REGISTRATION REPORT

Part B

Section 6

Mammalian Toxicology

Detailed summary of the risk assessment

Product code: F7B-39-30

Product name: Rinpode

Chemical active substance: Florpyrauxifen-benzyl 25 g/l

Southern/Central Zones

Zonal Rapporteur Member State: France/Poland zRMS

CORE ASSESSMENT

Applicant: Corteva Agriscience

Submission date: March 2023

zRMS Assessment date: 27/11/2023

Following commenting round: 11/04/2024

References correction: 31/07/2024

Version history

When	What
March 2023	Submission to zRMS and concerned Member States
November 2023	zRMS assessment
April 2024	Following commenting round
July 2024	References correction

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6 Mammalian Toxicology (KCP 7)

6.0 Introduction

This application was submitted by Corteva Agriscience in March 2023.
The application is for the first approval of the formulation F7B-39-30 (trademark: Rinpode) as new post-emergence herbicide developed by Corteva Agriscience. The formulation is an EC (emulsion concentrate) containing 25 g/L of florpyrauxifen-benzyl (19.870 g a.e./L) for use as an herbicide in sugar beets.
F7B-39-30 is submitted to Southern and Central zones with France and Poland acting as zRMS respectively. Concerned Member States are Spain, Italy, Portugal, Greece, Croatia in Southern zone and Belgium, The Netherlands, Luxembourg, Hungary, Germany, Austria, Romania, Czech Republic, Romania, Slovakia in Central zone.

Florpyrauxifen-benzyl (trademark: Rinskor® active) is a New Active Substance (NAS), developed by Corteva Agrisciences, approved in accordance with Regulation (EC) No 1107/2009 on July 3rd, 2019. Details of the approval Regulation, Commission Review Report and EFSA R.O. are provided in the below table:

Active Substance	*Approval Regulation*	*SANCO/SANTE Review Report*	*EFSA Scientific Report*
Florpyrauxifen-benzyl (trademark: Rinskor® active)	Commission Implementing Regulation (EU) 2019/1138 of 3 July 2019	SANTE/10658/2019 rev2 of 21 May 2019	EFSA Journal 2018;16(8):5378. doi: 10.2903/j.efsa.2018.5378.

The Regulation (EU) 2019/1138 for Florpyrauxifen-benzyl (trademark: Rinskor® active) provides specific provisions under Part B which need to be considered by the applicant in the preparation of their submission and by the MS prior to granting an authorisation: "*For the implementation of the uniform principles as referred to in Article 29(6) of Regulation (EC) No 1107/2009, the conclusions of the review report on 21 March 2019, and in particular Appendices I and II thereof, shall be taken into account. In this overall assessment Member States shall pay particular attention to: — the protection of aquatic and terrestrial non-target plants. Conditions of use shall include risk mitigation measures such as buffer zones and/or drift reduction nozzles, where appropriate*."
These concerns have been addressed within the current submission, where not otherwise stated.

Florpyrauxifen-benzyl (trademark: Rinskor® active) is a foliar post-emergence herbicide effective to control the most import weeds present in rice paddies; it is not yet authorized for sugar beets. Florpyrauxifen-benzyl is a member of the arylpicolinate family of chemistry, a new structural class of synthetic auxin herbicides, Group O (according to HRAC MOA classification). F7B-39-30 is active at low use rates in post-emergence applications against broadleaf weeds in sugar-beet.

F7B-39-30 (trademark: Rinpode) is very similar to GF-3206 (trademark Loyant 25 Neo EC), with the addition of a food-grade dye, included in the composition at 0.0005% w/w. F7B-39-30 and GF-3206 are the same formulation type (emulsion concentrate) and contain equal amounts of active ingredient, antifoam, emulsifiers, solvents and adjuvant. The minimal difference in composition between F7B-39-30 and GF-3206 lead to toxicological and ecotoxicological properties that can be considered equivalent and in comparable performance on crop safety or efficacy. Based on comparability of both formulations, data generated with GF-3206 are used in support of the claim for F7B-39-30. GF-3206, which is authorized formulation since 2019 in all Southern Europe rice countries, is the representative formulation considered for the florpyrauxifen-benzyl (trademark: Rinskor® active) approval, so it was fully evaluated in the active substance European process.

Information on the detailed composition of F7B-39-30 or of the GF-3206 formulation used as read-across can be found in the CONFIDENTIAL dossier of this submission (draft Registration Report - Part C).

