8, 9, 11,13,14.

Ophthalmoscopic examinations were not performed.

Blood samples were collected from three rats per sex per time point as per the sampling schedule. Blood was collected via retro-orbital plexus puncture under mild isoflurane anesthesia. The sampling intervals were as follows: 5 P.M (day 2 and 13) and 7 A.M, 10 A.M, 2 P.M and 5 P.M on days 3 and 14. Toxicokinetic evaluation was performed by validated Phoenix® WinNonlin® software (Pharsight Corporation, USA).

Blood was collected from all animals at the terminal sacrifice (Day 15) for haematology, coagulation and clinical chemistry evaluations. The animals were fasted overnight prior to terminal blood collection. Blood was collected via retro-orbital plexus puncture of right eye under mild isoflurane anaesthesia. The following haematology, coagulation and clinical chemistry parameters were measured: haematology – red blood cell count, haemoglobin concentration, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, platelet count, reticulocyte count, total white blood cell count, leukocyte differential (neutrophils, lymphocytes, monocytes, eosinophils and basophils); coagulation – active partial thromboplastin time, prothrombin time and fibrinogen; clinical chemistry – albumin/globulin ratio, alanine aminotransferase, albumin, alkaline phosphatase, aspartate aminotransferase, blood urea nitrogen, calcium, chloride, creatinine, creatinine kinase, gamma-glutamyl trans-peptidase, globulin, glucose, high-density lipoprotein, inorganic phosphorus, low-density lipoprotein, potassium, sodium, total bilirubin, total cholesterol, total protein and triglycerides.

Urine was collected from all animals at the end of dosing phase (Day 15). During urine collection, animals were fasted overnight in metabolic cages. The following parameters were measured: volume, colour, appearance, glucose, bilirubin, ketone, blood, pH, protein, urobilinogen, nitrite, leukocyte and specific gravity.

Gross necropsy was conducted on all animals at the end of dosing phase (Day 15). The following organs were collected, weighed, assessed histopathologically after fixation in 10% neutral buffered formalin and staining with haematoxylin and eosin: adrenals, brain, epididymites, heart, kidneys, liver, ovaries, pituitary, prostate, seminal vesicles and coagulating glands, spleen, testes, thymus, thyroid with parathyroid and uterus with cervix. The other organs or tissues such as aorta, biceps femoris muscles, cecum, colon, diaphragm, duodenum, oesophagus, eyes, femur bone with joint, gastrocnemius muscle, gross changes, harderian glands, ileum with Peyer's patches, jejunum, lungs, lymph nodes (mandibular and mesenteric), mammary gland, optic nerve, oviducts, pancreas, Quadriceps muscle, rectum, salivary glands (mandibular, sublingual and parotid), sciatic nerve, skin (inguinal region), soleus muscle, spinal cord, sternum with marrow, stomach, tongue, trachea, urinary bladder and vagina, were also examined histopathologically. Histopathology was performed on all tissues of animals from control and high dose groups. Peer review was performed by another in-house pathologist.

For comparative statistics, data were evaluated using Levene's Test for homogeneity of variance, with significance at 5% level. Data determined to be homogeneous was evaluated for analysis of variance (ANOVA). When the ANOVA verified significance at 5% level, pairwise comparisons of each treated group with the control group was made using a Dunnett Test, to identify statistical differences (at 5% level). When the data were found to be non-homogeneous, ANOVA was done using suitable transformation. When the homogeneity tests were significant even after transformation, data were evaluated using a Kruskal-Wallis Test for group factor significance. When significance (at 5% level) existed between groups, Dunn's pairwise comparison was performed.

Results and discussions

In control, 1000 ppm, 5000 ppm groups, there were no clinical signs of toxicity. In 16000/8000 ppm group, clinical signs of thin appearance were observed from day 7 to 10 in males and females. From days 11-14, there were no clinical signs of toxicity in both sexes in this group.

No mortalities or morbidity was observed in any dose group during the study.

In 1000 ppm group, no test substance-related changes were noticed in body weight and body weight gain parameters in any of the dose groups when compared to control group. In 5000 ppm group, statistically significant but mild reduction was noticed in overall body weight (7%) and body weight gain (38%) in males. Similarly, non-significant mild reduction in overall body weight (4%) and significant reduction in body weight gain (52%) was observed in females in females. The changes noted in 5000 ppm males and females were not considered adverse because the body weight decreases were <10%. Administration of RH-150721 at 16000 ppm for 7 days resulted in significant reduction in body weight gain (males -312%; females -894%) on day 1-7. However, upon reducing the dose to 8000 ppm from day 8 to day 14 of the study, a non-significant increase in body weight gain on days 7-11 (males -13%; females -74%) and significant increase in body weight gain on days 11-14 (males -69%; females -178%) was observed when compared to control group. The changes in the body weight parameters at 16000/8000 ppm group were considered test substance related and adverse in both sexes.

Table A 14: Body weight and body weight change

Group #		Dosing Phase					
		Day: 1	Day: 2	Day: 3	Day: 7	Day: 11	Day: 14
		Session 1	Session 1	Session 1	Session 1	Session 1	Session 1
Males							
Control	n	6	6	6	6	6	6
	Means	261.55	268.39	267.06	284.00	304.64	314.15
	SD	6.45	6.09	7.33	7.14	5.61	9.82
2	n	6	6	6	6	6	6
	Means	261.09	265.72	265.51	280.49	296.26	304.72
	SD	7.00	6.60	10.01	12.91	14.40	17.81
3	n	6	6	6	6	6	6
	Means	260.43	**252.48-**	**251.45-**	**271.50-**	290.49	**292.87-**
	SD	7.59	7.41	8.05	5.22	11.10	10.36
4	n	6	6	6	6	6	6
	Means	260.80	**245.45-**	**232.55-**	**213.28-**	**236.54-**	**252.60-**
	SD	10.08	11.46	9.95	6.64	11.84	14.52
Females							
Control	n	6	6	6	6	6	6
	Means	195.95	196.64	197.16	201.35	213.10	216.58
	SD	6.28	4.95	6.50	5.91	6.13	6.66
2	n	6	6	6	6	6	6
	Means	195.98	195.13	195.49	202.15	207.35	212.74
	SD	5.34	3.49	6.03	7.62	7.77	9.66
3	n	6	6	6	6	6	6
	Means	198.41	190.41	189.63	203.39	209.20	208.30
	SD	5.96	5.48	6.51	8.64	4.35	5.53
4	n	6	6	6	6	6	6
	Means	196.95	**184.02-**	**173.98-**	**154.07-**	**174.51-**	**184.17-**

	SD	5.01	5.76	5.19	5.46	5.63	5.47

Control: G1 (0 ppm in diet); 2: G2 (1000 ppm in diet); 3: G3 (5000 ppm in diet); 4: G4 (16000/8000 ppm in diet) -: Significantly lower than the control group at 5 % level.

Table A 15: Body weight change

Group #		D:1-D:2	D:2-D:3	D:3-D:7	D:1-D:7	D:7-D:11	D:11-D:14	D:1-D:14
Males								
Control	n	6	6	6	6	6	6	6
	Means	6.84	-1.34	16.94	22.45	20.64	9.50	52.60
	SD	1.38	3.49	4.02	2.78	2.44	4.36	6.50
2	n	6	6	6	6	6	6	6
	Means	4.62	-0.21	14.99	19.40	15.76	8.46	43.62
	SD	2.20	4.20	3.37	7.54	1.97	4.15	12.09
3	n	6	6	6	6	6	6	6
	Means	**-7.96^{-}**	-1.02	20.05	11.07	18.99	**2.39^{-}**	**32.44^{-}**
	SD	2.46	3.59	5.67	4.81	7.40	2.87	11.25
4	n	6	6	6	6	6	6	6
	Means	**-15.35-**	**-12.90-**	**-19.27-**	**-47.52-**	23.26	**16.06^{+}**	**-8.20-**
	SD	2.15	2.67	6.25	5.41	5.77	5.24	7.65
Females								
Control	n	6	6	6	6	6	6	6
	Means	0.69	0.51	4.19	5.40	11.75	3.48	20.63
	SD	1.86	2.09	3.96	4.29	4.63	3.08	3.67
2	n	6	6	6	6	6	6	6
	Means	-0.85	0.36	6.66	6.17	5.20	5.39	16.76
	SD	2.42	4.71	4.36	7.91	2.67	4.57	10.64
3	n	6	6	6	6	6	6	6
	Means	**-8.00^{-}**	-0.77	**13.76^{+}**	4.98	5.81	-0.90	**9.90^{-}**
	SD	2.61	2.63	5.39	6.55	5.59	2.50	3.39
4	n	6	6	6	6	6	6	6
	Means	**-12.93-**	**-10.04-**	**-19.91^{-}**	**-42.88^{-}**	20.43	**9.67^{+}**	**-12.77^{-}**
	SD	2.00	2.49	6.16	5.53	10.51	3.79	5.92

Group #: Control: G1 (0 ppm in diet); 2: G2 (1000 ppm in diet); 3: G3 (5000 ppm in diet); 4: G4 (16000/8000 ppm in diet) +/-: Significantly higher / lower than the control group at 5 % level.

In 1000 ppm group, no test substance-related changes were noticed in mean food when compared to control group. In 5000 ppm group, non-significant reduction was noticed in feed consumption (males -7%) when compared to control group. In 16000/8000 ppm group, significant reduction was noticed in overall mean food consumption (males -22%; females -21%) when compared to control group. The reduced food consumption correlated with changes in body weight parameters and might be attributed to palatability of test substance. The dose in ppm in diet for 1000 and 5000 ppm were equivalent to 66 and 334 mg/kg body weight/day in males and 77 and 382 mg/kg body weight/day in females, respectively. Test substance intake achieved over the treatment period (day 1-7) for 1600 ppm was equivalent to 911 mg/kg body weight/day in males and 1051 mg/kg body weight/day in females, respectively. Test substance intake achieved over the treatment period (day 8-14) for 8000 ppm was equivalent to 618 mg/kg body weight/day in males and 664 mg/kg body weight/day in females, respectively.

There were no test substance-related changes in haematology and coagulation parameters. In 16000/8000 ppm group, minimal, statistically non-significant decrease in mean total WBC (males: 10%, females:

18%), lymphocytes count (males: 19%, females: 21%) were noted. These changes correlated with the light microscopic findings of decreased lymphocytes in the thymus and spleen, and decreased cellularity in the bone marrow of femur and sternum bones. These hematologic changes were considered likely related to decreased food consumption and body weight gain observed during first week of the study (Levin et al., 1993[6], Hubert et al., 2000[7]). Similar changes were not evident at 1000 and 5000 ppm groups.

There were no test substance-related changes in clinical chemistry parameters. In 16000/8000 ppm group, statistically significant minimal decrease in mean serum BUN, total protein, albumin, globulin, increase in serum triglycerides, ALT, ALP was noticed. These changes were likely related to decreased food consumption and body weight gain observed the first week of the study (Levin et al., 1993[8], Hubert et al., 2000[9]).

There were no test substance-related changes in urinalysis parameters.

In 16000/8000 ppm group, decreased weights of thymus and spleen were observed. In 5000 and 16000/8000 ppm group, decreased weights of prostate, seminal vesicles, coagulation glands, ovaries were observed. These changes correlated with the light microscopic findings in these organs and were considered related to decreased food consumption and body weight gain during in-life phase (Rehm et al., 2008[10], Levin et al., 1993).

There were no test substance-related gross changes in 1000 and 5000 ppm groups. In 16000/8000 ppm group, gross finding of small size thymus, seminal vesicles, prostate, ovaries, uterus were observed. These changes correlated with decreased organ weights and/or light microscopic changes in these organs. These gross changes were considered likely related to the decreased food consumption and body weight gain during in-life phase. Uterine dilation observed randomly across the groups was a common change due to normal reproductive cycling in rats to this age. In 5000 and 16000/8000 ppm groups, acinar hypertrophy was observed in mandibular and parotid salivary glands. The acinar cell size was increased with increased secretary material. However, there was no increased salivation observed in these animals during in-life phase of the study. The biological significance of these findings is unknown (NTP Technical Report on Toxicity Studies of Glyphosate, 1992[11]); however, these findings were considered as adaptive change (Inoue et al., 2014[12]). The incidence and/or severity of this change was lower in 5000 ppm group than in 16000/8000 ppm group. This change was not observed in 1000 ppm group. In 16000/8000 ppm group, decreased lymphocytes in thymus and spleen, decreased secretion in prostate, seminal vesicles, coagulation glands, decreased size of *corpora lutea* in ovaries, decreased cellularity of bone marrow in sternum and femur bones, atrophy of mammary gland, decreased secretion in ducts of mandibular salivary gland were observed. All these changes were considered likely related to decreased food consump-

[6] Levin S, Semler D, Ruben Z. Effects of two weeks of feed restriction on some common toxicologic parameters in Sprague-Dawley rats. Toxicol Pathol.1993; 21 (1):1-14.
[7] Hubert MF, Laroque P, Gillet JP, Keenan KP. The effects of diet, ad Libitum feeding, and moderate and severe dietary restriction on body weight, survival, clinical pathology parameters, and cause of death in control Sprague-Dawley rats. Toxicol Sci. 2000 Nov;58(1):195-207.
[8] Levin S, Semler D, Ruben Z. Effects of two weeks of feed restriction on some common toxicologic parameters in Sprague-Dawley rats. Toxicol Pathol.1993; 21 (1):1-14.
[9] Hubert MF, Laroque P, Gillet JP, Keenan KP. The effects of diet, ad Libitum feeding, and moderate and severe dietary restriction on body weight, survival, clinical pathology parameters, and cause of death in control Sprague-Dawley rats. Toxicol Sci. 2000 Nov;58(1):195-207.
[10] Rehm S, White TE, Zahalka EA, Stanislaus DJ, Boyce RW, Wier PJ. Effects of food restriction on testis and accessory sex glands in maturing rats. Toxicol Pathol. 2008 Jul; 36(5):687-94.
[11] NTP Technical Report on Toxicity Studies of Glyphosate (CAS No. 1071836) Administered in Dosed Feed to F344/N Rats and B6C3F1 Mice, NIH Publication 92-3135, July 1992.
[12] Inoue K, Morikawa T, Matsuo S, Tamura K, Takahashi M, Yoshida M. Adaptive parotid gland hypertrophy induced by dietary treatment of GSE in rats. Toxicol Pathol. 2014 Aug;42 (6):1016-23.

tion [Levin et al. (1993), Rehm et al., (2008), Everds et al., (2013)[13]]. These light microscopic changes were not observed in 1000 and 5000 ppm groups.

RH-150721 plasma concentration at all-time points were below limit of quantification, hence, toxicokinetic evaluation was not performed.

Conclusion

Under the conditions of the study, the No-Observed-Adverse-Effect-Level (NOAEL) of RH-150721 in the Sprague Dawley rats is 5000 ppm, which is equivalent to appr. 334 mg/kg bw/day in males and 382 mg/kg bw/day in females. This NOAEL is based on reduction in body weight, food consumption, food efficiency at 16000/ 8000 ppm in males and females. Acinar hypertrophy of mandibular and parotid salivary glands at mid and high doses were considered likely related to test item. However, these findings were considered as adaptive change. The other changes noted in clinical pathology and anatomic pathology parameters at high and mid doses were considered related to decreased food consumption and/or body weights.

(xxx 2020)

[13] Everds NE, Snyder PW, Bailey KL, Bolon B, Creasy DM, Foley GL, Rosol TJ,Sellers T. Interpreting stress responses during routine toxicity studies: a review of the biology, impact, and assessment. Toxicol Pathol.2013; 41 (4):560-614.

A 2.11.14 Study 14

Comments of zRMS PL :	Confirmatory-like study which is under evaluation by the RMS for Zoxamide in an interzonal procedure, thus it was not evaluated in this assessment. The No-Observed-Adverse-Effect Level (NOAEL) of RH-150721 in 90-day oral dietary dose study with 28-day recovery period in Sprague Dawley rats was found to be 2000 ppm, which is equivalent to 111 mg/kg body weight/day in males and 134 mg/kg body weight/day in females.

Reference:	**KCP 7.4**
Report	xxx, 2020: RH-150721: 90-day oral dietary toxicity study and 28-day recovery period in Sprague Dawley Rats Gowan Crop Protection Ltd., UK xxx, Report No. U-19235, GLP, Not published
Guideline(s):	OECD 408 (2018) OPPTS 870.3100 (1998) ICH Harmonized Tripartite Guideline S3A (Toxicokinetics, 1994) Notification No. 12-Nousan-8147, MAFF, Japan (2000)
Deviations:	No
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test material (Lot/Batch No.)	RH-150721 (HHGCP004-00-3)
Purity:	99.9 % (w/w)
Vehicle:	Teklad Global 18% Protein Rodent Diet (meal) manufactured by Envigo
Stability:	Test material was stable for at least 2 days at room temperature.
Test organisms	Albino rat, Sprague Dawley, males and females purchased from Envigo, Netherlands
Age:	7-9 weeks
Body weight:	Males: 253.47 – 317.61 g Females: 177.08 – 205.74 g The weight variation of the animals at randomisation was within ± 20% of the mean weight for each sex.
Acclimation period:	5 days
Water:	Tap water, *ad libitum* (except overnight fasting prior to clinical pathology blood collection)
Diet:	Teklad Global 18% Protein Rodent Diet (meal) manufactured by Envigo

Housing:	2 per sex per cage in polycarbonate rat cages with bedding materials
Test design	
No. of groups:	6 (4 main groups + 2 recovery groups)
No. of animals/group:	10 rats/sex/group in main groups 6 rats/sex/group in recovery groups
Number of animals:	104 (52 Males + 52 Females)
Extra animals:	20 (10 Males + 10 Females)
Total number of animals:	124 (62 Males + 62 Females)
Environmental conditions	
Temperature:	19.1 – 23.7°C
Humidity:	Relative humidity ranged from 41 – 66 %
Air changes:	19 fresh air changes per hour
Photoperiod:	12-hour light/dark cycles
Test conentrations	670, 2000 and 6000 ppm in diet
Exposure time	90 days
Remarks	None

This study was designed to assess the potential sub-chronic toxicity of RH-150721 when fed in formulated diet to Sprague Dawley rats for 90 days. Rats were randomly assigned to 4 main groups (10 rats/sex/group) and 2 recovery groups (6 rats/sex/group), and – based on the results of a range-finding study - exposed to diet formulated with RH-150721 at 670, 2000 and 6000 ppm for 90 consecutive days, followed by a 28-day treatment-free period for the high dose group.

Males and females were randomised into 4 main groups (10 rats/sex/group), 2 recovery groups (6 rats/sex/group) using Pristima, based on body weight to control bias. RH-150721 was mixed with the diet and fed at concentration level of 670, 2000 and 6000 ppm for 90 days to Sprague Dawley rats. Care of animals compiled with the recommendations of Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC) and Committee for the Purpose of Control and Supervision of Experiments on Animals, Government of India. The principles of OECD Series on Testing and Assessment Document No. 19: Environment Directorate, Guidance Document on The Recognition, Assessment, and Use of Clinical Signs as Humane Endpoints for Experimental Animals Used in Safety Evaluation were followed.