F7B-39-30 critical and Country GAP within the zones is given in Part B, Section 0.

6.1 Summary

Table 6.1-1: Information on F7B-39-30 (trademark: Rinpode) *

Trademark and code	Rinpode / F7B-39-30
Formulation type	Emulsifiable concentrate [EC]
Active substance(s) (incl. content)	florpyrauxifen-benzyl; 25g/L
Function	herbicide
Product already evaluated as the 'representative formulation' during the approval of the active substance(s)	No. But F7B-39-30 is very similar to GF-3206 (representative formulation considered for the EU approval of florpyrauxifen-benzy), with the addition of a food-grade dye, included in the composition at 0.0005% w/w. F7B-39-30 and GF-3206 are the same formulation type (emulsion concentrate) and contain equal amounts of active ingredient, antifoam, emulsifiers solvents and adjuvant. The minimal difference in composition between F7B-39-30 and GF-3206 lead to toxicological and ecotoxicological properties that can be considered equivalent and in comparable performance on crop safety or efficacy. Based on comparability of both formulations, data generated with GF-3206 are used in support of the claim for F7B-39-30.
Product previously evaluated in another MS according to Uniform Principles	No

* Information on the detailed composition of F7B-39-30 (Rinpode) 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 F7B-39-30 (Rinpode) according to Regulation (EC) No 1272/2008

~~The classification of the product Rinpode (F7B-39-30) is the following:~~

~~Aquatic Acute 1 H400~~

~~Aquatic Chronic 1 H410.~~

~~This classification was triggered by study data.~~

The labelling, and hazard, precautionary and additional statements proposals and justification for F7B-39-30, in accordance with Regulation (EC) No 1272/2008 on Classification, Labelling and Packaging of Substances and Mixtures (CLP Regulation), are presented below:

Hazard class(es), categories	~~Aquatic Chronic Cat 1.~~
Hazard pictograms or Code(s) for hazard pictogram(s)	~~GHS 09~~
Signal word	~~Warning~~
Hazard statement(s)	~~H410 Very toxic to aquatic life with long lasting effects.~~ ~~Chronic aquatic Cat 1.~~
Precautionary statement	P391 Collect spillage. ~~P501 Dispose of contents/container in accordance with applicable regulations.~~
Additional labelling phrases	EUH208 Contains Florpyrauxifen Benzyl. May produce an allergic reaction. (*Mixtures not classified as sensitizing but containing at least one sensitizing substance: The label on the packaging of mixtures containing at least one substance classified as sensitizing and present in a concentration equal to or greater than 0.1% or in a concentration equal to or greater than that specified under a specific note for the substance in part 3 of Annex VI shall bear this statement*) EUH401 To avoid risks to human health and the environment, comply with the instructions for use.

Table 6.1-3: Summary of risk assessment for operators, workers, residents and bystanders for F7B-39-30 (Rinpode)

	Result	PPE / Risk mitigation measures
Operators	Acceptable	None Only the work wear (long sleeved shirt, long trousers) during loading, mixing and application. ~~Gloves during mixing/loading/application due to hazard classification of F7B-39-30 (RINPODE)~~
Workers	Acceptable	None
Residents	Acceptable	None
Bystanders	Acceptable	None

No unacceptable risk for operators, workers, residents and bystanders was identified when the product is used as intended. No specific PPE is necessary. ~~F7B-39-30 (RINPODE) is classified as a Category 1B Skin~~

~~Sensitiser in accordance with Regulation (EC) No 1272/2008. Based on the product classification, gloves are required to be worn during the mixing and loading of F7B-39-30.~~

A summary of the critical uses and the overall conclusion regarding exposure for operators, workers and residents/bystanders is presented in the following table.
A list of all intended uses within the ~~South~~ Central zone is given in Part B, Section 0.

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, resident or bystander 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**	**Residents**	**Bystander**
1 or 4	Sugar beet: *Beta vulgaris* (BEAVA). *Fodder beet* (BEAVC)	F	Overall, Broadcast foliar spray; LCTM	a) 1 b) 4 (5 days)	1 x 0.002 or Min at 4 x 0.0005	100-300	NA	A maximum single dose rate at 2.0 g a.s/ha, that can be splitted in max. of 4 x 0.5 g a.s./ha	A	A	A	A

* 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

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6.2 Toxicological Information on Active Substance

Information regarding classification of the active substance florpyrauxifen-benzyl (trademark: *Rinskor® active*) and on EU endpoints and critical areas of concern identified during the EU review are given in Table 6.2-1.

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

	Florpyrauxifen-benzyl
Common Name	florpyrauxifen-benzyl (trademark: Rinskor® active)
CAS-No.	1390661-72-9
With regard to toxicological endpoints (according to the criteria in Reg. 1272/2008, as amended)	Hazard classes (s), categories: ~~NONE~~ Skin Sens. 1B Code(s) for hazard pictogram(s):  Signal word: ~~May cause an allergic skin reaction. H317~~ Warning Hazard statement(s): ~~Skin sensitisation Cat 1B~~ H317 May cause an allergic skin reaction. Precautionary statement(s): P280 Wear protective gloves, ~~/~~protective clothing. P302 + ~~/~~352 IF ON SKIN: Wash with plenty of water.
Additional C&L proposal	EUH401 To avoid risks to human health and the environment, comply with the instructions for use.
AOEL systemic	0.13 mg/kg bw/day (corrected for 25% derived from 2 year chronic rat study at the NOAEL of 50 mg/kg bw/day)
Reference	~~e.g.~~ EFSA Conclusion (EFSA Journal 2018;16(8):5378)
According to Review Report/EFSA Conclusion for active substance	None

Note of the notifier: The active substance has the hazard statement H317 (May cause an allergic skin reaction) assigned. However, the formulated product isn't classified as H317 since the skin sensitization study of GF-3206 was negative.

6.3 Toxicological Evaluation of Plant Protection Product

A summary of the toxicological evaluation for F7B-39-30 (Rinpode) is given in the following tables. Full summaries of studies on the product are based on study results from GF-3206. F7B-39-30 has identical composition with GF-3206, except for adding Sensipro Blue Pond in the formulation. As the blue dye is only at 0.0005% w/w and its SDS indicates not being classified for any toxicity. As such toxicological study results for GF-3206 can be used for F7B-39-30 including acute and dermal absorption studies. Studies 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 F7B-39-30

Type of test, species, model system (Guideline)	Species/ strain (sex)	Results/Endpoint	Classification (acc. to the criteria in Reg. 1272/2008)	Acceptability	Reference
				Yes / No / Supplementary	
Oral / Gavage (OECD 423, EC B.1)	Rat / Wistar	LD50 >5000 mg/kg body weight	Not Classified	Yes	[redacted], 2014
Dermal / Topical (OECD 402, EC B.3)	Rat / Wistar	LD50 >5000 mg/kg body weight	Not Classified	Yes	[redacted], 2014
Inhalation / Nose only (OECD 403, EC B.2)	Rat / Wistar	LC50 > 5.40 mg/L air	Not Classified	Yes	[redacted], 2014
Dermal / Topical (OECD 404, EC B.4)	Rabbit / NZW	Not classified as a skin irritant	Not Classified	Yes	[redacted] 2014
Eye / Instillation (OECD 405, EC B.5)	Rabbit / NZW	Not classified as an eye irritant	Not Classified	Yes	[redacted], 2014
Dermal Sensitization (OECD 406, EC B.6)	Guinea pig / Hartley	Not classified as Skin Sensitizer	Not Classified	Yes	[redacted], 2014
Supplementary studies for combinations of plant protection products	No data – not required				

Table 6.3-2: Additional toxicological information relevant for classification/labelling of F7B-39-30 (Rinpode)

	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)	florpyrauxifen-benzyl CAS 1390661-72-9, 2.5542% (w/w)	Not applicable, classification based on formulation testing as listed in Table 6.3-1		
Toxicological properties of non-active substance(s) (relevant for classification of product)	Not applicable	Not applicable, no additional hazards presented by non-active ingredients	Not applicable	None
Further toxicological information	No data – not required			

* Please use concentration range or concentration limit (e.g. 1-10% or > 1%) as provided in MSDS.
** Material safety data sheet by the applicant

6.4 Toxicological Evaluation of Groundwater Metabolites

The following data on ground water metabolite X12483137 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 this metabolite is reported in Part B.10; the submitted toxicological studies are summarised

in this document.

6.4.1 Groundwater Metabolite: X12483137

Comments of zRMS:	Studies for assessment of florpyrauxifen-benzyl groundwater metabolite X12483137 should be evaluated at the EU level.

An overview of the results of the accepted toxicological studies for groundwater metabolite X12483137 is given in the following table.