Table A 16: Study design

Main Groups:

Test group	Concentration in diet (ppm)	Dose (mg/kg/day)		Animals assigned	
		Male	Female	Male	Female
1	0	0	0	10	10
2	670	38	44	10	10
3	2000	111	134	10	10
4	6000	335	404	10	10

Recovery Groups:

Test group	Concentration in diet (ppm)	Dose (mg/kg/day)		Animals assigned	
		Male	Female	Male	Female
1	0	0	0	6	6

2	6000	334	408	6	6

The required amount of test material was weighed and mixed with the diet using electric mixer. The test item was proofed to be stable in the diet for at least 2 days, and therefore diet was prepared every 2 days. Prepared diets were stored at room temperature before use. The concentration of the test substance in the feed were analysed on days 1, 31 and 63 of dosing period, and was verified.

The animals were observed for morbidity and mortality twice daily during study period and once on the day of terminal and recovery sacrifice. All rats were observed for clinical signs once daily during dosing period and recovery period. Detailed clinical examinations were done prior to initiation of treatment and weekly once thereafter all main and recovery group animals until scheduled necropsy. Detailed clinical examinations were not performed for the toxicokinetics groups.

Body weight were recorded for all animals on first day of treatment (say 1) and weekly thereafter. Terminal (fasting) body weights were measured on the days of scheduled necropsy (day 91 for main group and day 119 for recovery group).

Food input and food leftover was measured for all animals once in two days of start of treatment until scheduled sacrifice during dosing phase. Additional food leftover was measured on day 90 of dosing phase. For recovery group, food input and food leftover were measured once weekly during recovery phase.

Ophthalmological examination was performed using an ophthalmoscope once for all animals during acclimatization period and at the last week of dosing phase (day 88) for control and high dose groups. Mydriasis was induced before examination of eyes using a mydriatic agent.

Following examinations were conducted on Day 86 of the dosing phase in the control and test substance group. Neurological evaluation for individual animals, as applicable, were recorded in the form of scores as per test facility SOP.

The following neuromuscular observations were performed: 1. Grip strength, 2. Hind limb foot spray.

Rectal temperature was recorded in Fahrenheit (°F) using digital thermometer.

The following motor activity parameters were observed: 1. Distance travelled, 2. Horizontal counts, 3. Ambulatory counts, 4. Vertical counts.

Blood was collected from all animals of main groups at the end of dosing phase (day 91) and for recovery groups at the end of recovery phase (day 29) for haematology, coagulation and clinical chemistry evaluations. The animals were fasted overnight prior to terminal blood collection. Blood was collected via retro-orbital plexus puncture of right eye under mild isoflurane anaesthesia. The following haematology, coagulation and clinical chemistry parameters were measured: haematology – red blood cell count, haemoglobin concentration, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, platelet count, reticulocyte count, total white blood cell count, leukocyte differential (neutrophils, lymphocytes, monocytes, eosinophils and basophils); coagulation – active partial thromboplastin time, prothrombin time and fibrinogen; clinical chemistry – albumin/globulin ratio, alanine aminotransferase, albumin, alkaline phosphatase, aspartate aminotransferase, blood urea nitrogen, calcium, chloride, creatinine, creatinine kinase, gamma-glutamyl trans-peptidase, globulin, glucose, high-density lipoprotein, inorganic phosphorus, low-density lipoprotein, potassium, sodium, total bilirubin, total cholesterol, total protein, triglycerides and T3, T4 and TSH.

Urine was collected from all animals at the end of dosing period (day 91 of dosing phase) and at the end of recovery period (day 29 of recovery phase). During urine collection, animals were fasted overnight in metabolic cages. The following parameters were measured: volume, colour, appearance, glucose, bilirubin, ketone, blood, pH, protein, urobilinogen, nitrite, leukocyte and specific gravity.

Gross necropsy was conducted on all animals at the end of dosing phase for main groups (day 91; terminal sacrifice) and at the end of recovery phase of recovery group (day 29; recovery phase). The following organs were collected, weighed, assessed histopathologically after fixation in 10% neutral buffered formalin and staining with haematoxylin and eosin: adrenals, brain, epididymites, heart, kidneys, liver, ovaries, pituitary, prostate, seminal vesicles and coagulating glands, spleen, testes, thymus, thyroid with parathyroid and uterus with cervix. The other organs or tissues such as aorta, biceps femoris muscles, cecum, colon, diaphragm, duodenum, oesophagus, eyes, femur bone with joint, gastrocnemius muscle, gross changes, harderian glands, ileum with Peyer's patches, jejunum, lacrimal gland, lungs, lymph nodes (mandibular and mesenteric), mammary gland, optic nerve, oviducts, pancreas, Quadriceps muscle, rectum, salivary glands (mandibular, sublingual and parotid), sciatic nerve, skin (inguinal region), soleus muscle, spinal cord, sternum with marrow, stomach, tongue, trachea, urinary bladder and vagina, were also examined histopathologically. Histopathology was performed on all tissues of animals from control and high dose groups on main group. All gross changes in all animals were processed for histopathology and evaluated. Thyroid and parotid salivary gland were also evaluated from low dose, mid dose and recovery groups, because test substance-related microscopic changes were noted in the high dose group. Peer review was performed by another in-house pathologist.

For comparative statistics, data were evaluated using Levene's Test for homogeneity of variance, with significance at 5% level. Data determined to be homogeneous was evaluated for analysis of variance (ANOVA). When the ANOVA verified significance at 5% level, pairwise comparisons of each treated group with the control group was made using a Dunnett Test, to identify statistical differences (at 5% level). When the data were found to be non-homogeneous, ANOVA was done using suitable transformation. When the homogeneity tests were significant even after transformation, data were evaluated using a Kruskal-Wallis Test for group factor significance. When significance (at 5% level) existed between groups, Dunn's pairwise comparison was performed.

Results and discussions

There were no clinical signs were noticed in any of the treated groups during the study.

There was no mortality during the study.

In the 670 ppm group, no test substance-related changes were noticed in body weight and overall body weight (day 1-90) when compared to control group. In male 2000 ppm group, no test substance-related changes were noticed in body weight and overall body weight (day 1-90) when compared to control group. In female 2000 ppm group, statistically significant decrease in day 90 body weight (5%), was observed as compared to control group. In 6000 ppm group, statistically significant decrease in day 90 body weight (male: 11%; female: 11%) was observed. Similarly, statistically significant decrease in overall body weight gain (day 1-90) was also noticed in males (32%) and females (37%) in high dose group. In high dose recovery group, significant improvement in body weight gain (63%) was observed in males at the end of recovery phase. On recovery phase day 28, statistical decrease in body weight in female (8%) was noticed as compared to control recovery group. The changes in the body weight parameters at 6000 ppm group were considered test substance-related in both sexes and adverse in males and females. The changes noted in females at 2000 ppm were not considered adverse because the body weight decrease were <10% compared to controls.

In 670 and 2000 ppm group, no test substance-related changes were noticed in overall mean food consumption (day 1-90) when compared to control group. In 6000 ppm group, significant reduction was noticed in overall mean food consumption (day 1-90) in males (10%) and females (7%) when compared to control group. In high dose recovery group, the food consumption in recovery phase was comparable with that of control recovery group. The test substance intake achieved over the entire treatment period (day 1-90) for 670, 2000 and 6000 ppm concentration levels in diet was equivalent to 38, 111 and 335 mg/kg

body weight/day in males and 44, 134 and 404 mg/kg body weight/day in females, respectively. The test substance intake achieved over the entire treatment period (day 1-90) for high dose recovery group in diet was equivalent to 334 mg/kg body weight/day in males and 408 mg/kg body weight/day in females, respectively.

In 670 ppm and male 2000 ppm group, no test substance-related changes in overall food conversion efficiency (day 1-90) were noticed when compared with control group. In female 2000 ppm group, significant reduction (14%) was noticed in overall food conversion efficiency (day 1-90) as compared to control group. In 6000 ppm group, significant reduction was noticed in overall food conversion ratio (day 1-90) in males (25%) and female (32%) as compared to control group. In high dose recovery group, significant increase in food conversion efficiency (Recovery day 1-28) was noticed in males (69%) when compared with control group. The food conversion efficiency changes correlated with changes in body weight parameters and were considered test substance related. While decreases in males and females at 6000 ppm were considered adverse, decreases in 2000 ppm females were not considered adverse due to minimal nature of change.

No eye abnormalities were observed in ophthalmological examination performed towards the end of treatment period in males and females.

No test substance-related changes were observed in functional observation battery (FOB) parameters for main group animals as compared to control group.

No test substance-related changes were observed in grip strength and landing foot splay parameters as compared to control group.

No test substance-related changes were observed in rectal temperature as compared to control group.

No test substance-related changes were observed in motor activity parameters as compared to control group. Statistical significance increase (51%) in distance travelled was noticed in 670 ppm females as compared to control females. It was not considered test substance related as it occurred randomly in low dose females and lacked dose response relationship.

There were no test substance-related changes in blood coagulation parameters. In 6000 ppm group, minimal decrease in mean RBC and reticulocyte count in males, minimal decrease in haemoglobin and haematocrit in both sexes and mild decrease in eosinophil count in females were noticed. At recovery sacrifice, mean values of reticulocyte, haemoglobin and haematocrit were comparable with the concurrent control group. minimal decrease in mean RBC in both sexes and eosinophil count in females were noted at recovery sacrifice. These findings were considered likely related to persistence of lower magnitude of decreased food consumption and body weight during recovery phase. These minor changes were considered not toxicologically relevant due to their lower magnitude of change.

In 6000 ppm group, minimal increase in mean serum ALP, minimal decrease in total protein, albumin, globulin were noticed at terminal sacrifice. In 2000 and 6000 ppm group, minimal decrease in BUN, total cholesterol, HDL in males were noticed at terminal sacrifice. At the end of recovery period, changes in ALP, total cholesterol and HDL persisted, however at a lower magnitude compared to terminal sacrifice indicating trend towards recovery. Other changes in clinical chemistry parameters noted at terminal and recovery sacrifice were considered related to decreased food consumption (Levin et al., 1993[14]).

A dose-dependent minimal to mild decrease in mean serum T4 levels was noted at ≥ 2000 ppm at the terminal sacrifice in both sexes. Change in T4 was completely recovered to concurrent control levels at the end of recovery period. Magnitude of decrease in T4 at 2000 ppm was minimal (males 17%, females 21%) and mean values were within historical control data range. Mean value of T4 at 6000 ppm were

[14] Levin S, Semler D, Ruben Z. Effects of two weeks of feed restriction on some common toxicologic parameters in Sprague-Dawley rats. Toxicol Pathol.1993; 21 (1):1-14.

outside historical control data range. Only effects at 6000 ppm were associated with the microscopic findings of minimal to mild colloid alteration in the thyroid follicle. Decrease in T4 occurred in isolation without concurrent changes in T3 or TSH levels and there were no morphological changes in the thyroid follicular cells. At the terminal sacrifice, there was minimal decrease in the thyroid weights in females at 6000 ppm, which was considered likely due to decreased food consumption and body weight. Thyroid weights were recovered at the end of recovery period.

There were no test substance-related changes in urinalysis parameters.

At the terminal sacrifice, decrease in organ weights (absolute, relative to brain weight and/or body weight ratios) were noted in multiple organs (males: liver and heart; Females: ovaries, pituitary, thyroid with parathyroid, heart and kidney) in 6000 ppm group. All these changes in organ weights were without any microscopic correlate and were recovered at the end of recovery period except minimal decrease in liver weights in males. These changes in organ weights were considered related to decrease in body weight and food consumption (Levin et al., 1993, Moriyama et al., 2008[15]). Decreased thyroid with parathyroid weight was limited to females and completely recovered in the recovery period. Therefore, decreased thyroid with parathyroid weight was considered more likely related to decreased food consumption and body weight than direct test substance related effect.

There were no test substance-related gross changes at the terminal and recovery sacrifice. At terminal sacrifice, microscopic findings of minimal to mild colloid alteration in the thyroid follicle were observed at 6000 ppm. Colloid alteration was present without any other related morphological changes like hypertrophy or hyperplasia of follicles. Incidence and severity of colloid alteration was likely related to decrease in T4 levels as this change can occur secondary to rapid turnover of thyroid hormones (Baiocco et al., 2018[16]). This microscopic change was not present at 670 and 2000 ppm groups and was not observed at the end of recovery period indicating complete recovery. Acinar cell hypertrophy of minimal to mild severity was noted in parotid salivary glands in 6000 ppm at the terminal sacrifice. Hypertrophy was diffuse in distribution and characterized by increased acinar cell size with increased secretary material and basophilic cytoplasm. There was no evidence of cellular injury like degeneration or necrosis in parotid salivary gland and no increased salivation observation during in-life phase of the study. These microscopic changes were completely recovered at the end of recovery period. This microscopic finding was considered as adaptive change (Greim et al., 2015[17], Inoue et al., 2014[18]).

Table A 17: Body weights (g)

Group #		Dosing Phase							
		Day: 1	Day: 7	Day: 15	Day: 21	Day: 29	Day: 35	Day: 43	Day: 49
		Session 1	Session 1	Session 1	Session 1	Session 1	Session 1	Session 1	Session 1
Males									
Control	n	10	10	10	10	10	10	10	10
	Means	284.87	314.49	340.23	356.96	375.68	387.46	402.38	410.36
	SD	16.69	15.03	18.08	17.72	20.59	18.64	19.90	21.78

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2	n	10	10	10	10	10	10	10	10
	Means	290.53	313.52	338.76	353.77	370.29	379.07	390.71	395.79
	SD	16.28	15.49	18.57	19.78	370.29	21.63	21.34	23.04
3	n	10	10	10	10	10	10	10	10
	Means	289.97	311.84	339.00	355.15	370.69	380.51	393.16	402.58
	SD	17.05	15.15	15.72	17.63	20.63	21.14	23.77	25.50
4	n	10	10	10	10	10	10	10	10
	Means	288.09	**293.03-**	325.47	**332.75-**	**344.42-**	**352.47-**	**363.10-**	**366.26-**
	SD	16.37	14.01	17.85	16.30	16.30	16.42	16.51	18.52
Females									
Control	n	10	10	10	10	10	10	10	10
	Means	189.93	204.19	217.88	227.39	237.12	241.20	248.57	251.77
	SD	8.50	9.24	9.11	13.90	10.90	12.39	13.37	13.62
2	n	10	10	10	10	10	10	10	10
	Means	192.20	206.06	224.07	229.43	237.94	244.57	250.37	250.35
	SD	7.17	7.91	10.65	10.92	12.76	14.53	13.89	12.90
3	n	10	10	10	10	10	10	10	10
	Means	189.27	200.78	214.80	220.35	225.64	231.58	**234.63-**	**238.73-**
	SD	9.39	7.74	9.63	10.63	9.88	9.35	10.44	9.52
4	n	10	10	10	10	10	10	10	10
	Means	190.00	**190.16-**	210.58	**214.56-**	**217.97-**	**220.73-**	**225.16-**	**231.47-**
	SD	8.55	7.95	9.86	9.36	9.02	7.69	10.70	6.97

Group #: Control: G1 (0 ppm); 2: G2 (670 ppm); 3: G3 (2000 ppm); 4: G4 (6000 ppm)
-: Significantly lower than the control group at 5% level.

Table A 18: Body Weights (g) (continued)

Group #		**Dosing Phase**					
		Day: 57	**Day: 63**	**Day: 71**	**Day: 77**	**Day: 85**	**Day: 90**
		Session 1	**Session 1**	**Session 1**	**Session 1**	**Session 1**	**Session 1**
Males							
Control	n	10	10	10	10	10	10
	Means	422.61	428.00	434.57	441.53	446.11	449.02
	SD	25.20	25.38	24.32	26.30	26.14	27.95
2	n	10	10	10	10	10	10
	Means	411.18	415.51	423.72	429.11	432.45	436.61
	SD	22.38	22.93	24.98	27.16	27.87	25.09
3	n	10	10	10	10	10	10
	Means	414.41	420.70	430.18	433.55	437.13	440.70
	SD	24.68	26.37	26.30	28.21	28.98	30.15
5	n	10	10	10	10	10	10
	Means	**382.99-**	**386.27-**	**392.34-**	**396.19-**	**398.62-**	**400.04-**
	SD	19.03	19.59	18.68	18.54	17.41	18.55
Females							
Control	n	10	10	10	10	10	10
	Means	258.54	261.96	264.71	265.05	270.36	271.40
	SD	14.42	15.54	16.01	15.28	19.09	16.51
2	n	10	10	10	10	10	10

	Means	256.64	258.48	262.39	263.35	265.51	267.97
	SD	12.22	13.24	11.94	11.24	10.36	10.22
3	n	10	10	10	10	10	10
	Means	**245.40-**	249.95	253.75	255.32	256.42	**256.51-**
	SD	9.36	11.82	10.62	9.66	12.65	12.32
4	n	10	10	10	10	10	10
	Means	**239.98-**	**242.01-**	**242.47-**	**242.51-**	**240.52-**	**241.31-**
	SD	7.78	8.44	9.06	8.90	6.99	7.80

Group #: Control: G1 (0 ppm); 2: G2 (16000 ppm)
There were no statistically significant differences at 5% level

Table A 19: Body weight change (g)

Group #		D:1 – D:7	D:7 - D:15	D:15 – D:21	D:21 – D:29	D:29 – D:35	D:35 – D:43	D43 – D:49	D:59 – D:57
Males									
Control	n	10	10	10	10	10	10	10	10
	Means	29.62	25.74	16.73	18.72	11.78	14.92	7.97	12.25
	SD	9.96	5.78	5.01	5.79	4.01	2.89	4.41	5.39
2	n	10	10	10	10	10	10	10	10
	Means	22.99	25.24	15.01	16.52	8.78	11.65	5.07	15.39
	SD	8.92	7.11	3.16	3.93	3.92	2.44	3.29	4.20
3	n	10	10	10	10	10	10	10	10
	Means	21.87	27.16	16.15	15.54	9.82	12.65	9.42	11.83
	SD	5.95	4.72	3.04	5.59	3.66	4.51	3.04	3.34
4	n	10	10	10	10	10	10	10	10
	Means	**4.95-**	32.43	**7.28-**	**11.67-**	8.05	10.63	**3.16-**	16.73
	SD	6.08	11.95	4.94	5.47	2.95	4.78	4.64	4.00
Females									
Control	n	10	10	10	10	10	10	10	10
	Means	14.26	13.69	9.51	9.73	4.09	7.36	3.20	6.77
	SD	5.87	2.39	5.16	4.49	2.52	3.51	3.12	6.95
2	n	10	10	10	10	10	10	10	10
	Means	13.86	18.02	5.35	8.51	6.63	5.80	-0.02	6.29
	SD	6.28	6.52	4.37	4.13	6.13	4.41	2.39	4.99
3	n	10	10	10	10	10	10	10	10
	Means	11.51	14.02	5.55	**5.28-**	5.94	3.06	4.10	6.67
	SD	3.23	3.86	3.79	3.38	3.79	4.40	3.48	3.21
4	n	10	10	10	10	10	10	10	10
	Means	**0.15-**	**20.42+**	**3.98-**	**3.41-**	2.76	4.43	6.31	8.51
	SD	3.91	5.09	2.78	3.30	2.14	5.35	5.64	6.42