Table 6.4-1: Summary of the results of toxicity studies for X12483137

Type of test, species (Guideline)	Result	Acceptability	Reference*
Bacterial AMES (OECD 471)	Non-genotoxic	YES	Study ID 201938; Davis, 2020
HLCAT (In vitro Mammalian Cell Micronucleus Assay in Human Peripheral Blood Lymphocytes (HPBL) Chromosome Aberration Test) (OECD 487)	Non-genotoxic	YES	Study ID 201936; Xie, 2021
HGPRT (In vitro Mammalian Cell Gene Mutation Test) (OECD 476)	Non-genotoxic	YES	Study ID 201937; Miller, 2020

6.5 Dermal Absorption (KCP 7.3)

A summary of the dermal absorption rates for the active substances in F7B-39-30 (Rinpode) (Emulsifiable concentrate [EC]) are presented in the following table.

Table 6.5-1: Dermal absorption rates for active substances in F7B-39-30 (Rinpode)

	Florpyrauxifen-benzyl	
	Value	**Reference**
Concentrate	0.45%	EFSA Conclusion and CP-B.6.2 of DAR
Dilution (1:1000)	11%	EFSA Conclusion and CP-B.6.2 of DAR

6.5.1 Justification for proposed values - Florpyrauxifen-benzyl

Proposed dermal absorption rates for florpyrauxifen-benzyl are based on human skin in vitro dermal absorption study on GF-3206 which has identical composition of F7B-39-30 (Rinpode) except for adding the blue dye at 0.0005% w/w. The study results are summarised in the following table. The results of the experiments with GF-3206 are applicable for the risk assessment of the present application.

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

Test	Concentrate	Spray dilution (1:1000)	Formulation in study	Acceptability of study	Justification provided on representativity of study formulation for current product	Acceptability of justification	Reference*
In vitro (human)	0.45%	11%	GF-3206	Yes	Not required as data is generated on the current product	Endpoint can be used for current product.	151074; Brufau Donés, G. 2016

* indicates that a study was reviewed at EU level

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

F7B-39-30 (RINPODE)	
Formulation type	EC
Category	Herbicice
Active substance(s) (incl. content)	**Florpyrauxifen-benzyl** 25 g/L
AOEL systemic	0.13 mg/kg bw/d
Inhalation absorption	100%
Oral absorption	100%
Dermal absorption	Concentrate: 0.45% Dilution (0.025 g/L or 1:1000): 11% (not used in RA) Dilution (0.0067 g/L or 1:3750): 41%* Dilution (0.0017 g/L or 1:15000): 70% Cut-off value for dermal absorption** (Based on formulation GF-3206, study 151074 and considering linear extrapolation)

* Linear extrapolation was made based on the highest dilution for the max application rate (2 g a.s./ha in 300 L/ha, 0.0067 g/L or 1:3750); [0.025 g a.s./L (highest dilution tested) /0.0067 g a.s./L (target dilution)]*11% (highest dilution tested) = 41%).

**Following the highly conservative approach regarding the dermal absorption value taken in the New EFSA OPEX model version 1.0.0, the highest dermal absorption EFSA default should be taken for the cut-off; EFSA (2017) Guidance on Dermal Absorption, EFSA Journal 2017; 15(6):4873.

F7B-39-30 (RINPODE) is not classified on the basis of acute toxicity and florpyrauxifen-benzyl has no acute reference dose (ARfD).

6.6.1 Selection of critical use(s) and justification

The critical GAP(s) used for the exposure assessment of the plant protection product is shown in Table 6.1-4. A list of all intended uses within the South zone is given in Part B, Section 0.

6.6.2 Operator exposure (KCP 7.2.1)

Exposures resulting from the proposed uses of F7B-39-30 (RINPODE) have been evaluated using the 2022

EFSA model. Predicted exposures based on the highest proposed application rate and assuming no gloves are worn for application, mixing and loading, were 0.2%, of the AOEL for Florpyrauxifen-benzyl for tractor mounted applications using the EFSA model. ~~Additionally, the formulation F7B-39-30 is classified as a Category 1B Skin Sensitiser in accordance with criteria in Regulation EC/1272/2008. Therefore, based on the product classification, gloves are required to be worn during the mixing and loading of F7B-39-30, and on this case the exposure would be reduced even further.~~ There is no evidence for operator risk concerns.