Group #: Control: G1 (0 ppm); 2: G2 (670 ppm); 3: G3 (2000 ppm); 4: G4 (6000 ppm)
-: Significantly lower than the control group at 5% level

Table A 20: Body weight change (g) (continued)

Group #		D:57-D:63	D:63-D:71	D:71-D:77	D:77-D:85	D:85-D:90	D:1-D:90
Males							
Control	n	10	10	10	10	10	10
	Means	5.39	6.58	6.95	4.58	2.91	164.15
	SD	2.01	4.19	3.49	3.53	3.27	25.87
2	n	10	10	10	10	10	10
	Means	4.34	8.21	5.39	3.34	4.16	146.07
	SD	1.95	4.10	3.87	4.91	5.22	23.37
3	n	10	10	10	10	10	10
	Means	6.29	9.48	3.37	3.58	3.57	150.73
	SD	5.86	3.10	3.46	5.18	4.03	26.25
4	n	10	10	10	10	10	10
	Means	3.28	6.07	3.85	2.43	1.42	**111.95-**
	SD	3.40	4.80	4.22	7.22	4.17	21.23
Female							
Control	n	10	10	10	10	10	10
	Means	3.42	2.75	0.34	5.31	1.04	81.47
	SD	3.83	1.96	4.41	8.17	3.72	11.66
2	n	10	10	10	10	10	10
	Means	1.84	3.92	0.96	2.16	2.46	75.77
	SD	3.40	2.50	1.26	4.39	3.04	5.67
3	n	10	10	10	10	10	10
	Means	4.55	3.81	1.56	1.11	0.09	67.24
	SD	4.13	2.46	1.89	6.80	3.80	10.49
4	n	10	10	10	10	10	10
	Means	2.02	0.47	0.04	-1.99	0.79	**51.30-**
	SD	6.42	2.29	1.70	3.49	2.59	5.78

Group #: Control: G1 (0 ppm); 2: G2 (670 ppm); 3: G3 (2000 ppm); 4: G4 (6000 ppm)
-: Significantly lower than the control group at 5% level

Table A 21: Test item intake (mg/kg/day)

Group #		Day No.						
		1-7	**7-15**	**15-21**	**21-29**	**29-35**	**35-43**	**43-49**
Males								
G2 670	Mean	45.33	42.08	41.21	39.38	39.10	37.85	37.31
	SD	2.41	2.38	2.44	2.21	2.31	1.88	2.08
	n	10	10	10	10	10	10	10
G3 2000	Mean	135.55	125.88	123.53	118.14	115.53	109.98	108.90
	SD	6.81	5.01	4.99	6.32	4.61	5.40	7.28
	n	10	10	10	10	10	10	10
G4 6000	Mean	357.32	379.26	383.45	371.63	357.35	337.21	334.98
	SD	31.64	19.22	19.82	17.07	15.45	14.67	19.60
	n	10	10	10	10	10	10	10
G4R 6000	Mean	340.71	385.56	372.74	371.56	360.20	333.48	327.98
	SD	14.02	8.27	15.91	12.07	14.22	17.56	18.20
	n	6	6	6	6	6	6	6
Females								
G2 670	Mean	50.98	47.92	47.84	45.81	45.70	43.59	44.21
	SD	2.08	1.40	1.96	1.50	2.18	2.68	2.97
	n	10	10	10	10	10	10	10
G3 2000	Mean	154.88	147.80	148.05	144.23	143.63	137.63	128.59
	SD	6.52	6.10	7.68	7.41	7.24	9.98	5.40
	n	10	10	10	10	10	10	10
G4 6000	Mean	384.94	463.17	439.07	448.93	450.42	428.16	398.53
	SD	31.98	28.65	21.91	17.64	16.46	15.87	13.12
	n	10	10	10	10	10	10	10
G4R 6000	Mean	377.73	450.12	443.87	459.52	458.13	423.31	406.92
	SD	13.67	23.85	31.05	18.83	19.84	21.19	13.60
	n	6	6	6	6	6	6	6

Table A 22: Test item intake (mg/kg/day) (continued)

Group #		Day No.					
		57-63	**63-71**	**71-77**	**77-85**	**85-90**	**1-90**
Males							
G2 670	Mean	36.04	34.84	33.88	33.76	34.04	37.75
	SD	1.83	1.88	2.20	1.92	1.98	1.93
	n	10	10	10	10	10	10
G3 2000	Mean	103.03	101.50	98.63	99.06	97.77	110.74
	SD	6.34	5.32	5.88	6.75	5.69	5.27
	n	10	6	6	6	6	6
G4 6000	Mean	314.90	307.51	303.91	310.15	297.00	334.85
	SD	19.48	14.83	14.19	11.77	17.91	13.78
	n	10	10	10	10	10	10
G4R 6000	Mean	315.15	308.39	311.05	306.62	301.98	333.90
	SD	18.20	20.10	16.81	20.07	21.03	12.51
	n	6	6	6	6	6	6
Females							
G2 670	Mean	41.98	41.52	40.77	40.88	40.65	44.23
	SD	2.68	1.94	1.81	1.17	1.42	1.69
	n	10	10	10	10	10	10
G3 2000	Mean	124.91	121.77	120.70	121.84	117.54	134.14
	SD	6.90	5.20	5.43	5.42	5.02	4.80
	n	10	10	10	10	10	10
G4 6000	Mean	375.05	368.27	373.76	373.13	366.77	403.85
	SD	14.31	15.82	13.30	11.93	12.96	13.47
	n	10	10	10	10	10	10
G4R 6000	Mean	377.72	377.37	386.14	378.22	377.48	408.01
	SD	15.95	18.66	18.31	27.15	18.06	14.95
	N	6	6	6	6	6	6

Conclusion

Based on the results, the No-Observed-Adverse-Effect-Level (NOAEL) of RH-150721 in the Sprague Dawley rats is 2000 ppm, which is equivalent to 111 mg/kg body weight/day in males and 134 mg/kg body weight/day in females. This NOAEL is based on reduction in food consumption and food efficiency, triggering changes in bodyweight, hematological and clinical chemistry parameters and multiple organ weights as a result thereof at the Lowest-Observed-Adverse-Effect-Level (LOAEL) of 6000 ppm in males and females.

Effects at 6000 ppm were partially recovered at recovery. A dose-dependent minimal to mild decrease in mean serum T4 levels was noted at ≥ 2000 ppm at the terminal sacrifice in both sexes, which were within historical control at 2000 ppm and associated with the microscopic finding of minimal to mild colloid alteration in the thyroid follicle only at 6000 ppm. Decreases in T4 and microscopic findings in the thyroid were completely recovered at the end of recovery period.

(xxx 2020)

A 2.11.15 Study 15

Comments of zRMS PL:	Confirmatory-like study which is under evaluation by the RMS for Zoxamide in an interzonal procedure, thus it was not evaluated in this assessment. Noting that 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 while setting ADI for dietary risk assessment.

Reference:	**KCP 7.4**
Report	Strupp, Ch., 2020: Zoxamide Metabolite RH-150721 - Proposal of ADI setting for dietary risk assessment Gowan Crop Protection Ltd., UK, Report No. CS13072020, No GLP, Not published
Guideline(s):	None.
Deviations:	No
Acceptability:	Yes
Duplication (if vertebrate study)	No

Discussion and results

Sprague Dawley rats (6/sex/group) were exposed to RH-150721 via the diet for 14 days at dietary levels of 0, 1000, 5000 and 16000 ppm. Observations included body weight, clinical signs, hematology, clinical chemistry (blood and urine), gross pathology, organ weights and histopathology.
The test material was described as having a strong odour. The top dose was not palatable, and had to be reduced to 8000 ppm after the first week. Achieved doses were:

Dietary inclusion level	**1000 ppm**	**5000 ppm**	**8000 ppm** (days 8-14)	**16000 ppm** (days 1-7)
Males	66 mg/kg bw/d	334 mg/kg bw/d	618 mg/kg bw/d	911 mg/kg bw/d
Females	77 mg/kg bw/d	382 mg/kg bw/d	664 mg/kg bw/d	1051 mg/kg bw/d

Blood levels of RH-150721 could not be measured, as the metabolism was too quick. There were no mortalities. Clinical signs were limited to thin appearance of females at 16000 ppm, which recovered after reduction of the top dose. Body weight and food consumption was reduced in the mid and high dose group, reaching statistical significance in males at the mid dose. There were no significant effects on hematology or urine analysis. Clinical chemistry effects were limited to the high dose and most likely linked to the strongly reduced food consumption in the initial phase of the study (BUN↓, total protein↓, albumin↓, globulin↓, phosphorus↓; ALT↑, ALP↑). Several organ weights were slightly reduced vs. control, which was judged secondary to the low food consumption. The only histopathological finding that was not judged to be related to low food consumption was acinar hypertrophy of the salivary glands, a finding described before on other compounds as an adaptive change.

In a 90-day dietary study in rats with 28 days recovery, Sprague Dawley rats (10/sex/group) were exposed to RH-150721 via the diet for 90 days at dietary levels of 0, 670, 2000 and 6000 ppm. In parallel, recovery groups (6/sex/group) were exposed to control and low dose, followed by a 28-day treatment free period to judge on adversity of effects. Observations included body weight, clinical signs, opthalmoscopy, functional observation battery (FOB), hematology, clinical chemistry (blood and urine), gross pathology, organ weights and histopathology. Achieved doses were:

Dietary inclusion level	670 ppm	2000 ppm	6000 ppm
Males	38 mg/kg bw/d	111 mg/kg bw/d	335 mg/kg bw/d (recovery: 334)
Females	44 mg/kg bw/d	134 mg/kg bw/d	404 mg/kg bw/d (recovery: 408)

There were no mortalities, clinical signs, opthalmoscopic changes, FOB effects or gross pathology findings. Body weight and food consumption were reduced in the high dose group, reaching statistical significance. During recovery, body weights improved but not to the level of control. Hematology effects were limited to the top dose, and mainly consisted of rather mild decreases of red blood cells, hemoglobin and hematocrit with increased MCV and MCH; RBC, MCH and MCV were not fully recovered after recovery. Due to the low magnitude, effects were not judged as adverse in nature. Clinical chemistry effects were identical to the effects seen in the 14-d study, correlate with the body weight, and were not considered adverse. Thyroid weights were decreased in the top dose, and T4 levels at the mid and top dose. The effects were considered adverse in the top dose as they correlated with minimal to mild colloid alteration in males only; effects on T4 were completely recovered to control level in both sexes after recovery period. Effects in the mid dose were not considered adverse due to the low magnitude and the absence of a histopathological correlate. Multiple organ weights were reduced at end of treatment, correlated with body weight, and recovered during recovery period; they were not considered adverse. Liver weights were minimally reduced at the end of recovery, however without a histopathological correlation and thus judged as being not adverse. Minimal to mild acinar hypertrophy of the salivary glands was found at the high dose, considered adaptive, but related to treatment. The NOALE was set at 2000 ppm (111 mg/kg bw/d in males) based on reductions in body weight and food consumption, colloid changes in the thyroid and acinar hypertrophy of the salivary glands at the next higher dose level.

Conclusion

The toxicological profile of RH-150721 is mainly driven by body weight and food consumption effects, most other effects are secondary in nature or adaptive. The NOAEL of the 90-d 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. This proposal is based on the REACH guidance[19] .

(Strupp Ch. 2020)

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

Appendix 3 Exposure calculations

A 3.1 Operator exposure calculations (KCP 7.2.1.1)

A 3.1.1 Calculations for zoxamide

Table A 23: Estimation of operator exposure using EFSA model; grapes, tractor-mounted air-blast sprayer, upwards; 3x 0.45 kg product/ha in 200 L/ha water at 7 days interval

Application rate of active substance	0.1485	kg a.s./ha	*i_AppRate*
Assumed area treated	10	ha/day	*d_AreaTreated*
Amount of active substance applied	1.485	kg a.s./day	*i_AmoutAS*
Dermal absorption of the product	0.21%		*i_AbsorpProduct*
Dermal absorption of in-use dilution	5.90%		*i_AbsorInuse*
Formulation type	Wettable granules, soluble granules		
Indoor or Outdoor application	Outdoor		
Application method	Upward spraying		
Application equipment	Vehicle-mounted		
Season	not relevant		

Outdoor|Wettable granules, soluble granules|Upward spraying|Vehicle-mounted

	Exposure values	µg exposure/day mixed and loaded 75th centile	95th centile	Reference	Comment
Mixing and loading	Hands	1777	8506	AOEM	
	Body	1631	18027	AOEM	
	Head	10	133	AOEM	
	Protected hands (gloves)	21	47	AOEM	
	Protected body (workwear or protective garment and sturdy footwear)	26	92	AOEM	
	Protected head (hood and face shield)	0	8	AOEM	
	Inhalation	42	262	AOEM	
	Protective Equipment	Select for inclusion		Penetration factor	Inhalation Protection factor
	Gloves	No			
	Clothing	Work wear - arms, body and legs covered		Incl. in AOEM model	
	Head and respiratory PPE	None		1	1
	Water soluble bag	No		1	

	Exposure values	µg exposure/day applied 75th centile	95th centile	Reference	Comment
Application	Hands	3583	9262	AOEM	No data available for a drift reduction scenario
	Body	13085	76353	AOEM	
	Head	1720	10554	AOEM	
	Protected hands (gloves)	52	1365	AOEM	
	Protected body (workwear or protective garment and sturdy footwear)	171	334	AOEM	
	Inhalation	80	123	AOEM	
	Protective Equipment	Select for inclusion		Penetration factor	Inhalation Protection factor
	Gloves	No			
	Clothing	Work wear - arms, body and legs covered		Incl. in AOEM model	
	Head and respiratory PPE	None		1	1
	Closed cab	No		vehicle mounted upward spraying only	

Operator Model	Mixing, loading and application AOEM			
Potential exposure	Longer term systemic exposure mg/kg bw/day	0.0202	% of RVNAS	6.74%
	Acute systemic exposure mg/kg bw/day	0.1019	% of RVAAS	
Mixing and Loading	Gloves = No	Clothing = Work wear - arms, body and legs covered	RPE = None	Soluble bags = No
Application	Gloves = No	Clothing = Work wear - arms, body and legs covered	RPE = None	Closed cabin = No
Exposure (including PPE options above)	Longer term systemic exposure mg/kg bw/day	0.0075	% of RVNAS	2.49%
	Acute systemic exposure mg/kg bw/day	0.0265	% of RVAAS	

Table A 24: Estimation of operator exposure using EFSA model; grapes, tractor-mounted air-blast sprayer, upwards; 3x 0.45 kg product/ha in 200 L/ha water at 7 days interval plus PPE

Application rate of active substance	0.1485	kg a.s./ha	*i_AppRate*
Assumed area treated	10	ha/day	*d_AreaTreated*
Amount of active substance applied	1.485	kg a.s./day	*i_AmoutAS*
Dermal absorption of the product	0.21%		*i_AbsorpProduct*
Dermal absorption of in-use dilution	5.90%		*i_AbsorInuse*
Formulation type	Wettable granules, soluble granules		
Indoor or Outdoor application	Outdoor		
Application method	Upward spraying		
Application equipment	Vehicle-mounted		
Season	not relevant		

OutdoorWettable granules, soluble granulesUpward sprayingVehicle-mounted

	Exposure values	µg exposure/day mixed and loaded 75th centile	95th centile	Reference	Comment
Mixing and loading	Hands	1777	8506	AOEM	
	Body	1631	18027	AOEM	
	Head	10	133	AOEM	
	Protected hands (gloves)	21	47	AOEM	
	Protected body (workwear or protective garment and sturdy footwear)	26	92	AOEM	
	Protected head (hood and face shield)	0	8	AOEM	
	Inhalation	42	262	AOEM	
	Protective Equipment	Select for inclusion		Penetration factor	Inhalation Protection factor
	Gloves	Yes		Incl. in AOEM model	
	Clothing	Work wear - arms, body and legs covered		Incl. in AOEM model	
	Head and respiratory PPE	None		1	1
	Water soluble bag	No		1	

	Exposure values	µg exposure/day applied 75th centile	95th centile	Reference	Comment
Application	Hands	3583	9262	AOEM	No data available for a drift reduction scenario
	Body	13085	76353	AOEM	
	Head	1720	10554	AOEM	
	Protected hands (gloves)	52	1365	AOEM	
	Protected body (workwear or protective garment and sturdy footwear)	171	334	AOEM	
	Inhalation	80	123	AOEM	
	Protective Equipment	Select for inclusion		Penetration factor	Inhalation Protection factor
	Gloves	Yes		Incl. in AOEM model	
	Clothing	Work wear - arms, body and legs covered		Incl. in AOEM model	
	Head and respiratory PPE	Hood and visor		0.05	1
	Closed cab	No		vehicle mounted upward spraying only	

Operator Model		Mixing, loading and application AOEM		
Potential exposure	Longer term systemic exposure mg/kg bw/day	0.0202	% of RVNAS	6.74%
	Acute systemic exposure mg/kg bw/day	0.1019	% of RVAAS	
Mixing and Loading	Gloves = Yes	Clothing = Work wear - arms, body and legs covered	RPE = None	Soluble bags = No
Application	Gloves = Yes	Clothing = Work wear - arms, body and legs covered	RPE = Hood and visor	Closed cabin = No
Exposure (including PPE options above)	Longer term systemic exposure mg/kg bw/day	0.0023	% of RVNAS	0.78%
	Acute systemic exposure mg/kg bw/day	0.0086	% of RVAAS	

Table A 25: Estimation of operator exposure using EFSA model; grapes, manual, hand-held; 3x 0.45 kg product/ha in 200 L/ha water at 7 days interval

Application rate of active substance	0.1485	kg a.s./ha	*i_AppRate*
Assumed area treated	4	ha/day	*d_AreaTreated*
Amount of active substance applied	0.594	kg a.s./day	*i_AmoutAS*
Dermal absorption of the product	0.21%		*i_AbsorpProduct*
Dermal absorption of in-use dilution	5.90%		*i_AbsorInuse*
Formulation type	Wettable granules, soluble granules		
Indoor or Outdoor application	Outdoor		
Application method	Upward spraying		
Application equipment	Manual-Hand held		
Season	not relevant		

OutdoorWettable granules, soluble granulesUpward sprayingManual-Hand held

	Exposure values	µg exposure/day mixed and loaded		Reference	Comment
		75th centile	95th centile		
Mixing and loading	Hands	878	4167	AOEM	
	Body	856	13814	AOEM	
	Head	4	53	AOEM	
	Protected hands (gloves)	12	19	AOEM	
	Protected body (workwear or protective garment and sturdy footwear)	12	37	AOEM	
	Protected head (hood and face shield)	0	3	AOEM	
	Inhalation	32	256	AOEM	
	Protective Equipment	Select for inclusion		Penetration factor	Inhalation Protection factor
	Gloves	No			
	Clothing	Work wear - arms, body and legs covered		Incl. in AOEM model	
	Head and respiratory PPE	None		1	1
	Water soluble bag	No		1	

	Exposure values	µg exposure/day applied		Reference	Comment
		75th centile	95th centile		
Application	Hands	1760	5908	AOEM	No data available for a drift reduction scenario
	Body	56650	177878	AOEM	
	Head	129	693	AOEM	
	Protected hands (gloves)	14	74	AOEM	
	Protected body (workwear or protective garment and sturdy footwear)	1033	1938	AOEM	
	Inhalation	52	134	AOEM	
	Protective Equipment	Select for inclusion		Penetration factor	Inhalation Protection factor
	Gloves	No			
	Clothing	Work wear - arms, body and legs covered		Incl. in AOEM model	
	Head and respiratory PPE	None		1	1
	Closed cab	No		vehicle mounted upward spraying only	

Operator Model	Mixing, loading and application AOEM			
Potential exposure	Longer term systemic exposure mg/kg bw/day	0.0590	% of RVNAS	19.68%
	Acute systemic exposure mg/kg bw/day	0.1885	% of RVAAS	
Mixing and Loading	Gloves = No	Clothing = Work wear - arms, body and legs covered	RPE = None	Soluble bags = No
Application	Gloves = No	Clothing = Work wear - arms, body and legs covered	RPE = None	Closed cabin = No
Exposure (including PPE options above)	Longer term systemic exposure mg/kg bw/day	0.0043	% of RVNAS	1.44%
	Acute systemic exposure mg/kg bw/day	0.0150	% of RVAAS	

Table A 26: Estimation of operator exposure using EFSA model; grapes, manual, hand-held; 3x 0.45 kg product/ha in 200 L/ha water at 7 days interval plus PPE

Application rate of active substance	0.1485	kg a.s./ha	*i_AppRate*
Assumed area treated	4	ha/day	*d_AreaTreated*
Amount of active substance applied	0.594	kg a.s./day	*i_AmoutAS*
Dermal absorption of the product	0.21%		*i_AbsorpProduct*
Dermal absorption of in-use dilution	5.90%		*i_AbsorInuse*
Formulation type	Wettable granules, soluble granules		
Indoor or Outdoor application	Outdoor		
Application method	Upward spraying		
Application equipment	Manual-Hand held		
Season	not relevant		

OutdoorWettable granules, soluble granulesUpward sprayingManual-Hand held

	Exposure values	µg exposure/day mixed and loaded 75th centile	95th centile	Reference	Comment
Mixing and loading	Hands	878	4167	AOEM	
	Body	856	13814	AOEM	
	Head	4	53	AOEM	
	Protected hands (gloves)	12	19	AOEM	
	Protected body (workwear or protective garment and sturdy footwear)	12	37	AOEM	
	Protected head (hood and face shield)	0	3	AOEM	
	Inhalation	32	256	AOEM	
	Protective Equipment	Select for inclusion		Penetration factor	Inhalation Protection factor
	Gloves	Yes		Incl. in AOEM model	
	Clothing	Work wear - arms, body and legs covered		Incl. in AOEM model	
	Head and respiratory PPE	None		1	1
	Water soluble bag	No		1	

	Exposure values	µg exposure/day applied 75th centile	95th centile	Reference	Comment
Application	Hands	1760	5908	AOEM	No data available for a drift reduction scenario
	Body	56650	177878	AOEM	
	Head	129	693	AOEM	
	Protected hands (gloves)	14	74	AOEM	
	Protected body (workwear or protective garment and sturdy footwear)	1033	1938	AOEM	
	Inhalation	52	134	AOEM	
	Protective Equipment	Select for inclusion		Penetration factor	Inhalation Protection factor
	Gloves	Yes		Incl. in AOEM model	
	Clothing	Work wear - arms, body and legs covered		Incl. in AOEM model	
	Head and respiratory PPE	None		1	1
	Closed cab	No		vehicle mounted upward spraying only	

Operator Model		Mixing, loading and application AOEM			
Potential exposure	Longer term systemic exposure mg/kg bw/day		0.0590	% of RVNAS	19.68%
	Acute systemic exposure mg/kg bw/day		0.1885	% of RVAAS	
Mixing and Loading		Gloves = Yes	Clothing = Work wear - arms, body and legs covered	RPE = None	Soluble bags = No
Application		Gloves = Yes	Clothing = Work wear - arms, body and legs covered	RPE = None	Closed cabin = No
Exposure (including PPE options above)	Longer term systemic exposure mg/kg bw/day		0.0026	% of RVNAS	0.85%
	Acute systemic exposure mg/kg bw/day		0.0092	% of RVAAS	

Table A 27: Estimation of operator exposure using EFSA model; grapes, manual, Knapsack; 3x 0.45 kg product/ha in 200 L/ha water at 7 days interval interval

Application rate of active substance	0.1485	kg a.s./ha	*i_AppRate*
Assumed area treated	1	ha/day	*d_AreaTreated*
Amount of active substance applied	0.1485	kg a.s./day	*i_AmoutAS*
Dermal absorption of the product	0.21%		*i_AbsorpProduct*
Dermal absorption of in-use dilution	5.90%		*i_AbsorInuse*
Formulation type	Wettable granules, soluble granules		
Indoor or Outdoor application	Outdoor		
Application method	Upward spraying		
Application equipment	Manual-Knapsack		
Season	not relevant		

OutdoorWettable granules, soluble granulesUpward sprayingManual-Knapsack

	Exposure values	µg exposure/day mixed and loaded		Reference	Comment
		75th centile	95th centile		
Mixing and loading	Hands	9495	25482	AOEM	
	Body	803	2787	AOEM	
	Head	5	11	AOEM	
	Protected hands (gloves)	18	164	AOEM	
	Protected body (workwear or protective garment and sturdy footwear)	25	103	AOEM	
	Protected head (hood and face shield)	5	11	AOEM	
	Inhalation	25	26	AOEM	
	Protective Equipment	Select for inclusion		Penetration factor	Inhalation Protection factor
	Gloves	No			
	Clothing	Work wear - arms, body and legs covered		Incl. in AOEM model	
	Head and respiratory PPE	None		1	1
	Water soluble bag	No		1	

	Exposure values	µg exposure/day applied		Reference	Comment
		75th centile	95th centile		
Application	Hands	552	2046	AOEM	No data available for a drift reduction scenario
	Body	45600	175585	AOEM	
	Head	83	440	AOEM	
	Protected hands (gloves)	4	18	AOEM	
	Protected body (workwear or protective garment and sturdy footwear)	1033	1938	AOEM	
	Inhalation	17	59	AOEM	
	Protective Equipment	Select for inclusion		Penetration factor	Inhalation Protection factor
	Gloves	No			
	Clothing	Work wear - arms, body and legs covered		Incl. in AOEM model	
	Head and respiratory PPE	None		1	1
	Closed cab	No		vehicle mounted upward spraying only	

Operator Model		Mixing, loading and application AOEM			
Potential exposure	Longer term systemic exposure mg/kg bw/day		0.0465	% of RVNAS	15.51%
	Acute systemic exposure mg/kg bw/day		0.1775	% of RVAAS	
Mixing and Loading		Gloves = No	Clothing = Work wear - arms, body and legs covered	RPE = None	Soluble bags = No
Application		Gloves = No	Clothing = Work wear - arms, body and legs covered	RPE = None	Closed cabin = No
Exposure (including PPE options above)	Longer term systemic exposure mg/kg bw/day		0.0027	% of RVNAS	0.89%
	Acute systemic exposure mg/kg bw/day		0.0067	% of RVAAS	

Table A 28: Estimation of operator exposure using EFSA model; grapes, manual, Knapsack; 3x 0.45 kg product/ha in 200 L/ha water at 7 days interval interval plus PPE

Application rate of active substance	0.1485	kg a.s./ha	*i_AppRate*
Assumed area treated	1	ha/day	*d_AreaTreated*
Amount of active substance applied	0.1485	kg a.s./day	*i_AmoutAS*
Dermal absorption of the product	0.21%		*i_AbsorpProduct*
Dermal absorption of in-use dilution	5.90%		*i_AbsorInuse*
Formulation type	Wettable granules, soluble granules		
Indoor or Outdoor application	Outdoor		
Application method	Upward spraying		
Application equipment	Manual-Knapsack		
Season	not relevant		

Outdoor/Wettable granules, soluble granules/Upward spraying/Manual/Knapsack

	Exposure values	µg exposure/day mixed and loaded 75th centile	95th centile	Reference	Comment
Mixing and loading	Hands	9495	25482	AOEM	
	Body	803	2787	AOEM	
	Head	5	11	AOEM	
	Protected hands (gloves)	18	164	AOEM	
	Protected body (workwear or protective garment and sturdy footwear)	25	103	AOEM	
	Protected head (hood and face shield)	5	11	AOEM	
	Inhalation	25	26	AOEM	
	Protective Equipment	Select for inclusion		Penetration factor	Inhalation Protection factor
	Gloves	Yes		Incl. in AOEM model	
	Clothing	Work wear - arms, body and legs covered		Incl. in AOEM model	
	Head and respiratory PPE	None		1	1
	Water soluble bag	No		1	

	Exposure values	µg exposure/day applied 75th centile	95th centile	Reference	Comment
Application	Hands	552	2046	AOEM	No data available for a drift reduction scenario
	Body	45600	175585	AOEM	
	Head	83	440	AOEM	
	Protected hands (gloves)	4	18	AOEM	
	Protected body (workwear or protective garment and sturdy footwear)	1033	1938	AOEM	
	Inhalation	17	59	AOEM	
	Protective Equipment	Select for inclusion		Penetration factor	Inhalation Protection factor
	Gloves	Yes		Incl. in AOEM model	
	Clothing	Work wear - arms, body and legs covered		Incl. in AOEM model	
	Head and respiratory PPE	None		1	1
	Closed cab	No		vehicle mounted upward spraying only	

Operator Model		Mixing, loading and application AOEM			
Potential exposure	Longer term systemic exposure mg/kg bw/day		0.0465	% of RVNAS	15.51%
	Acute systemic exposure mg/kg bw/day		0.1775	% of RVAAS	
Mixing and Loading		Gloves = Yes	Clothing = Work wear - arms, body and legs covered	RPE = None	Soluble bags = No
Application		Gloves = Yes	Clothing = Work wear - arms, body and legs covered	RPE = None	Closed cabin = No
Exposure (including PPE options above)	Longer term systemic exposure mg/kg bw/day		0.0018	% of RVNAS	0.60%
	Acute systemic exposure mg/kg bw/day		0.0038	% of RVAAS	

Table A 29: Estimation of operator exposure using EFSA model; potatoes, tractor-mounted boom sprayer; 3x 0.45 kg product/ha in 200 L/ha water at 7 days interval

Application rate of active substance	0.1485	kg a.s./ha	*i_AppRate*
Assumed area treated	50	ha/day	*d_AreaTreated*
Amount of active substance applied	7.425	kg a.s./day	*i_AmoutAS*
Dermal absorption of the product	0.21%		*i_AbsorpProduct*
Dermal absorption of in-use dilution	5.90%		*i_AbsorInuse*
Formulation type	Wettable granules, soluble granules		
Indoor or Outdoor application	Outdoor		
Application method	Downward spraying		
Application equipment	Vehicle-mounted		
Season	not relevant		

OutdoorWettable granules, soluble granulesDownward sprayingVehicle-mounted

	Exposure values	µg exposure/day mixed and loaded		Reference	Comment
		75th centile	95th centile		
Mixing and loading	Hands	6136	29784	AOEM	
	Body	5055	28773	AOEM	
	Head	48	665	AOEM	
	Protected hands (gloves)	61	234	AOEM	
	Protected body (workwear or protective garment and sturdy footwear)	110	462	AOEM	
	Protected head (hood and face shield)	1	38	AOEM	
	Inhalation	68	272	AOEM	
	Protective Equipment	Select for inclusion		Penetration factor	Inhalation Protection factor
	Gloves	No			
	Clothing	Work wear - arms, body and legs covered		Incl. in AOEM model	
	Head and respiratory PPE	None		1	1
	Water soluble bag	No		1	

	Exposure values	µg exposure/day applied		Reference	Comment
		75th centile	95th centile		
Application	Hands	1101	9951	AOEM	
	Body	616	3174	AOEM	
	Head	29	88	AOEM	
	Protected hands (gloves)	126	4211	AOEM	
	Protected body (workwear or protective garment and sturdy footwear)	17	41	AOEM	
	Inhalation	3	9	AOEM	
	Protective Equipment	Select for inclusion		Penetration factor	Inhalation Protection factor
	Gloves	No			
	Clothing	Work wear - arms, body and legs covered		Incl. in AOEM model	
	Head and respiratory PPE	None		1	1
	Closed cab	No		vehicle mounted upward spraying only	

Operator Model		Mixing, loading and application AOEM			
Potential exposure	Longer term systemic exposure mg/kg bw/day		0.0033	% of RVNAS	1.10%
	Acute systemic exposure mg/kg bw/day		0.0198	% of RVAAS	
Mixing and Loading		Gloves = No	Clothing = Work wear - arms, body and legs covered	RPE = None	Soluble bags = No
Application		Gloves = No	Clothing = Work wear - arms, body and legs covered	RPE = None	Closed cabin = No
Exposure (including PPE options above)	Longer term systemic exposure mg/kg bw/day		0.0025	% of RVNAS	0.84%
	Acute systemic exposure mg/kg bw/day		0.0157	% of RVAAS	

A 3.1.2 Calculations for cymoxanil

Table A 30: Estimation of operator exposure using EFSA model; grapes, tractor-mounted air-blast sprayer, upwards; 3x 0.45 kg product/ha in 200 L/ha water at 7 days interval

Operator exposure for Lieto WG outdoor spray applications

Application rate of active substance	0.1485	kg a.s./ha	*i_AppRate*
Assumed area treated	10	ha/day	*d_AreaTreated*
Amount of active substance applied	1.485	kg a.s./day	*i_AmoutAS*
Dermal absorption of the product	0.13%		*i_AbsorpProduct*
Dermal absorption of in-use dilution	35.00%		*i_AbsorInuse*
Formulation type	Wettable granules, soluble granules		
Indoor or Outdoor application	Outdoor		
Application method	Upward spraying		
Application equipment	Vehicle-mounted		
Season	not relevant		

OutdoorWettable granules, soluble granulesUpward sprayingVehicle-mounted

	Exposure values	µg exposure/day mixed and loaded		Reference	Comment
		75th centile	95th centile		
Mixing and loading	Hands	1777	8506	AOEM	
	Body	1631	18027	AOEM	
	Head	10	133	AOEM	
	Protected hands (gloves)	21	47	AOEM	
	Protected body (workwear or protective garment and sturdy footwear)	26	92	AOEM	
	Protected head (hood and face shield)	0	8	AOEM	
	Inhalation	42	262	AOEM	
	Protective Equipment	Select for inclusion		Penetration factor	Inhalation Protection factor
	Gloves	No			
	Clothing	Work wear - arms, body and legs covered		Incl. in AOEM model	
	Head and respiratory PPE	None		1	1
	Water soluble bag	No		1	

	Exposure values	µg exposure/day applied		Reference	Comment
		75th centile	95th centile		
Application	Hands	3583	9262	AOEM	No data available for a drift reduction scenario
	Body	13085	76353	AOEM	
	Head	1720	10554	AOEM	
	Protected hands (gloves)	52	1365	AOEM	
	Protected body (workwear or protective garment and sturdy footwear)	171	334	AOEM	
	Inhalation	80	123	AOEM	
	Protective Equipment	Select for inclusion		Penetration factor	Inhalation Protection factor
	Gloves	No			
	Clothing	Work wear - arms, body and legs covered		Incl. in AOEM model	
	Head and respiratory PPE	None		1	1
	Closed cab	No		vehicle mounted upward spraying only	

Operator Model		Mixing, loading and application AOEM			
Potential exposure	Longer term systemic exposure mg/kg bw/day		0.1094	% of RVNAS	1093.68%
	Acute systemic exposure mg/kg bw/day		0.5680	% of RVAAS	
Mixing and Loading		Gloves = No	Clothing = Work wear - arms, body and legs covered	RPE = None	Soluble bags = No
Application		Gloves = No	Clothing = Work wear - arms, body and legs covered	RPE = None	Closed cabin = No
Exposure (including PPE options above)	Longer term systemic exposure mg/kg bw/day		0.0340	% of RVNAS	339.98%
	Acute systemic exposure mg/kg bw/day		0.1241	% of RVAAS	

Table A 31: Estimation of operator exposure using EFSA model; grapes, tractor-mounted air-blast sprayer, upwards; 3x 0.45 kg product/ha in 200 L/ha water at 7 days interval plus PPE

Operator exposure for Lieto WG outdoor spray applications

Application rate of active substance	0.1485	kg a.s./ha	*i_AppRate*
Assumed area treated	10	ha/day	*d_AreaTreated*
Amount of active substance applied	1.485	kg a.s./day	*i_AmoutAS*
Dermal absorption of the product	0.13%		*i_AbsorpProduct*
Dermal absorption of in-use dilution	35.00%		*i_AbsorInuse*
Formulation type	Wettable granules, soluble granules		
Indoor or Outdoor application	Outdoor		
Application method	Upward spraying		
Application equipment	Vehicle-mounted		
Season	not relevant		

Outdoor|Wettable granules, soluble granules|Upward spraying|Vehicle-mounted

	Exposure values	µg exposure/day mixed and loaded		Reference	Comment
		75th centile	95th centile		
Mixing and loading	Hands	1777	8506	AOEM	
	Body	1631	18027	AOEM	
	Head	10	133	AOEM	
	Protected hands (gloves)	21	47	AOEM	
	Protected body (workwear or protective garment and sturdy footwear)	26	92	AOEM	
	Protected head (hood and face shield)	0	8	AOEM	
	Inhalation	42	262	AOEM	
	Protective Equipment	Select for inclusion		Penetration factor	Inhalation Protection factor
	Gloves	Yes		Incl. in AOEM model	
	Clothing	Work wear - arms, body and legs covered		Incl. in AOEM model	
	Head and respiratory PPE	None		1	1
	Water soluble bag	No		1	

	Exposure values	µg exposure/day applied		Reference	Comment
		75th centile	95th centile		
Application	Hands	3583	9262	AOEM	No data available for a drift reduction scenario
	Body	13085	76353	AOEM	
	Head	1720	10554	AOEM	
	Protected hands (gloves)	52	1365	AOEM	
	Protected body (workwear or protective garment and sturdy footwear)	171	334	AOEM	
	Inhalation	80	123	AOEM	
	Protective Equipment	Select for inclusion		Penetration factor	Inhalation Protection factor
	Gloves	Yes		Incl. in AOEM model	
	Clothing	Work wear - arms, body and legs covered		Incl. in AOEM model	
	Head and respiratory PPE	Hood and visor		0.05	1
	Closed cab	No		vehicle mounted upward spraying only	