6.6.2.1 Estimation of operator exposure

A summary of the exposure models used for estimation of operator exposure to the active substance during application of F7B-39-30 (RINPODE) according to the critical use(s) is presented in Table 6.6-2. The outcome of the estimation is presented in Table 6.6-3 (longer term exposure). Detailed calculations are in A 2.1. The EFSA model has been used to predict exposure for the standard tractor-mounted boom sprayer application.

Table 6.6-2: Exposure models for intended uses

Critical use(s)	Sugar beet (max. 1 x 0.08 L product/ha)
Model(s)	Guidance on the assessment of exposure of operators, workers, residents and bystanders in risk assessment for plant protection products; EFSA Journal 2022;20(1):7032 calculator version: 1.0.0 (new on-line EFSA OPEX calculator)

Table 6.6-3: Estimated operator exposure (longer term exposure)

		Florpyrauxifen-benzyl	
Model data	**Level of PPE**	**Total absorbed dose (mg/kg/day)**	**% of systemic AAOEL**
Tractor mounted boom spray application outdoors, downwards to Low vegetables Drift Reduction: 0% Crop Density: Normal Dermal absorption (concentrate): 0.45 % Dermal absorption (in-use dilution): 41.0 %			
Application rate		1 x 0.002 kg a.s./ha	
Spray application (AOEM**;** 75th percentile) Body weight: 60 kg Spray Volume 150 L/ha	Work wear (arms, body and legs covered) M/L and A	0.0003	0.2
	Work wear (arms, body and legs covered) M/L and A Gloves and body coverall	Not Available*	0.09

*The new on-line EFSA OPEX calculator version 1.0.0 doesn't report the total systemic exposure per kg body weight (mg/kg bw/day) beyond the 'passing scenario' in general.

6.6.2.2 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 use, a study to provide measurements of operator exposure was not necessary and was, therefore, not performed.

<table>
<tr><td>Study comment 6.6.2:</td><td>The applicant presented calculations for the application of Rinpode (F7B-39-30) on sugar beet: max dose 0.08 L/ha (Field). zRMS calculated of operator exposure for dose 4x0.02l/ha (Field).
<table>
<tr><td rowspan="2">Spray application (AOEM; 75th percentile)
Body weight: 60 kg
Spray Volume 150 L/ha</td><td>Work wear (arms, body and legs covered) M/L and A</td><td>0.0002</td><td>0.1</td></tr>
<tr><td>Work wear (arms, body and legs covered) M/L and A
Gloves and body coverall</td><td>Not Available*</td><td>0.09</td></tr>
</table>
The exposure calculations were conducted using the EFSA online calculator 2022 v 1.0.0. (OPEX).
The calculations provided by Applicant were done correctly.</td></tr>
<tr><td>Agreed endpoint 6.6.2:</td><td>According to EFSA OPEX calculations, it can be concluded that the risk of operator exposure during mixing & loading and application using the tractor-mounted on field on sugar beet is acceptable under conditions of intended use when the work wear (long sleeved shirt, long trousers) is worn during loading, mixing and application.

Thus, the operator using Rinpode (F7B-39-30) will be safe as long as he wears work wear.</td></tr>
</table>

6.6.3 Worker exposure (KCP 7.2.3)

No unacceptable risk for workers from the supported uses of F7B-39-30 (RINPODE) was identified based on exposure estimates from the 2022 EFSA model. The predicted worker exposure to Florpyrauxifen-benzyl was 0.1% of the respective AOEL, based on normal work wear and no additional PPE.
F7B-39-30 (RINPODE) will be applied to sugar beet up to a maximum growth stage BBCH 10-19, when the plants are very small. However, a conservative assessment of re-entry worker exposure has been undertaken according to the EFSA model. Due to the proposed timing of application, any expected re-entry into the treated crop will be for the purpose of crop inspection/irrigation only.
This assessment considers the potential for exposure resulting from most critical exposure scenario, on this case (4 x 0.0005 kg a.s./ha) and immediate re-entry and assumes PPE is not used. The major potential route of exposure on re-entry is contact with residues via the skin.
It should be noted that this critical use presented for the worker re-entry assessment (4 x 0.0005 kg a.s./ha) is being considered the highest exposure scenario due to the highly conservative approach regarding the dermal absorption value taken in the new EFSA OPEX calculator v. 1.0.0, which will be resulting the in the highest exposure estimates even not considering the maximum application rate in the label.