Operator Model		Mixing, loading and application AOEM			
Potential exposure	Longer term systemic exposure mg/kg bw/day		0.1094	% of RVNAS	1093.68%
	Acute systemic exposure mg/kg bw/day		0.5680	% of RVAAS	
Mixing and Loading		Gloves = Yes	Clothing = Work wear - arms, body and legs covered	RPE = None	Soluble bags = No
Application		Gloves = Yes	Clothing = Work wear - arms, body and legs covered	RPE = Hood and visor	Closed cabin = No
Exposure (including PPE options above)	Longer term systemic exposure mg/kg bw/day		0.0038	% of RVNAS	38.33%
	Acute systemic exposure mg/kg bw/day		0.0194	% of RVAAS	

Table A 32: Estimation of operator exposure using EFSA model; grapes, manual, hand-held; 3x 0.45 kg product/ha in 200 L/ha water at 7 days interval plus PPE

Operator exposure for Lieto WG outdoor spray applications

Application rate of active substance	0.1485	kg a.s./ha	*i_AppRate*
Assumed area treated	4	ha/day	*d_AreaTreated*
Amount of active substance applied	0.594	kg a.s./day	*i_AmoutAS*
Dermal absorption of the product	0.13%		*i_AbsorpProduct*
Dermal absorption of in-use dilution	35.00%		*i_AbsorInuse*
Formulation type	Wettable granules, soluble granules		
Indoor or Outdoor application	Outdoor		
Application method	Upward spraying		
Application equipment	Manual-Hand held		
Season	not relevant		

OutdoorWettable granules, soluble granulesUpward sprayingManual-Hand held

	Exposure values	µg exposure/day mixed and loaded 75th centile	95th centile	Reference	Comment
Mixing and loading	Hands	878	4167	AOEM	
	Body	856	13814	AOEM	
	Head	4	53	AOEM	
	Protected hands (gloves)	12	19	AOEM	
	Protected body (workwear or protective garment and sturdy footwear)	12	37	AOEM	
	Protected head (hood and face shield)	0	3	AOEM	
	Inhalation	32	256	AOEM	
	Protective Equipment	Select for inclusion		Penetration factor	Inhalation Protection factor
	Gloves	No			
	Clothing	Work wear - arms, body and legs covered		Incl. in AOEM model	
	Head and respiratory PPE	None		1	1
	Water soluble bag	No		1	

	Exposure values	µg exposure/day applied 75th centile	95th centile	Reference	Comment
Application	Hands	1760	5908	AOEM	No data available for a drift reduction scenario
	Body	56650	177878	AOEM	
	Head	129	693	AOEM	
	Protected hands (gloves)	14	74	AOEM	
	Protected body (workwear or protective garment and sturdy footwear)	1033	1938	AOEM	
	Inhalation	52	134	AOEM	
	Protective Equipment	Select for inclusion		Penetration factor	Inhalation Protection factor
	Gloves	No			
	Clothing	Work wear - arms, body and legs covered		Incl. in AOEM model	
	Head and respiratory PPE	None		1	1
	Closed cab	No		vehicle mounted upward spraying only	

Operator Model		Mixing, loading and application AOEM		
Potential exposure	Longer term systemic exposure mg/kg bw/day	0.3429	% of RVNAS	3429.22%
	Acute systemic exposure mg/kg bw/day	1.0830	% of RVAAS	
Mixing and Loading	Gloves = No	Clothing = Work wear - arms, body and legs covered	RPE = None	Soluble bags = No
Application	Gloves = No	Clothing = Work wear - arms, body and legs covered	RPE = None	Closed cabin = No
Exposure (including PPE options above)	Longer term systemic exposure mg/kg bw/day	0.0185	% of RVNAS	184.69%
	Acute systemic exposure mg/kg bw/day	0.0564	% of RVAAS	

Table A 33: Estimation of operator exposure using EFSA model; grapes, manual, hand-held; 3x 0.45 kg product/ha in 200 L/ha water at 7 days interval plus PPE

Operator exposure for Lieto WG outdoor spray applications

Application rate of active substance	0.1485	kg a.s./ha	*i_AppRate*
Assumed area treated	4	ha/day	*d_AreaTreated*
Amount of active substance applied	0.594	kg a.s./day	*i_AmoutAS*
Dermal absorption of the product	0.13%		*i_AbsorpProduct*
Dermal absorption of in-use dilution	35.00%		*i_AbsorInuse*
Formulation type	Wettable granules, soluble granules		
Indoor or Outdoor application	Outdoor		
Application method	Upward spraying		
Application equipment	Manual-Hand held		
Season	not relevant		

OutdoorWettable granules, soluble granulesUpward sprayingManual-Hand held

	Exposure values	µg exposure/day mixed and loaded 75th centile	95th centile	Reference	Comment
Mixing and loading	Hands	878	4167	AOEM	
	Body	856	13814	AOEM	
	Head	4	53	AOEM	
	Protected hands (gloves)	12	19	AOEM	
	Protected body (workwear or protective garment and sturdy footwear)	12	37	AOEM	
	Protected head (hood and face shield)	0	3	AOEM	
	Inhalation	32	256	AOEM	
	Protective Equipment	Select for inclusion		Penetration factor	Inhalation Protection factor
	Gloves	Yes		Incl. in AOEM model	
	Clothing	Work wear - arms, body and legs covered		Incl. in AOEM model	
	Head and respiratory PPE	None		1	1
	Water soluble bag	No		1	

	Exposure values	µg exposure/day applied 75th centile	95th centile	Reference	Comment
Application	Hands	1760	5908	AOEM	No data available for a drift reduction scenario
	Body	56650	177878	AOEM	
	Head	129	693	AOEM	
	Protected hands (gloves)	14	74	AOEM	
	Protected body (workwear or protective garment and sturdy footwear)	1033	1938	AOEM	
	Inhalation	52	134	AOEM	
	Protective Equipment	Select for inclusion		Penetration factor	Inhalation Protection factor
	Gloves	Yes		Incl. in AOEM model	
	Clothing	Work wear - arms, body and legs covered		Incl. in AOEM model	
	Head and respiratory PPE	None		1	1
	Closed cab	No		vehicle mounted upward spraying only	

Operator Model		Mixing, loading and application AOEM			
Potential exposure	Longer term systemic exposure mg/kg bw/day		0.3429	% of RVNAS	3429.22%
	Acute systemic exposure mg/kg bw/day		1.0830	% of RVAAS	
Mixing and Loading		Gloves = Yes	Clothing = Work wear - arms, body and legs covered	RPE = None	Soluble bags = No
Application		Gloves = Yes	Clothing = Work wear - arms, body and legs covered	RPE = None	Closed cabin = No
Exposure (including PPE options above)	Longer term systemic exposure mg/kg bw/day		0.0083	% of RVNAS	82.66%
	Acute systemic exposure mg/kg bw/day		0.0223	% of RVAAS	

Table A 34: Estimation of operator exposure using EFSA model; grapes, Knapsack, hand-held; 3x 0.45 kg product/ha in 200 L/ha water at 7 days interval

Operator exposure for Lieto WG outdoor spray applications

Application rate of active substance	0.1485	kg a.s./ha	*i_AppRate*
Assumed area treated	1	ha/day	*d_AreaTreated*
Amount of active substance applied	0.1485	kg a.s./day	*i_AmoutAS*
Dermal absorption of the product	0.13%		*i_AbsorpProduct*
Dermal absorption of in-use dilution	35.00%		*i_AbsorInuse*
Formulation type	Wettable granules, soluble granules		
Indoor or Outdoor application	Outdoor		
Application method	Upward spraying		
Application equipment	Manual-Knapsack		
Season	not relevant		

OutdoorWettable granules, soluble granulesUpward sprayingManual-Knapsack

	Exposure values	µg exposure/day mixed and loaded		Reference	Comment
		75th centile	95th centile		
Mixing and loading	Hands	9495	25482	AOEM	
	Body	803	2787	AOEM	
	Head	5	11	AOEM	
	Protected hands (gloves)	18	164	AOEM	
	Protected body (workwear or protective garment and sturdy footwear)	25	103	AOEM	
	Protected head (hood and face shield)	5	11	AOEM	
	Inhalation	25	26	AOEM	
	Protective Equipment	Select for inclusion		Penetration factor	Inhalation Protection factor
	Gloves	No			
	Clothing	Work wear - arms, body and legs covered		Incl. in AOEM model	
	Head and respiratory PPE	None		1	1
	Water soluble bag	No		1	

	Exposure values	µg exposure/day applied		Reference	Comment
		75th centile	95th centile		
Application	Hands	552	2046	AOEM	No data available for a drift reduction scenario
	Body	45600	175585	AOEM	
	Head	83	440	AOEM	
	Protected hands (gloves)	4	18	AOEM	
	Protected body (workwear or protective garment and sturdy footwear)	1033	1938	AOEM	
	Inhalation	17	59	AOEM	
	Protective Equipment	Select for inclusion		Penetration factor	Inhalation Protection factor
	Gloves	No			
	Clothing	Work wear - arms, body and legs covered		Incl. in AOEM model	
	Head and respiratory PPE	None		1	1
	Closed cab	No		vehicle mounted upward spraying only	

Operator Model		Mixing, loading and application AOEM			
Potential exposure	Longer term systemic exposure mg/kg bw/day		0.2706	% of RVNAS	2706.20%
	Acute systemic exposure mg/kg bw/day		1.0408	% of RVAAS	
Mixing and Loading		Gloves = No	Clothing = Work wear - arms, body and legs covered	RPE = None	Soluble bags = No
Application		Gloves = No	Clothing = Work wear - arms, body and legs covered	RPE = None	Closed cabin = No
Exposure (including PPE options above)	Longer term systemic exposure mg/kg bw/day		0.0106	% of RVNAS	106.27%
	Acute systemic exposure mg/kg bw/day		0.0278	% of RVAAS	

Table A 35: Estimation of operator exposure using EFSA model; grapes, Knapsack, hand-held; 3x 0.45 kg product/ha in 200 L/ha water at 7 days interval plus PPE

Operator exposure for Lieto WG outdoor spray applications

Application rate of active substance	0.1485	kg a.s./ha	*i_AppRate*
Assumed area treated	1	ha/day	*d_AreaTreated*
Amount of active substance applied	0.1485	kg a.s./day	*i_AmoutAS*
Dermal absorption of the product	0.13%		*i_AbsorpProduct*
Dermal absorption of in-use dilution	35.00%		*i_AbsorInuse*
Formulation type	Wettable granules, soluble granules		
Indoor or Outdoor application	Outdoor		
Application method	Upward spraying		
Application equipment	Manual-Knapsack		
Season	not relevant		

OutdoorWettable granules, soluble granulesUpward sprayingManual-Knapsack

	Exposure values	µg exposure/day mixed and loaded		Reference	Comment
		75th centile	95th centile		
Mixing and loading	Hands	9495	25482	AOEM	
	Body	803	2787	AOEM	
	Head	5	11	AOEM	
	Protected hands (gloves)	18	164	AOEM	
	Protected body (workwear or protective garment and sturdy footwear)	25	103	AOEM	
	Protected head (hood and face shield)	5	11	AOEM	
	Inhalation	25	26	AOEM	
	Protective Equipment	Select for inclusion		Penetration factor	Inhalation Protection factor
	Gloves	Yes		Incl. in AOEM model	
	Clothing	Work wear - arms, body and legs covered		Incl. in AOEM model	
	Head and respiratory PPE	None		1	1
	Water soluble bag	No		1	

	Exposure values	µg exposure/day applied		Reference	Comment
		75th centile	95th centile		
Application	Hands	552	2046	AOEM	No data available for a drift reduction scenario
	Body	45600	175585	AOEM	
	Head	83	440	AOEM	
	Protected hands (gloves)	4	18	AOEM	
	Protected body (workwear or protective garment and sturdy footwear)	1033	1938	AOEM	
	Inhalation	17	59	AOEM	
	Protective Equipment	Select for inclusion		Penetration factor	Inhalation Protection factor
	Gloves	Yes		Incl. in AOEM model	
	Clothing	Work wear - arms, body and legs covered		Incl. in AOEM model	
	Head and respiratory PPE	None		1	1
	Closed cab	No		vehicle mounted upward spraying only	

Operator Model		Mixing, loading and application AOEM			
Potential exposure	Longer term systemic exposure mg/kg bw/day		0.2706	% of RVNAS	2706.20%
	Acute systemic exposure mg/kg bw/day		1.0408	% of RVAAS	
Mixing and Loading		Gloves = Yes	Clothing = Work wear - arms, body and legs covered	RPE = None	Soluble bags = No
Application		Gloves = Yes	Clothing = Work wear - arms, body and legs covered	RPE = None	Closed cabin = No
Exposure (including PPE options above)	Longer term systemic exposure mg/kg bw/day		0.0072	% of RVNAS	72.22%
	Acute systemic exposure mg/kg bw/day		0.0154	% of RVAAS	

Table A 36: Estimation of operator exposure using EFSA model; potatoes, tractor-mounted boom sprayer; 3x 0.45 kg product/ha in 200 L/ha water at 7 days interval

Operator exposure for Lieto WG outdoor spray applications

Application rate of active substance	0.1485	kg a.s./ha	*i_AppRate*
Assumed area treated	50	ha/day	*d_AreaTreated*
Amount of active substance applied	7.425	kg a.s./day	*i_AmoutAS*
Dermal absorption of the product	0.13%		*i_AbsorpProduct*
Dermal absorption of in-use dilution	35.00%		*i_AbsorInuse*
Formulation type	Wettable granules, soluble granules		
Indoor or Outdoor application	Outdoor		
Application method	Downward spraying		
Application equipment	Vehicle-mounted		
Season	not relevant		

OutdoorWettable granules, soluble granulesDownward sprayingVehicle-mounted

	Exposure values	µg exposure/day mixed and loaded		Reference	Comment
		75th centile	95th centile		
Mixing and loading	Hands	6136	29784	AOEM	
	Body	5055	28773	AOEM	
	Head	48	665	AOEM	
	Protected hands (gloves)	61	234	AOEM	
	Protected body (workwear or protective garment and sturdy footwear)	110	462	AOEM	
	Protected head (hood and face shield)	1	38	AOEM	
	Inhalation	68	272	AOEM	
	Protective Equipment	Select for inclusion		Penetration factor	Inhalation Protection factor
	Gloves	No			
	Clothing	Work wear - arms, body and legs covered		Incl. in AOEM model	
	Head and respiratory PPE	None		1	1
	Water soluble bag	No		1	

	Exposure values	µg exposure/day applied		Reference	Comment
		75th centile	95th centile		
Application	Hands	1101	9951	AOEM	
	Body	616	3174	AOEM	
	Head	29	88	AOEM	
	Protected hands (gloves)	126	4211	AOEM	
	Protected body (workwear or protective garment and sturdy footwear)	17	41	AOEM	
	Inhalation	3	9	AOEM	
	Protective Equipment	Select for inclusion		Penetration factor	Inhalation Protection factor
	Gloves	No			
	Clothing	Work wear - arms, body and legs covered		Incl. in AOEM model	
	Head and respiratory PPE	None		1	1
	Closed cab	No		vehicle mounted upward spraying only	

Operator Model		Mixing, loading and application AOEM			
Potential exposure	Longer term systemic exposure mg/kg bw/day		0.0116	% of RVNAS	116.07%
	Acute systemic exposure mg/kg bw/day		0.0830	% of RVAAS	
Mixing and Loading		Gloves = No	Clothing = Work wear - arms, body and legs covered	RPE = None	Soluble bags = No
Application		Gloves = No	Clothing = Work wear - arms, body and legs covered	RPE = None	Closed cabin = No
Exposure (including PPE options above)	Longer term systemic exposure mg/kg bw/day		0.0080	% of RVNAS	80.06%
	Acute systemic exposure mg/kg bw/day		0.0642	% of RVAAS	

A 3.2 Worker exposure calculations (KCP 7.2.3.1)

A 3.2.1 Calculations for zoxamide

Table A 37: Estimation of worker exposure using EFSA model; grapes, tractor-mounted air-blast sprayer, upwards; 3x 0.45 kg product/ha at 7 days interval

Substance	Zoxamide	Formulation = Wettable granules, soluble granules	Application rate-0.1485 kg a.s. /ha	Spray dilution = 0.7425 g a.s./l	Vapour pressure = low volatile substances having a vapour pressure of <5*10-3Pa
Scenario	Grapes / Outdoor / Upward spraying / Vehicle-mounted			Buffer = 5	Number applications = 3, Application interval = 7 days
Percentage Absoprtion	Dermal for product = 0.21	Dermal for in use diluation = 5.9	Oral = 60	Inhalation = 100	
RVNAS	0.3 mg/kg bw/day		RVAAS	mg/kg bw/day	
DFR	3 µg a.s./cm2 per kg a.s./ha		DT50	30 days	

Worker - Hand harvesting	Potential exposure mg/kg bw/day	0.2707	% of RVNAS	90.22%
	Working clothing mg/kg bw/day	0.0911	% of RVNAS	30.37%
	Working clothing and gloves mg/kg bw/day		% of RVNAS	

Table A 38: Estimation of worker exposure using EFSA model; potatoes, tractor-mounted boom sprayer, downwards; 3x 0.45 kg product/ha at 7 days interval

Substance	Zoxamide	Formulation = Wettable granules, soluble granules	Application rate-0.1485 kg a.s. /ha	Spray dilution = 0.7425 g a.s./l	Vapour pressure = low volatile substances having a vapour pressure of <5*10-3Pa
Scenario	Root and tuber vegetables / Outdoor / Downward spraying / Vehicle-mounted			Buffer = 2-3	Number applications = 3, Application interval = 7 days
Percentage Absoprtion	Dermal for product = 0.21	Dermal for in use diluation = 5.9	Oral = 60	Inhalation = 100	
RVNAS	0.3 mg/kg bw/day		RVAAS	mg/kg bw/day	
DFR	3 µg a.s./cm2 per kg a.s./ha		DT50	30 days	

Worker - Inspection, irrigation	Potential exposure mg/kg bw/day	0.0282	% of RVNAS	9.40%
	Working clothing mg/kg bw/day	0.0032	% of RVNAS	1.05%
	Working clothing and gloves mg/kg bw/day		% of RVNAS	

A 3.2.2 Calculations for cymoxanil

Table A 39: Estimation of worker exposure using EFSA model; grapes, tractor-mounted air-blast sprayer, upwards; 3x 0.45 kg product/ha at 7 days interval

Worker exposure from residues on foliage for Lieto WG			
Crop type	Grapes		
Indoor or outdoor	Outdoor		
Application method	Upward spraying		
Application equipment	Vehicle-mounted		
Worker's task	Hand harvesting		
Main body parts in contact with foliage	Hand and body		
Application rate of active substance	0.1485	kg a.s./ha	*i_AppRate*
Number of applications	3		*i_AppNo*
Interval between multiple applications	7	days	*i_AppInt*
Half-life of active substance	1	days	*d_HalflifeAS*
Multiple application factor	1.0		*d_MAF*
Dermal absorption of the product	0.13%		*i_AbsorpProduct*
Dermal absorption of the in-use dilution	35.00%		*i_AbsorpInuse*
Dislodgeable foliar residue (i_AppRate*i_DFR)	0.2376	µg a.s./cm^2	*d_DFR*
Working hours	8	hr	*d_WorkHr*
Dermal transfer coefficient - Total potential exposure	30000	cm^2/hr	*d_DermTcUCV*
Dermal transfer coefficient - arms, body and legs covered	10100	cm^2/hr	*d_DermTcCV1*
Dermal transfer coefficient - hands, arms, body and legs covered	no TC available for this assessment	cm^2/hr	*d_DermTcCV2*
Inhalation transfer coefficient for automated applications	NA	ha/hr*10^(-3)	*d_InhalTcAut*
Inhalation transfer coefficient for cutting ornamentals	NA	ha/hr*10^(-3)	*d_InhalTcCut*
Inhalation transfer coefficient for sorting / bundling ornamentals	NA	ha/hr*10^(-3)	*d_InhalTcSort*

Worker - Hand harvesting	Potential exposure mg/kg bw/day	0.3353	% of RVNAS	3352.59%
	Working clothing mg/kg bw/day	0.1129	% of RVNAS	1128.71%
	Working clothing and gloves mg/kg bw/day		% of RVNAS	

Table A 40: Estimation of worker exposure using EFSA model; grapes, tractor-mounted air-blast sprayer, upwards; 3x 0.45 kg product/ha at 7 days interval. Refinement DA Dry Residue 5.7%

Worker exposure from residues on foliage for Lieto WG			
Crop type	Grapes		
Indoor or outdoor	Outdoor		
Application method	Upward spraying		
Application equipment	Vehicle-mounted		
Worker's task	Hand harvesting		
Main body parts in contact with foliage	Hand and body		
Application rate of active substance	0.1485	kg a.s./ha	*i_AppRate*
Number of applications	3		*i_AppNo*
Interval between multiple applications	7	days	*i_AppInt*
Half-life of active substance	1	days	*d_HalflifeAS*
Multiple application factor	1.0		*d_MAF*
Dermal absorption of the product	0.13%		*i_AbsorpProduct*
Dermal absorption of the in-use dilution	5.70%		*i_AbsorpInuse*
Dislodgeable foliar residue (i_AppRate*i_DFR)	0.2376	µg a.s./cm^2	*d_DFR*
Working hours	8	hr	*d_WorkHr*
Dermal transfer coefficient - Total potential exposure	30000	cm^2/hr	*d_DermTcUCV*
Dermal transfer coefficient - arms, body and legs covered	10100	cm^2/hr	*d_DermTcCV1*
Dermal transfer coefficient - hands, arms, body and legs covered	no TC available for this assessment	cm^2/hr	*d_DermTcCV2*
Inhalation transfer coefficient for automated applications	NA	ha/hr*10^(-3)	*d_InhalTcAut*
Inhalation transfer coefficient for cutting ornamentals	NA	ha/hr*10^(-3)	*d_InhalTcCut*
Inhalation transfer coefficient for sorting / bundling ornamentals	NA	ha/hr*10^(-3)	*d_InhalTcSort*

Worker - Hand harvesting	Potential exposure mg/kg bw/day	0.0546	% of RVNAS	545.99%
	Working clothing mg/kg bw/day	0.0184	% of RVNAS	183.82%
	Working clothing and gloves mg/kg bw/day		% of RVNAS	

Residue decline calculated using a moving time window approach in determining time weighted average concentrations according to EFSA (2015) guidance "Risk Assessment for Birds and Mammals"[20] considering Dermal Absorption 5.7% for dry residue, DT 50 1d ~~and 1d re-entry~~ and re-entry on 2nd day after application~~-~~:

$$C = C_0 \times e^{-kt}$$

With:

C = actual concentration at time t

C_0 = initial concentration

k = rate constant where $k = \ln 2/DT_{50}$

	Dermal absorption*		Inhalation absorption		Total absorption*	
	(mg/kg bw/day)	(% AOEL)	(mg/kg bw/day)	(% AOEL)	(mg/kg bw/day)	(% AOEL)
Without re-entry	0.0184	183.82	--	--	0.0184	183.82
2d re-entry	0.00460	45.95	--	--	0.00460	45.95

* considering worker wearing normal work clothes

Residue decline calculated using a moving time window approach in determining time weighted average concentrations according to EFSA (2015) guidance "Risk Assessment for Birds and Mammals"[21] considering Dermal Absorption 35% for dry residue, TC 3500 or TC 4861 (see main section), DT 50 1d and re-entry on 2nd day after application:

$$C = C_0 \times e^{-kt}$$

With:

C = actual concentration at time t

C_0 = initial concentration

k = rate constant where $k = \ln 2/DT_{50}$

	Dermal absorption*		Inhalation absorption		Total absorption*	
	(mg/kg bw/day)	(% AOEL)	(mg/kg bw/day)	(% AOEL)	(mg/kg bw/day)	(% AOEL)
Without re-entry	0.1129	1128.71	--	--	0.1129	1128.71
TC 3500 2d re-entry	0.00978	97.78	--	--	0.00978	97.78
TC 4861 3d re-entry	0.00683	68.33	--	--	0.00683	68.33

* considering worker wearing normal work clothes

[20] Appendix H: EFSA Journal 2009; 7(12):1438
[21] Appendix H: EFSA Journal 2009; 7(12):1438

Table A 41: Estimation of worker exposure using EFSA model; potatoes, tractor-mounted boom sprayer, downwards; 3x 0.45 L product/ha at 7 days interval

Worker exposure from residues on foliage for Lieto WG			
Crop type	Root and tuber vegetables		
Indoor or outdoor	Outdoor		
Application method	Downward spraying		
Application equipment	Vehicle-mounted		
Worker's task	Inspection, irrigation		
Main body parts in contact with foliage	Hand and body		
Application rate of active substance	0.1485	kg a.s./ha	*i_AppRate*
Number of applications	3		*i_AppNo*
Interval between multiple applications	7	days	*i_AppInt*
Half-life of active substance	1	days	*d_HalflifeAS*
Multiple application factor	1.0		*d_MAF*
Dermal absorption of the product	0.13%		*i_AbsorpProduct*
Dermal absorption of the in-use dilution	35.00%		*i_AbsorpInuse*
Dislodgeable foliar residue (i_AppRate*i_DFR)	0.297	µg a.s./cm^2	*d_DFR*
Working hours	2	hr	*d_WorkHr*
Dermal transfer coefficient - Total potential exposure	12500	cm^2/hr	*d_DermTcUCV*
Dermal transfer coefficient - arms, body and legs covered	1400	cm^2/hr	*d_DermTcCV1*
Dermal transfer coefficient - hands, arms, body and legs covered	no TC available for this assessment	cm^2/hr	*d_DermTcCV2*
Inhalation transfer coefficient for automated applications	NA	ha/hr*10^(-3)	*d_InhalTcAut*
Inhalation transfer coefficient for cutting ornamentals	NA	ha/hr*10^(-3)	*d_InhalTcCut*
Inhalation transfer coefficient for sorting / bundling ornamentals	NA	ha/hr*10^(-3)	*d_InhalTcSort*

Worker - Inspection, irrigation	Potential exposure mg/kg bw/day	0.0437	% of RVNAS	436.54%
	Working clothing mg/kg bw/day	0.0049	% of RVNAS	48.89%
	Working clothing and gloves mg/kg bw/day		% of RVNAS	

A 3.3 Bystander and resident exposure calculations (KCP 7.2.2.1)

A 3.3.1 Calculations for zoxamide

Table A 42: Estimation of resident exposure using EFSA model; grapes, tractor-mounted air-blast sprayer, upwards; 3x 0.45 kg product/ha at 7 days interval

Substance	Zoxamide	Formulation = Wettable granules, soluble granules	Application rate-0.1485 kg a.s. /ha	Spray dilution = 0.7425 g a.s./l	Vapour pressure = low volatile substances having a vapour pressure of <5*10-3Pa
Scenario	Grapes / Outdoor / Upward spraying / Vehicle-mounted			Buffer = 5	Number applications = 3, Application interval = 7 days
Percentage Absoprtion	Dermal for product = 0.21	Dermal for in use diluation = 5.9	Oral = 60	Inhalation = 100	
RVNAS	0.3 mg/kg bw/day		RVAAS	mg/kg bw/day	
DFR	3 µg a.s./cm2 per kg a.s./ha		DT50	30 days	

Resident - child	Spray drift (75th percentile) mg/kg bw/day	0.0062	% of RVNAS	2.06%
	Vapour (75th percentile) mg/kg bw/day	0.0011	% of RVNAS	0.36%
	Surface deposits (75th percentile) mg/kg bw/day	0.0003	% of RVNAS	0.09%
	Entry into treated crops (75th percentile) mg/kg bw/day	0.0038	% of RVNAS	1.27%
	All pathways (mean) mg/kg bw/day	0.0084	% of RVNAS	2.80%
Resident - adult	Spray drift (75th percentile) mg/kg bw/day	0.0034	% of RVNAS	1.13%
	Vapour (75th percentile) mg/kg bw/day	0.0002	% of RVNAS	0.08%
	Surface deposits (75th percentile) mg/kg bw/day	0.0001	% of RVNAS	0.03%
	Entry into treated crops (75th percentile) mg/kg bw/day	0.0021	% of RVNAS	0.70%
	All pathways (mean) mg/kg bw/day	0.0042	% of RVNAS	1.40%

Table A 43: Estimation of resident exposure using EFSA model; potatoes field, tractor-mounted boom sprayer; 3x 0.45 kg product/ha at 7 days interval

Substance	Zoxamide	Formulation = Wettable granules, soluble granules	Application rate-0.1485 kg a.s. /ha	Spray dilution = 0.7425 g a.s./l	Vapour pressure = low volatile substances having a vapour pressure of <5*10-3Pa
Scenario	Root and tuber vegetables / Outdoor / Downward spraying / Vehicle-mounted			Buffer = 2-3	Number applications = 3, Application interval = 7 days
Percentage Absoprtion	Dermal for product = 0.21	Dermal for in use diluation = 5.9	Oral = 60	Inhalation = 100	
RVNAS	0.3 mg/kg bw/day		RVAAS	mg/kg bw/day	
DFR	3 µg a.s./cm2 per kg a.s./ha		DT50	30 days	

Resident - child	Spray drift (75th percentile) mg/kg bw/day	0.0012	% of RVNAS	0.40%
	Vapour (75th percentile) mg/kg bw/day	0.0011	% of RVNAS	0.36%
	Surface deposits (75th percentile) mg/kg bw/day	0.0005	% of RVNAS	0.17%
	Entry into treated crops (75th percentile) mg/kg bw/day	0.0038	% of RVNAS	1.27%
	All pathways (mean) mg/kg bw/day	0.0051	% of RVNAS	1.71%
Resident - adult	Spray drift (75th percentile) mg/kg bw/day	0.0003	% of RVNAS	0.09%
	Vapour (75th percentile) mg/kg bw/day	0.0002	% of RVNAS	0.08%
	Surface deposits (75th percentile) mg/kg bw/day	0.0002	% of RVNAS	0.05%
	Entry into treated crops (75th percentile) mg/kg bw/day	0.0021	% of RVNAS	0.70%
	All pathways (mean) mg/kg bw/day	0.0022	% of RVNAS	0.72%

A 3.3.2 Calculations for cymoxanil

Table A 44: Estimation of resident exposure using EFSA model; grapes, tractor-mounted air-blast sprayer, upwards; 3x 0.45 kg product/ha at 7 days interval

Substance	Cymoxanil	Formulation = Wettable granules, soluble granules	Application rate-0.1485 kg a.s. /ha	Spray dilution = 0.7425 g a.s./l	Vapour pressure = low volatile substances having a vapour pressure of <5*10-3Pa
Scenario	Grapes / Outdoor / Upward spraying / Vehicle-mounted			Buffer = 5	Number applications = 3, Application interval = 7 days
Percentage Absoprtion	Dermal for product = 0.13	Dermal for in use diluation = 35	Oral = 75	Inhalation = 100	
RVNAS	0.01 mg/kg bw/day		RVAAS	0 mg/kg bw/day	
DFR	1.6 µg a.s./cm2 per kg a.s./ha		DT50	1 days	

Resident - child	Spray drift (75th percentile) mg/kg bw/day	0.0361	% of RVNAS	361.14%
	Vapour (75th percentile) mg/kg bw/day	0.0011	% of RVNAS	10.70%
	Surface deposits (75th percentile) mg/kg bw/day	0.0005	% of RVNAS	4.68%
	Entry into treated crops (75th percentile) mg/kg bw/day	0.0047	% of RVNAS	47.15%
	All pathways (mean) mg/kg bw/day	0.0289	% of RVNAS	289.35%
Resident - adult	Spray drift (75th percentile) mg/kg bw/day	0.0200	% of RVNAS	200.22%
	Vapour (75th percentile) mg/kg bw/day	0.0002	% of RVNAS	2.30%
	Surface deposits (75th percentile) mg/kg bw/day	0.0002	% of RVNAS	1.96%
	Entry into treated crops (75th percentile) mg/kg bw/day	0.0026	% of RVNAS	26.19%
	All pathways (mean) mg/kg bw/day	0.0156	% of RVNAS	155.57%

Table A 45: Estimation of resident exposure using EFSA model; grapes, tractor-mounted air-blast sprayer, upwards; 3x 0.45 kg product/ha at 7 days interval, 10 m buffer zone, DRT, 400 L water min, DA dry residue 5.7% for re-entry only

Substance	Cymoxanil	Formulation = Wettable granules, soluble granules	Application rate- 0.1485 kg a.s. /ha	Spray dilution = 0.37125 g a.s./l	Vapour pressure = low volatile substances having a vapour pressure of <5*10-3Pa
Scenario	Grapes / Outdoor / Upward spraying / Vehicle-mounted-Drift Reduction			Buffer = 10	Number applications = 3, Application interval = 7 days
Percentage Absoprtion	Dermal for product = 0.13	Dermal for in use dilution = 5.7/35	Oral = 75	Inhalation = 100	
RVNAS	0.01 mg/kg bw/day		RVAAS	0 mg/kg bw/day	
DFR	1.6 µg a.s./cm2 per kg a.s./ha		DT50	1 days	

Resident - child	Spray drift (75th percentile) mg/kg bw/day	0.0090	% of RVNAS	90.29%
	Vapour (75th percentile) mg/kg bw/day	0.0011	% of RVNAS	10.70%
	Surface deposits (75th percentile) mg/kg bw/day	0.0001	% of RVNAS	0.78%
	Entry into treated crops (75th percentile) mg/kg bw/day	0.0008	% of RVNAS	7.68%
	All pathways (mean) mg/kg bw/day	0.0077	% of RVNAS	76.79%
Resident - adult	Spray drift (75th percentile) mg/kg bw/day	0.0050	% of RVNAS	50.05%
	Vapour (75th percentile) mg/kg bw/day	0.0002	% of RVNAS	2.30%
	Surface deposits (75th percentile) mg/kg bw/day	0.0000	% of RVNAS	0.33%
	Entry into treated crops (75th percentile) mg/kg bw/day	0.0004	% of RVNAS	4.27%
	All pathways (mean) mg/kg bw/day	0.0078	% of RVNAS	76.79

Table A 46A: Estimation of resident exposure using EFSA model; grapes, tractor-mounted air-blast sprayer, upwards; 3x 0.45 kg product/ha at 7 days interval, 5 m buffer zone, DRT, 400 L water min, DA dry residue 5.7% for re-entry only

Resident exposure for Reboot				
Croptype	Grapes			
Application method	Upward spraying			
Application equipment	Vehicle-mounted-Drift Reduction			*i_AppEquip*
Formulation type	Wettable granules, soluble granules			*i_FormVal*
Buffer strip	5	m		*i_Buffer*
Application rate of the product	0,1485	kg a.s./ha		*i_AppRate*
Concentration of active substance (in-use dilution for liquid applications)	0,37125	g a.s./l		*d_ConcAS*
Dermal absorption of product	0,13%			*i_AbsorpProduct*
Dermal absorption of in-use dilution	5,70%			*i_AbsorpInuse*
Oral absorption	75,00%			*i_AbsorpOralInuse*
Dislodgeable foliar residue (i_AppRate*i_DFR)	0,4455	µg a.s./cm^2		*d_DFR*
Vapour pressure of in-use dilution	low volatile substances having a vapour pressure of <5*10-3Pa	Pa		*i_Volat*
Concentration in air	0,001	mg/m^3		*d_AirCon*
Resident dermal spray drift exposure 75th percentile - adult	5,63	ml spray dilution/person		
Resident dermal spray drift exposure 75th percentile - child	1,689	ml spray dilution/person		
Resident inhal. spray drift exposure 75th percentile - adult	0,00210	ml spray dilution/person		
Resident inhal. spray drift exposure 75th percentile - child	0,00164	ml spray dilution/person		
Resident dermal spray drift exposure mean - adult	3,68	ml spray dilution/person		
Resident dermal spray drift exposure mean - child	1,11	ml spray dilution/person		
Resident inhal. spray drift exposure mean - adult	0,00170	ml spray dilution/person		
Resident inhal. spray drift exposure mean - child	0,00133	ml spray dilution/person		
Exposure duration dermal	2	hours		*d_ReExpDur*
Exposure duration inhalation	24	hours		*d_ReExpDurInhal*
Exposure duration entry into treated crops	0,25	hours		*d_ExpDurTreatCrop*
Light clothing adjustment factor	18,0%			*d_ClothAF*
Breathing rate adult	0,23	m^3/day/kg		*d_BreathRAd*
Breathing rate child (1-3 year old)	1,07	m^3/day/kg		*d_BreathRCh*
Drift percentage on surface (75th percentile)	3,07%			
Drift percentage on surface (mean)	2,32%			
Turf transferable residues percentage	5,00%			*d_Turf*
Transfer coeff. of surface deposits-adult	7300	cm^2/hour		*d_ReTCAd*
Transfer coeff. of surface deposits-child (1-3 year old)	2600	cm^2/hour		*d_ReTCCh*
Saliva extraction percentage	50,00%			*d_SalExt*
Surface area of hands mouthed	20	cm^2		*d_AreaHM*
Frequency of hand to mouth activity	9,5	events/hour		*d_ReFreqHM*
Ingestion rate for mouthing of grass per day	25	cm^2		*d_MouthGrass*
Dislodgeable residues percentage transferability for object to mouth	20,00%			*d_DRP*
Transfer coefficient for entry into treated crops (75th percentile) - adı	7500	cm^2/h		*d_TcEntryAd*
Transfer coefficient for entry into treated crops (75th percentile) - chi	2250	cm^2/h		*d_TcEntryCh*
Transfer coefficient for entry into treated crops (mean) - adult	5980	cm^2/h		*d_TcEntryAd*
Transfer coefficient for entry into treated crops (mean) - child	1794	cm^2/h		*d_TcEntryCh*

1. Total

1.1 1-3 year old child

	Spray drift (75th percentile)	Vapour (75th percentile)	Surface deposits (75th percentile)	Entry into treated crops (75th percentile)	All pathways (mean)
Total systemic exposure (mg a.s./day)	0,0149590	0,0107000	0,0015078	0,0367709	0,0510356
Total systemic exposure per kg body weight	0,0014959	0,0010700	0,0001508	0,0036771	0,0051036
% of RVNAS	14,96%	10,70%	1,51%	36,77%	51,04%