6.6.3.1 Estimation of worker exposure

The presented assessment shows negligible risk for the worker wearing adequate clothing, (normal workwear) when re-entering crops treated with F7B-39-30 (RINPODE).

Table 6.6- shows the exposure model used for estimation of worker exposure after entry into a previously

treated area or handling a crop treated with F7B-39-30 according to the critical use(s). The outcome of the estimation is presented in Table 6.6-5 (longer term exposure). Detailed calculations are in A 2.1.

Table 6.6-4: Exposure models for intended uses

Critical use(s)	Sugar beet (max. 4 x 0.02 L product/ha)
Model	Guidance on the assessment of exposure of operators, workers, residents and bystanders in risk assessment of plant protection products; EFSA Journal 2022;20(1):7032 calculator version: 1.0.0 (new on-line EFSA OPEX calculator)

Table 6.6-5: Estimated worker exposure (longer term exposure)

		Florpyrauxifen-benzyl		
Model data	**Level of PPE**	**Total absorbed dose (mg/kg bw/day)**	**% of systemic AOEL**	**Re-entry restriction [days]**
Low Vegetables Crop inspection and irrigation (All) Outdoor Work rate: 2 hours/day Dermal absorption: 70%* DT_{50}: 30 days DFR: 3 µg/cm²/kg a.s./ha Interval between treatments: 5 days				
Number of applications and application rate		4 x 0.0005 kg a.s./ha		
Body weight: 60 kg	Potential TC: 12500 cm²/person/h	0.001	1.1	0
	Work wear (arms, body and legs covered) TC: 1400 cm²/person/h	0.0002	0.1	0
	Work wear (arms, body and legs covered) and gloves TC: 1250 cm²/person/h	0.0001	0.1	0
	Hands covered, no workwear TC: no TC available cm²/person/h	NA	NA	NA

New on-line EFSA OPEX calculator version: 1.0.0
*This was manually corrected in the online tool (once it´s being automatically considered at 75% DA) to be consistent with the highest dermal absorption default value in the EFSA (2017) Dermal Absorption guideline and should be taken for the cut-off;

6.6.3.2 Refinement of generic DFR value (KCP 7.2)

Measurement of worker exposure is required where, on the basis of estimated exposure, the AOEL may be exceeded. Estimations of worker exposure indicate that the AOEL will not be exceeded by proposed uses of F7B-39-30 (RINPODE). Therefore, measurement of worker exposure is not required and has not been performed.
The preceding assessment provides a sufficient margin of safety to re-entry workers. Therefore, an estimation of worker exposure assuming dislodgeable residue data from proposed conditions of use is not required and has not been performed.

6.6.3.3 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, a study to provide measurements of worker exposure was not necessary and was therefore not performed.

Study comment 6.6.34:

~~**Extension of use on stone fruit**~~

The evaluator agrees with Applicant's estimation of worker exposure after entry into a previously treated area or handling a crop treated with Rinpode (F7B-39-30) according to the critical use on sugar beet. The calculations were made by means of EFSA OPEX calculator for dose 4 x 0.02 L product/ha~~as the worst case~~.

The calculations were done correctly.

Additionally, the evaluator calculated worker exposure using dose 1x0,08l product/ha. The results there are below.

Number of applications and application rate		1 x 2 g a.s./ha		
Body weight: 60 kg	Potential TC: 12500 cm^2/person/h	0.001	0,8	0
	Work wear (arms, body and legs covered) TC: 1400 cm^2/person/h	0.0001	0.09	0
	Work wear (arms, body and legs covered) and gloves TC: 1250 cm^2/person/h	0.0001	0.08	0
	Hands covered, no workwear TC: no TC available cm^2/person/h	NA	NA	NA

Agreed endpoint 6.6.34:

According to the calculations, it can be concluded that the risk of worker exposure during re-entry activities is acceptable.

The risk for worker exposure during re-entry activities on area treated with Rinpode (F7B-39-30) is acceptable without the PPE but worker should be wearing the adequate work clothing for its intended use within good agricultural practice.

6.6.4 Resident and bystander exposure (KCP 7.2.2)

6.6.4.1 Estimation of resident and bystander exposure

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.

The recommended uses of F7B-39-30 (RINPODE) may potentially result in the incidental exposure of bystanders and residential, but the extent of exposure effectively represents no adverse risk. The highest predicted exposure for both incidental and residential bystanders was 0.8% of the AOEL, for tractor-mounted spray boom application (EFSA 2022 Model). The GAPs and rationale for the risk envelope assessed for resident/bystanders are provided in Appendix 3.

Table 6.6- shows the exposure model(s) used for estimation of resident/bystander exposure to Florpyrauxifen-benzyl. The outcome of the estimation is presented in **Błąd! Nie można odnaleźć źródła odwołania.** (longer-term resident exposure).

It should be noted that the critical use presented for the resident/ bystander assessment, with 4 x 0.0005 kg a.s./ha, revealed the highest exposure scenario for re-entry and deposits parameters, but for drift parameters the highest exposure is reached by the maximum single dose rate. (1 x 2 g a.s./ha). However, the relevant highest predicted exposure values, the 'Sum (mean)' are the same for both application scenario assessments. Detailed calculations for both application scenario assessment are in A 2.1.

Table 6.6-6: Exposure models for intended uses

Critical use(s)	Sugar beet (max. 4 x 0.02 L product/ha)
Model	Guidance on the assessment of exposure of operators, workers, residents and bystanders in risk assessment of plant protection products; EFSA Journal 2022;20(1):7032

Table 6.6-7: Estimated resident exposure (longer term exposure)

		Florpyrauxifen-benzyl	
Model data		**Total absorbed dose (mg/kg bw/day)**	**% of systemic AOEL**
Tractor mounted boom spray application outdoors, downwards to low crops Buffer zone: 2-3 (m) Drift reduction technology: 0% DT_{50}: 30 days DFR: 3 µg/cm^2/kg a.s./ha Interval between treatments: 5 days Minimum water Volume: 100 L/ha Dermal Absorption: 70%			
Number of applications and application rate		4 x 0.0005 kg a.s./ha	
Resident child Body weight: 10 kg	Drift (75th perc.)	9e-05	0.07
	Vapour (75th perc.)	0.0008	0.6
	Deposits (75th perc.)	2e-05	0.01
	Re-entry (75th perc.)	0.0002	0.2
	Sum (mean)	0.001	**0.8**
Resident adult Body weight: 60 kg	Drift (75th perc.)	2e-05	0.02
	Vapour (75th perc.)	0.0003	0.2
	Deposits (75th perc.)	8e-06	0.006
	Re-entry (75th perc.)	0.0001	0.09
	Sum (mean)	0.0004	0.3

6.6.4.2 Measurement of resident and/or bystander exposure

Since the resident and/or bystander exposure estimations carried out indicated that the acceptable operator exposure level (AOEL) for Florpyrauxifen-benzyl will not be exceeded under conditions of intended uses, a study to provide measurements of resident/bystander exposure was not necessary and was therefore not performed.

Study comment 6.6.4:	The evaluator agrees with estimation of resident exposure after application of Rinpode (F7B-39-30) on sugar beet. The exposure estimation of resident (adult and child) to florpyrauxifen-benzyl, applied on a field of sugar beet at dose of 4x0.02 L product/ha, using tractor-mounted, calculated with the the EFSA OPEX calculator demonstrates that such a exposure for adult and child resident amounted 0.3 % to 0.8% of respective AOEL, thus not causing an unacceptable risk.

<table>
<tr><td></td><td colspan="4">The calculations were done correctly. The exposure assessment for residents also covers bystander exposure.

Additionally, the evaluator calculated resident exposure using dose 1x0,08l product/ha. The results there are below.
<table>
<tr><td colspan="2">Number of applications and application rate</td><td colspan="2">1x 2 g a.s./ha</td></tr>
<tr><td rowspan="5">Resident child Body weight: 10 kg</td><td>Drift (75th perc.)</td><td>0.0002</td><td>0.2</td></tr>
<tr><td>Vapour (75th perc.)</td><td>0.0008</td><td>0.6</td></tr>
<tr><td>Deposits (75th perc.)</td><td>1e-05</td><td>0.01</td></tr>
<tr><td>Re-entry (75th perc.)</td><td>0.0001</td><td>0.1</td></tr>
<tr><td>Sum (mean)</td><td>0.001</td><td>0.8</td></tr>
<tr><td rowspan="5">Resident adult Body weight: 60 kg</td><td>Drift (75th perc.)</td><td>5e-05</td><td>0.04</td></tr>
<tr><td>Vapour (75th perc.)</td><td>0.0003</td><td>0.2</td></tr>
<tr><td>Deposits (75th perc.)</td><td>6e-06</td><td>0.004</td></tr>
<tr><td>Re-entry (75th perc.)</td><td>8e-05</td><td>0.06</td></tr>
<tr><td>Sum (mean)</td><td>0.0004</td><td>0.3</td></tr>
</table></td></tr>
<tr><td>Agreed endpoint 6.6.4:</td><td colspan="4">According to calculations, it can be concluded that there is no unacceptable risk to any resident (child and adult) and bystander after application product Rinpode (F7B-39-30) on sugar beet.</td></tr>
</table>

6.6.5 Combined exposure

Not relevant. The product contains only one active substance.