1.2 Adult

	Spray drift	Vapour	Surface deposits	Entry into treated crops	All pathways (mean)
Total systemic exposure (mg a.s./day)	0,0492363	0,0138000	0,0024417	0,1225697	0,1456178
Total systemic exposure per kg body weight	0,0008206	0,0002300	0,0000407	0,0020428	0,0024270
% of RVNAS	8,21%	2,30%	0,41%	20,43%	24,27%

Table A 47A: Estimation of resident exposure using EFSA model; grapes, tractor-mounted air-blast sprayer, upwards; 3x 0.45 kg product/ha at 7 days interval, 10 m buffer zone, DRT, 400 L water min, DA dry residue 5.7% for re-entry only

Resident exposure for Reboot				
Croptype	Grapes			
Application method	Upward spraying			
Application equipment	Vehicle-mounted-Drift Reduction			*i_AppEquip*
Formulation type	Wettable granules, soluble granules			*i_FormVal*
Buffer strip	10	m		*i_Buffer*
Application rate of the product	0,1485	kg a.s./ha		*i_AppRate*
Concentration of active substance (in-use dilution for liquid applications)	0,37125	g a.s./l		*d_ConcAS*
Dermal absorption of product	0,13%			*i_AbsorpProduct*
Dermal absorption of in-use dilution	5,70%			*i_AbsorpInuse*
Oral absorption	75,00%			*i_AbsorpOralInuse*
Dislodgeable foliar residue (i_AppRate*i_DFR)	0,4455	µg a.s./cm^2		*d_DFR*
Vapour pressure of in-use dilution	low volatile substances having a vapour pressure of <5*10-3Pa	Pa		*i_Volat*
Concentration in air	0,001	mg/m^3		*d_AirCon*
Resident dermal spray drift exposure 75th percentile - adult	5,63	ml spray dilution/person		
Resident dermal spray drift exposure 75th percentile - child	1,689	ml spray dilution/person		
Resident inhal. spray drift exposure 75th percentile - adult	0,00210	ml spray dilution/person		
Resident inhal. spray drift exposure 75th percentile - child	0,00164	ml spray dilution/person		
Resident dermal spray drift exposure mean - adult	3,68	ml spray dilution/person		
Resident dermal spray drift exposure mean - child	1,11	ml spray dilution/person		
Resident inhal. spray drift exposure mean - adult	0,00170	ml spray dilution/person		
Resident inhal. spray drift exposure mean - child	0,00133	ml spray dilution/person		
Exposure duration dermal	2	hours		*d_ReExpDur*
Exposure duration inhalation	24	hours		*d_ReExpDurInhal*
Exposure duration entry into treated crops	0,25	hours		*d_ExpDurTreatCrop*
Light clothing adjustment factor	18,0%			*d_ClothAF*
Breathing rate adult	0,23	m^3/day/kg		*d_BreathRAd*
Breathing rate child (1-3 year old)	1,07	m^3/day/kg		*d_BreathRCh*
Drift percentage on surface (75th percentile)	1,02%			
Drift percentage on surface (mean)	0,77%			
Turf transferable residues percentage	5,00%			*d_Turf*
Transfer coeff. of surface deposits-adult	7300	cm^2/hour		*d_ReTCAd*
Transfer coeff. of surface deposits-child (1-3 year old)	2600	cm^2/hour		*d_ReTCCh*
Saliva extraction percentage	50,00%			*d_SalExt*
Surface area of hands mouthed	20	cm^2		*d_AreaHM*
Frequency of hand to mouth activity	9,5	events/hour		*d_ReFreqHM*
Ingestion rate for mouthing of grass per day	25	cm^2		*d_MouthGrass*
Dislodgeable residues percentage transferability for object to mouth	20,00%			*d_DRP*
Transfer coefficient for entry into treated crops (75th percentile) - adı	7500	cm^2/h		*d_TcEntryAd*
Transfer coefficient for entry into treated crops (75th percentile) - chi	2250	cm^2/h		*d_TcEntryCh*
Transfer coefficient for entry into treated crops (mean) - adult	5980	cm^2/h		*d_TcEntryAd*
Transfer coefficient for entry into treated crops (mean) - child	1794	cm^2/h		*d_TcEntryCh*

1. Total

1.1 1-3 year old child

	Spray drift (75th percentile)	Vapour (75th percentile)	Surface deposits (75th percentile)	Entry into treated crops (75th percentile)	All pathways (mean)
Total systemic exposure (mg a.s./day)	0,0149590	0,0107000	0,0005010	0,0367709	0,0502743
Total systemic exposure per kg body weight	0,0014959	0,0010700	0,0000501	0,0036771	0,0050274
% of RVNAS	14,96%	10,70%	0,50%	36,77%	50,27%

1.2 Adult

	Spray drift	Vapour	Surface deposits	Entry into treated crops	All pathways (mean)
Total systemic exposure (mg a.s./day)	0,0492363	0,0138000	0,0008112	0,1225697	0,1443850
Total systemic exposure per kg body weight	0,0008206	0,0002300	0,0000135	0,0020428	0,0024064
% of RVNAS	8,21%	2,30%	0,14%	20,43%	24,06%

Table A 48: Estimation of resident exposure using EFSA model; grapes, tractor-mounted air-blast sprayer, upwards; 3x 0.45 kg product/ha at 7 days interval, 10 m buffer zone, DRT, 475 L water min

Substance	Cymoxanil	Formulation = Wettable granules, soluble granules	Application rate- 0.1485 kg a.s. /ha	Spray dilution = 0.312631579 g a.s./l	Vapour pressure = low volatile substances having a vapour pressure of <5*10-3Pa
Scenario	Grapes / Outdoor / Upward spraying / Vehicle-mounted-Drift Reduction			Buffer = 10	Number applications = 3, Application interval = 7 days
Percentage Absoprtion	Dermal for product = 0.13	Dermal for in use dilution = 35	Oral = 75	Inhalation = 100	
RVNAS	0.01 mg/kg bw/day		RVAAS	0 mg/kg bw/day	
DFR	1.6 µg a.s./cm2 per kg a.s./ha		DT50	1 days	

Resident - child	Spray drift (75th percentile) mg/kg bw/day	0.0076	% of RVNAS	76.03%
	Vapour (75th percentile) mg/kg bw/day	0.0011	% of RVNAS	10.70%
	Surface deposits (75th percentile) mg/kg bw/day	0.0001	% of RVNAS	0.78%
	Entry into treated crops (75th percentile) mg/kg bw/day	0.0047	% of RVNAS	47.15%
	All pathways (mean) mg/kg bw/day	0.0099	% of RVNAS	98.88%
Resident - adult	Spray drift (75th percentile) mg/kg bw/day	0.0042	% of RVNAS	42.15%
	Vapour (75th percentile) mg/kg bw/day	0.0002	% of RVNAS	2.30%
	Surface deposits (75th percentile) mg/kg bw/day	0.0000	% of RVNAS	0.33%
	Entry into treated crops (75th percentile) mg/kg bw/day	0.0026	% of RVNAS	26.19%
	All pathways (mean) mg/kg bw/day	0.0051	% of RVNAS	50.99%

Table A 49: **Estimation of resident exposure using EFSA model; potatoes open field, tractor-mounted boom sprayer; 3x 0.45 L product/ha at 7 days interval**

Substance	Cymoxanil	Formulation = Wettable granules, soluble granules	Application rate- 0.1485 kg a.s. /ha	Spray dilution = 0.7425 g a.s./l	Vapour pressure = low volatile substances having a vapour pressure of <5*10-3Pa
Scenario	Root and tuber vegetables / Outdoor / Downward spraying / Vehicle-mounted			Buffer = 2-3	Number applications = 3, Application interval = 7 days
Percentage Absoprtion	Dermal for product = 0.13	Dermal for in use dilution = 35	Oral = 75	Inhalation = 100	
RVNAS	0.01 mg/kg bw/day		RVAAS	0 mg/kg bw/day	
DFR	2 µg a.s./cm2 per kg a.s./ha		DT50	1 days	

Resident - child	Spray drift (75th percentile) mg/kg bw/day	0.0070	% of RVNAS	69.85%
	Vapour (75th percentile) mg/kg bw/day	0.0011	% of RVNAS	10.70%
	Surface deposits (75th percentile) mg/kg bw/day	0.0009	% of RVNAS	8.54%
	Entry into treated crops (75th percentile) mg/kg bw/day	0.0059	% of RVNAS	58.93%
	All pathways (mean) mg/kg bw/day	0.0102	% of RVNAS	102.42%
Resident - adult	Spray drift (75th percentile) mg/kg bw/day	0.0017	% of RVNAS	16.71%
	Vapour (75th percentile) mg/kg bw/day	0.0002	% of RVNAS	2.30%
	Surface deposits (75th percentile) mg/kg bw/day	0.0004	% of RVNAS	3.57%
	Entry into treated crops (75th percentile) mg/kg bw/day	0.0033	% of RVNAS	32.74%
	All pathways (mean) mg/kg bw/day	0.0039	% of RVNAS	38.96%

Table A 50: Estimation of resident exposure using EFSA model; potatoes open field, tractor-mounted boom sprayer; 3x 0.45 L product/ha at 7 days interval, DA dry residue 5.7% for re-entry only

Substance	Cymoxanil	Formulation = Wettable granules, soluble granules	Application rate- 0.1485 kg a.s. /ha	Spray dilution = 0.7425 g a.s./l	Vapour pressure = low volatile substances having a vapour pressure of <5*10-3Pa
Scenario	Root and tuber vegetables / Outdoor / Downward spraying / Vehicle-mounted			Buffer = 2-3	Number applications = 3, Application interval = 7 days
Percentage Absoprtion	Dermal for product = 0.13	Dermal for in use dilution = 5.7/35	Oral = 75	Inhalation = 100	
RVNAS	0.01 mg/kg bw/day		RVAAS	0 mg/kg bw/day	
DFR	2 µg a.s./cm2 per kg a.s./ha		DT50	1 days	

Resident - child	Spray drift (75th percentile) mg/kg bw/day	0.0070	% of RVNAS	69.85%
	Vapour (75th percentile) mg/kg bw/day	0.0011	% of RVNAS	10.70%
	Surface deposits (75th percentile) mg/kg bw/day	0.0009	% of RVNAS	8.54%
	Entry into treated crops (75th percentile) mg/kg bw/day	0.0010	% of RVNAS	9.60%
	All pathways (mean) mg/kg bw/day	0.0063	% of RVNAS	63.09%
Resident - adult	Spray drift (75th percentile) mg/kg bw/day	0.0017	% of RVNAS	16.71%
	Vapour (75th percentile) mg/kg bw/day	0.0002	% of RVNAS	2.30%
	Surface deposits (75th percentile) mg/kg bw/day	0.0004	% of RVNAS	3.57%
	Entry into treated crops (75th percentile) mg/kg bw/day	0.0005	% of RVNAS	5.33%
	All pathways (mean) mg/kg bw/day	0.0017	% of RVNAS	17.10%

Table A 51: Estimation of resident exposure using EFSA model; potatoes open field, tractor-mounted boom sprayer; 3x 0.45 L product/ha at 7 days interval, water 250 L min

Substance	Cymoxanil	Formulation = Wettable granules, soluble granules	Application rate- 0.1485 kg a.s. /ha	Spray dilution = 0.594 g a.s./l	Vapour pressure = low volatile substances having a vapour pressure of <5*10-3Pa
Scenario	Root and tuber vegetables / Outdoor / Downward spraying / Vehicle-mounted			Buffer = 2-3	Number applications = 3, Application interval = 7 days
Percentage Absoprtion	Dermal for product = 0.13	Dermal for in use dilution = 35	Oral = 75	Inhalation = 100	
RVNAS	0.01 mg/kg bw/day		RVAAS	0 mg/kg bw/day	
DFR	2 µg a.s./cm2 per kg a.s./ha		DT50	1 days	

Resident - child	Spray drift (75th percentile) mg/kg bw/day	0.0056	% of RVNAS	55.88%
	Vapour (75th percentile) mg/kg bw/day	0.0011	% of RVNAS	10.70%
	Surface deposits (75th percentile) mg/kg bw/day	0.0009	% of RVNAS	8.54%
	Entry into treated crops (75th percentile) mg/kg bw/day	0.0059	% of RVNAS	58.93%
	All pathways (mean) mg/kg bw/day	0.0095	% of RVNAS	94.73%
Resident - adult	Spray drift (75th percentile) mg/kg bw/day	0.0013	% of RVNAS	13.36%
	Vapour (75th percentile) mg/kg bw/day	0.0002	% of RVNAS	2.30%
	Surface deposits (75th percentile) mg/kg bw/day	0.0004	% of RVNAS	3.57%
	Entry into treated crops (75th percentile) mg/kg bw/day	0.0033	% of RVNAS	32.74%
	All pathways (mean) mg/kg bw/day	0.0037	% of RVNAS	37.37%

Appendix 4 Detailed evaluation of exposure and/or DFR studies relied upon (KCP 7.2, KCP 7.2.1.1, KCP 7.2.2.1, KCP 7.2.3.1)

Report:	**KCP 7.2/01: Jullian E., 2014**
Title:	Cymoxanil: Quantification of dislodgeable foliar residues following five applications of Vitene Ultra to grapevines in northern France and Italy, 2013
Report no.:	S13-01291
GLP:	Yes
Guidelines:	EU 1999: 1607/VI/97, OECD Test Guideline 504; SANCO/3029/99 rev. 4 SANCO/825/00 rev. 8.1; Guideline 7029/VI/95 (rev. 5) to Directive 91/414/EEC and Regulations (EU) 283/2013 and 284/2013 implementing Regulation (EC) 1107/2009 (for residue studies); OECD Series on Testing and Assessment No. 9 ”Guidance document on the conduct of studies of occupational exposure to pesticides during agricultural application ”, Paris 1997. OCDE/GD(97)148; U.S. EPA Series 875.2100 Occupational and Residential Exposure Test Guidelines. Foliar Dislodgeable Residue Dissipation
Published:	No
Deviations:	None
Comment:	All study validity criteria were considered to have been met

Executive Summary

The objective of the study was to quantify the amount of cymoxanil residue that can be dislodged from grapevine leaves following five applications of Vitene Ultra. (cymoxanil 225 g/L), applied at 0.66 L product/ha, diluted with water immediately prior to application to a target spray volume of 500-1000 L/ha.

Two dislodgeable foliar residue trials were conducted on grapevine during 2013 in Northern France (S13-01291-01) and Italy (S13-01291-02).
Since the dislodgeable residue results were in good accordance between the two trials, the ten values obtained just after treatment were considered together, and a median value was calculated as it was considered the more representative parameter. A median value of 0.16 µg/cm2 per 148.5 g a.s./ha, corresponding to about 1.1 µg/cm2 kg a.s./ha was obtained, to be used in re-entry evaluation. Mean DT50 between the two trials was 0.93 days.

I. MATERIALS AND METHODS

Two dislodgeable foliar residue trials were conducted on grapevine during 2013 in Northern France (S13-01291-01) and Italy (S13-01291-02).

Following each application of Vitene Ultra, an SC formulation containing 225 g/L cymoxanil, leaf disc samples were taken at pre-defined sampling times pre- and post-application. The foliar residues were dislodged using an aqueous solution of a surfactant and the dislodged solution specimens were analyzed for cymoxanil.

Leaf disc specimens were collected from the untreated and treated plots and were taken using a birkestrand precision leaf punch, with leaf punch diameter of 2.523cm and leaf surface area of 10cm^2 (5cm^2 x 2 surfaces). Leaf disc specimens were collected from the untreated plot, before the first application, after the last application and 26-28 days after the last application. Leaf disc specimens were collected from the treated plot before and after each application, and 1, 2, 3, 5, 7, 10, 14 and 26-28 days after the last application.

After sampling, the leaf discs were transported to a field lab where the foliage was mechanically shaken for 15 minutes with two sequential 100mL washes with an aqueous solution of 0.01% Aerosol OT100 (dislodging solution). Following each wash and shake, the wash solution was transferred into a single glass 500mL jar of 'Total washings' and the remaining leaf disc foliage discarded. Before freezing 1mL of 1% formic acid was added to each 'Total washings' specimens.

The analytical method applied in this analytical phase was validated under GLP compliance in the Huntingdon Life science Project Identity LRP0003 (30th November 2009 – Author Howard Harper – Sponsor Sipcam Oxon S.p.A.). The whole frozen sample was de-frozen at room temperature and mixed using a glass rod, a portion of the homogeneous sample (20mL) was extracted with dichloromethane, concentrated to dryness and reconstituted with a mixture water/acetone/formic acid 75/25/0.05 v/v/v prior to be analysed by an HPLC system coupled with a triple quadropole mass analyser (LC-MS/MS).

The limit of quantitation for cymoxanil was set at 0.01 µg/mL / 0.005 µg/cm^2. Procedural recoveries run concurrently with test specimen at levels of 0.01, 0.10 and 1.0 µg/mL gave an overall mean recovery of 93.8%.

II. RESULTS

Residues of cymoxanil were undetectable (less than 0.01 µg/cm^2) in all untreated leaf washing specimens. Cymoxanil showed a rapid degradation with residues falling below detection limit starting from two days after the last application at trial S13-1291-02 (Italy) or five days after the last application at trial S12-01291-01 (Northern France). DT 50 based on SFO kinetics were calculated resulting in 0.771 and 1.08 days for S12-01291-01 (Northern France) and S13-1291-02 (Italy) trials respectively.

Table A 50: Summary of cymoxanil residues in leaf washing specimens (Northern France)

Sampling code	Sample timing	Plot	Specimen code	Cymoxanil Residue Level Mean Value of Replicates	
				(µg/mL)	(µg/cm^2)**
S2	0DAA1	P2	L13-01291-01-022	0.4608	0.2389
			L13-01291-01-023		
			L13-01291-01-024		
S3	0DBA2	P2	L13-01291-01-028	<0.01 (n.d.)	<0.005 (n.d.)
			L13-01291-01-029		
			L13-01291-01-030		
S4	0DAA2	P2	L13-01291-01-034	0.3193	0.1597
			L13-01291-01-035		
			L13-01291-01-036		
S5	0DBA3	P2	L13-01291-01-040	<0.01 (n.d.)	<0.005 (n.d.)
			L13-01291-01-041		
			L13-01291-01-042		
S6	0DAA3	P2	L13-01291-01-046	0.4561	0.2281
			L13-01291-01-047		
			L13-01291-01-048		
S7	0DBA4	P2	L13-01291-01-052	<0.01 (n.d.)	<0.005 (n.d.)

Sampling code	Sample timing	Plot	Specimen code	Cymoxanil Residue Level Mean Value of Replicates	
				(µg/mL)	(µg/cm²)**
			L13-01291-01-053		
			L13-01291-01-054		
S8	0DAA4	P2	L13-01291-01-058*	0.2385	0.1193
			L13-01291-01-059*		
			L13-01291-01-060*		
S9	0DBA5	P2	L13-01291-01-064	<0.01 (n.d.)	<0.005 (n.d.)
			L13-01291-01-065		
			L13-01291-01-066		
S10	0DAA5	P2	L13-01291-01-088	0.4429	0.2215
			L13-01291-01-089		
			L13-01291-01-090		
S12	2DAA5	P2	L13-01291-01-100	0.0256	0.0128
			L13-01291-01-101		
			L13-01291-01-102		
S13	3DAA5	P2	L13-01291-01-106	0.0232	0.0116
			L13-01291-01-107		
			L13-01291-01-108		
S14	5DAA5	P2	L13-01291-01-112	<0.01 (n.d.)	<0.005 (n.d.)
			L13-01291-01-113		
			L13-01291-01-114		
S15	7DAA5	P2	L13-01291-01-118	<0.01 (n.d.)	<0.005 (n.d.)
			L13-01291-01-119		
			L13-01291-01-120		
S16	10DAA5	P2	L13-01291-01-124	<0.01 (n.d.)	<0.005 (n.d.)
			L13-01291-01-125		
			L13-01291-01-126		
S17	14DAA5	P2	L13-01291-01-130	<0.01 (n.d.)	<0.005 (n.d.)
			L13-01291-01-131		
			L13-01291-01-132		
S18	20DAA5	P2	L13-01291-01-136	<0.01 (n.d.)	<0.005 (n.d.)
			L13-01291-01-137		
			L13-01291-01-138		
S19	26DAA5	P2	L13-01291-01-160	<0.01 (n.d.)	<0.005 (n.d.)
			L13-01291-01-161		
			L13-01291-01-162		
S11	1DAA5	P2	L13-01291-01-094	0.2281	0.1141
			L13-01291-01-095		
			L13-01291-01-096		

Note: DBA = days before application; DAA = days after application; n.d. = not determined
* Samples re-extracted a second time due to high residue level, for this reason following Test site SOPs they compare on analytical raw data as L13-01291-01-058/1a ; L13-01291-01-059/1a ; L13-01291-01-060/1a
** µg/cm²: data calculated considering that 200mL of washing solution were used to wash 400 cm² of leaves, therefore µg/cm²= (µg/mL)*200mL / 400cm²

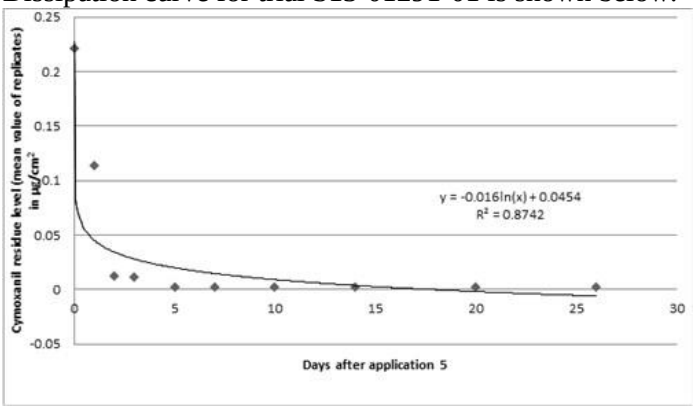
Table A 51: Summary of cymoxanil residues in leaf washing specimens (Italy)

Sam-pling code	Sam-ple timing	Plot	Specimen code	Cymoxanil Residue Level Mean Value of Replicates	
				(µg/mL)	(µg/cm^2)**
S2	0DAA 1	P2	L13-01291-02-022 L13-01291-02-023 L13-01291-02-024	0.1982	0.0991
S3	0DBA 2	P2	L13-01291-02-028 L13-01291-02-029 L13-01291-02-030	<0.01 (n.d.)	<0.005 (n.d.)
S4	0DAA 2	P2	L13-01291-02-034 L13-01291-02-035 L13-01291-02-036	0.2529	0.1265
S5	0DBA 3	P2	L13-01291-02-040 L13-01291-02-041 L13-01291-02-042	<0.01 (n.d.)	<0.005 (n.d.)
S6	0DAA 3	P2	L13-01291-02-046 L13-01291-02-047 L13-01291-02-048	0.3323	0.1662
S7	0DBA 4	P2	L13-01291-02-052 L13-01291-02-053 L13-01291-02-054	<0.01 (n.d.)	<0.005 (n.d.)
S8	0DAA 4	P2	L13-01291-02-058 L13-01291-02-059 L13-01291-02-060	0.2723	0.1362
S9	0DBA 5	P2	L13-01291-02-064 L13-01291-02-065 L13-01291-02-066	<0.01 (n.d.)	<0.005 (n.d.)
S10	0DAA 5	P2	L13-01291-02-088 L13-01291-02-089 L13-01291-02-090	0.4214	0.2107
S11	1DAA 5	P2	L13-01291-02-094 L13-01291-02-095 L13-01291-02-096	0.4358	0.2179
S12	2DAA 5	P2	L13-01291-02-100 L13-01291-02-101 L13-01291-02-102	<0.01 (0.0069)	<0.005 (0.0035)
S13	3DAA 5	P2	L13-01291-02-106 L13-01291-02-107 L13-01291-02-108	<0.01 (n.d.)	<0.005 (n.d.)
S14	5DAA 5	P2	L13-01291-02-112 L13-01291-02-113 L13-01291-02-114	<0.01 (n.d.)	<0.005 (n.d.)
S15	7DAA 5	P2	L13-01291-02-118 L13-01291-02-119 L13-01291-02-120	<0.01 (n.d.)	<0.005 (n.d.)
S16	10DAA5	P2	L13-01291-02-124 L13-01291-02-125 L13-01291-02-126	<0.01 (n.d.)	<0.005 (n.d.)
S17	14DA	P2	L13-01291-02-130	<0.01 (n.d.)	<0.005 (n.d.)

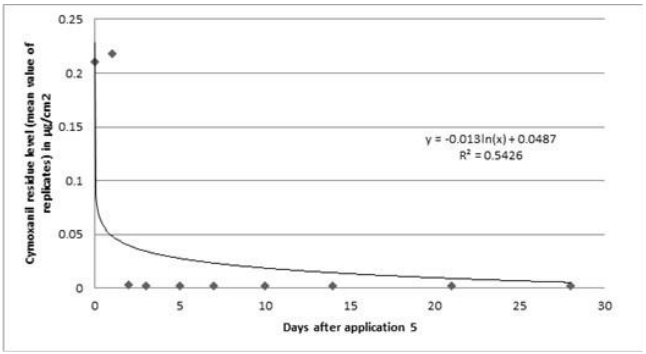
Sampling code	Sample timing	Plot	Specimen code	Cymoxanil Residue Level Mean Value of Replicates	
				(µg/mL)	(µg/cm^2)**
	A5		L13-01291-02-131		
			L13-01291-02-132		
S18	20DA A5	P2	L13-01291-02-136	<0.01 (n.d.)	<0.005 (n.d.)
			L13-01291-02-137		
			L13-01291-02-138		
S19	26DA A5	P2	L13-01291-02-160	<0.01 (n.d.)	<0.005 (n.d.)
			L13-01291-02-161		
			L13-01291-02-162		

Note: DBA = days before application ;DAA = days after application; n.d. = not determined
** µg/cm^2: data calculated considering that 200mL of washing solution were used to wash 400 cm^2 of leaves, therefore µg/cm^2= (µg/mL)*200mL / 400cm^2

Dissipation curve for trial S13-01291-01 is shown below.



Dissipation curve for trial S13-01291-02 is shown below.



III. CONCLUSION

Since the dislodgeable residue results were in good accordance between the two trials, the ten values obtained just after treatment were considered together, and a median value was calculated as it was considered the more representative parameter. A median value of 0.16 µg/cm2 per 148.5 g a.s./ha, corresponding to about 1.1 µg/cm2 kg a.s./ha was obtained, to be used in re-entry evaluation. Mean DT50 between the two trials was 0.93 days.

zRMS: The dislogable residue of cymoxanil on grape leaves was found to be 0.16 µg/cm2 after application of Vitene Ultra, an SC formulation containing 225 g/L cymoxanil at a rate of 0.1485 kg of cymoxanil /ha, thus at the same rate this active substance is applied according to GAP of Cymoxanil 33% + Zox-

amide 33% WG (Lieto 66 WG). Thus DFR for cymoxanil is equal 1.1 µg/cm2 kg a.s./ha. It is noted that DT_{50} of cymoxanil residue is below 1 day. The study is performed according to internationally recognized guidelines and is acceptable. The DFR of 1.1 µg/cm^2 kg a.s./ha can be used for calculation of worker exposure after application of formulation Cymoxanil 33% + Zoxamide 33% WG (Lieto 66 WG)
However, the worst-case residue result (0.2389 corresponding to 1.6 µg/cm^2 kg a.s./ha is being proposed in place of the median value to be more conservative.

Report:	**KCP 7.2/02: Jullian E., 2014**
Title:	Cymoxanil Quantification of dislodgeable foliar residues following six applications of Vitene Ultra to potato in the United Kingdom, 2013
Report no.:	S13-01293
GLP:	Yes
Guidelines:	EU 1999: 1607/VI/97, OECD Test Guideline 504; SANCO/3029/99 rev. 4 SANCO/825/00 rev. 8.1; Guideline 7029/VI/95 (rev. 5) to Directive 91/414/EEC and Regulations (EU) 283/2013 and 284/2013 implementing Regulation (EC) 1107/2009 (for residue studies); OECD Series on Testing and Assessment No. 9 ”Guidance document on the conduct of studies of occupational exposure to pesticides during agricultural application ”, Paris 1997. OCDE/GD(97)148; U.S. EPA Series 875.2100 Occupational and Residential Exposure Test Guidelines. Foliar Dislodgeable Residue Dissipation
Published:	No
Deviations:	None
Comment:	All study validity criteria were considered to have been met

Executive summary

The objective of the study was to quantify the amount of cymoxanil residue that can be dislodged from potato leaves following six applications of Vitene Ultra. One dislodgeable foliar residue trial was conducted on potato during 2013 in the UK (S13-01293-01). Six applications of Vitene Ultra (cymoxanil 225 g/L) were applied at 0.66 L product/ha, diluted with water immediately prior to application to a target spray volume of 300 L/ha.

Residues of cymoxanil were undetectable (less than 0.01 µg/cm^2) in all untreated leaf washing specimens. Cymoxanil showed a rapid degradation with residues falling below the detection limit starting from five days after the last application. DT 50 based on SFO kinetics were calculated resulting in 0.771 and 1.08 days for S12-01291-01 (Northern France) and S13-1291-02 (Italy) trials respectively.
DT50 was 0.9535 days and will be approximated to 1 for risk assessment purpose.

I. MATERIAL AND METHODS

The objective of the study was to quantify the amount of cymoxanil residue that can be dislodged from potato leaves following six applications of Vitene Ultra.

One dislodgeable foliar residue trial was conducted on potato during 2013 in the UK (S13-01293-01). Six applications of Vitene Ultra (cymoxanil 225 g/L) were applied at 0.66 L product/ha, diluted with water immediately prior to application to a target spray volume of 300 L/ha.

Following each application of Vitene Ultra, an SC formulation containing 225 g/L cymoxanil, leaf disc samples were taken at pre-defined sampling times pre and post application. The foliar residues were dislodged using an aqueous solution of a surfactant and the dislodged solution specimens were analyzed for cymoxanil.

Leaf disc specimens were collected from the untreated and treated plots and were taken using a birkestrand precision leaf punch, with leaf punch diameter of 2.523cm and leaf surface area of 10cm^2 (5cm^2 x 2 surfaces). Leaf disc specimens were collected from the untreated plot, before the first and the last application, and 14 days after the last application. Leaf disc specimens were collected from the treated plot after the first application, before and after each other applications and 1, 2, 3, 5, 7, 10, and 14 days after the last application

After sampling, the leaf discs were transported to a field lab where the foliage was mechanically shaken for 15 minutes with two sequential 100mL washes with an aqueous solution of 0.01% Aerosol OT100 (dislodging solution). Following each wash and shake, the wash solution was transferred into a single glass 500mL jar of 'Total washings' and the remaining leaf disc foliage discarded. Before freezing 1mL of 1% formic acid was added to each 'Total washings' specimens.

The analytical method applied in this analytical phase was validated under GLP compliance in the Huntingdon Life science Project Identity LRP0003 (30th November 2009 – Author Howard Harper – Sponsor Sipcam Oxon S.p.A.). The whole frozen sample was de-frozen at room temperature and mixed using a glass rod, a portion of the homogeneous sample (20mL) was extracted with dichloromethane, concentrated to dryness and reconstituted with a mixture water/acetone/formic acid 75/25/0.05 v/v/v prior to be analysed by an HPLC system coupled with a triple quadrupole mass analyser (LC-MS/MS).

The limit of quantitation for cymoxanil was set at 0.01 µg/mL / 0.005 µg/cm^2.Procedural recoveries run concurrently with test specimen at levels of 0.01, 0.10 and 1.0 µg/mL gave an overall mean recovery of 86.04%.

II.RESULTS

Residues of cymoxanil were undetectable (less than 0.01 µg/cm^2) in all untreated leaf washing specimens. Cymoxanil showed a rapid degradation with residues falling below the detection limit starting from five days after the last application. DT 50 based on SFO kinetics were calculated resulting in 0.771 and 1.08 days for S12-01291-01 (Northern France) and S13-1291-02 (Italy) trials respectively.

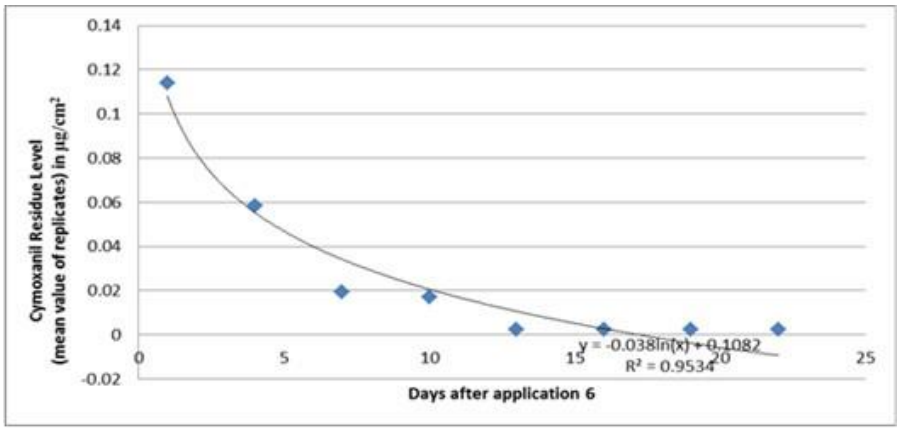
Table A 52: Summary of cymoxanil residues in leaf washing specimens

Sampling code	Sample timing	Plot	Specimen code	Cymoxanil Residue Level Mean Value of Replicates	
				(µg/mL)	(µg/cm^2)**
S2	0DAA1	P2	L13-01293-01-022	0.5602	0.2801
			L13-01293-01-023		
			L13-01293-01-024		
S3	0DBA2	P2	L13-01293-01-028	<0.01 (n.d.)	<0.005 (n.d.)
			L13-01293-01-029		
			L13-01293-01-030		
S4	0DAA2	P2	L13-01293-01-034	0.5964	0.2982
			L13-01293-01-035		
			L13-01293-01-036		
S5	0DBA3	P2	L13-01293-01-040	<0.01 (n.d.)	<0.005 (n.d.)
			L13-01293-01-041		
			L13-01293-01-042		

S6	0DAA3	P2	L13-01293-01-046	0.0804	0.0402
			L13-01293-01-047		
			L13-01293-01-048		
S7	0DBA4	P2	L13-01293-01-052	<0.01 (n.d.)	<0.005 (n.d.)
			L13-01293-01-053		
			L13-01293-01-054		
S8	0DAA4	P2	L13-01293-01-058	0.2855	0.1428
			L13-01293-01-059		
			L13-01293-01-060		
S9	0DBA5	P2	L13-01293-01-064	<0.01 (n.d.)	<0.005 (n.d.)
			L13-01293-01-065		
			L13-01293-01-066		
S10	0DAA5	P2	L13-01293-01-070	0.1403	0.0702
			L13-01293-01-071		
			L13-01293-01-072		
S11	0DBA6	P2	L13-01293-01-076	<0.01 (n.d.)	<0.005 (n.d.)
			L13-01293-01-077		
			L13-01293-01-078		
S12	0DAA6	P2	L13-01293-01-100	0.2281	0.1141
			L13-01293-01-101		
			L13-01293-01-102		
S13	1DAA6	P2	L13-01293-01-106	0.1165	0.0583
			L13-01293-01-107		
			L13-01293-01-108		
S14	2DAA6	P2	L13-01293-01-112	0.0390	0.0195
			L13-01293-01-113		
			L13-01293-01-114		
S15	3DAA6	P2	L13-01293-01-118	0.0339	0.0170
			L13-01293-01-119		
			L13-01293-01-120		
S16	5DAA6	P2	L13-01293-01-124	<0.01 (n.d.)	<0.005 (n.d.)
			L13-01293-01-125		
			L13-01293-01-126		
S17	7DAA6	P2	L13-01293-01-130	<0.01 (n.d.)	<0.005 (n.d.)
			L13-01293-01-131		
			L13-01293-01-132		
S18	10DAA6	P2	L13-01293-01-136	<0.01 (n.d.)	<0.005 (n.d.)
			L13-01293-01-137		
			L13-01293-01-138		
S19	14DAA6	P2	L13-01293-01-142	<0.01 (n.d.)	<0.005 (n.d.)
			L13-01293-01-143		
			L13-01293-01-144		

Note:DBA = days before application ;DAA = days after application; n.d. = not determined
** µg/cm^2: data calculated considering that 200mL of washing solution were used to wash 400 cm^2 of leaves, therefore µg/cm^2= (µg/mL)*200mL / 400cm^2

Dissipation curve for trial S13-01291-03 is shown below.



III. CONCLUSIONS

The highest DFR value of 0.2982 µg/cm2 per 148.5 g a.s./ha, corresponding to about 2 µg/cm2 kg a.s./ha was obtained just after the second application, to be used in re-entry evaluation. DT50 was 0.9535 days and will be approximated to 1 for risk assessment purpose. The trial was conducted in northern Europe, however, due to climatic conditions, this is normally representing a worst case for degradation. the information can, therefore, be used to refine risk assessment also for the southern zone.

zRMS: The dislogable residue of cymoxanil on potato leaves was found to be 0.2982 µg/cm^2 after application of Vitene Ultra, an SC formulation containing 225 g/L cymoxanil at a rate of 0.1485 kg of cymoxanil /ha, thus at the same rate this active substance is applied according to GAP of Cymoxanil 33% + Zoxamide 33% WG (Lieto 66 WG). Thus, DFR for cymoxanil on potato leaves is equal 2 µg/cm2 kg a.s./ha. It is noted that DT_{50} of cymoxanil residue is ca. 1 day. The study is performed according to internationally recognized guidelines and is acceptable. The DFR of 2.0 µg/cm2 kg a.s./ha can be used for calculation of worker exposure after application of formulation Cymoxanil 33% + Zoxamide 33% WG (Lieto 66 WG) on potatoes.