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
CA 5.4.1/4	Xie, H.	2021	X12483137: In Vitro Mammalian Cell Micronucleus Assay in Human Peripheral Blood Lymphocytes (HPBL). Corteva Report No. 201936 BioReliance Corporation, Rockville, Maryland, USA GLP/GEP (Y/N): Yes Published (Y/N): No	N	Corteva Agriscience (bringing together the global heritage businesses of Pioneer, DuPont Crop Protection, and Dow AgroSciences)
CA 5.4.1/5	Davis, X. F	2020	X12483137: Bacterial Reverse Mutation Test. Corteva Report No. 201938 Haskell R&D Center, E.I. du pont de Nemours and Company, Member of Corteva Agriscience Group of Companies, Newark, Delaware, USA GLP/GEP (Y/N): Yes Published (Y/N): No	N	Corteva Agriscience (bringing together the global heritage businesses of Pioneer, DuPont Crop Protection, and Dow AgroSciences)
CA 5.4.1/6	Miller, A.	2021	X12483137: In Vitro Mammalian Cell Forward Gene Mutation (CHO/HPRT) Assay with Duplicate Cultures. Corteva Report No. 201937 BioReliance Corporation, Rockville, Maryland, USA DAS GLP/GEP (Y/N): Yes Published (Y/N): No	N	Corteva Agriscience (bringing together the global heritage businesses of Pioneer, DuPont Crop Protection, and Dow AgroSciences)

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

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
CP 7.1.1/1	[redacted]	2014	Acute Oral Toxicity Study of GF-3206 in Rats [redacted] GLP/GEP (Y/N): Yes Published (Y/N): No	Y	Corteva Agriscience (bringing together the global heritage businesses of Pioneer, DuPont Crop Protection, and Dow AgroSciences)
CP 7.1.2/1	[redacted]	2014	Acute Dermal Toxicity Study of GF-3206 in Rats [redacted] GLP/GEP (Y/N): Yes Published (Y/N): No	Y	Corteva Agriscience (bringing together the global heritage businesses of Pioneer, DuPont Crop Protection, and Dow AgroSciences)
CP 7.1.3/1	[redacted]	2014	Acute Inhalation Toxicity Study of GF-3206 in Rats [redacted] GLP/GEP (Y/N): Yes Published (Y/N): No	Y	Corteva Agriscience (bringing together the global heritage businesses of Pioneer, DuPont Crop Protection, and Dow AgroSciences)
CP 7.1.4/1	[redacted]	2014	Acute Dermal Irritation Study of GF-3206 in Rabbits [redacted] GLP/GEP (Y/N): Yes Published (Y/N): No	Y	Corteva Agriscience (bringing together the global heritage businesses of Pioneer, DuPont Crop Protection, and Dow AgroSciences)
CP 7.1.5/1	[redacted]	2014	Acute Eye Irritation Study of GF-3206 in Rabbits [redacted] GLP/GEP (Y/N): Yes Published (Y/N): No	Y	Corteva Agriscience (bringing together the global heritage businesses of Pioneer, DuPont Crop Protection, and Dow AgroSciences)
CP 7.1.6/1	[redacted]	2014	Skin Sensitisation Study of GF-3206 in Guinea Pigs [BUEHLER Test] [redacted] GLP/GEP (Y/N): Yes Published (Y/N): No	Y	Corteva Agriscience (bringing together the global heritage businesses of Pioneer, DuPont Crop Protection, and Dow AgroSciences)

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
CP 7.3/1	[redacted]	2016	*In vitro* dermal absorption of XDE-848 Benzyl Ester, formulated in F7B-39-30, and one spray dilution through human split-thickness skin using flow through diffusion cells [redacted] GLP/GEP (Y/N): Yes Published (Y/N): No	Y	Corteva Agriscience (bringing together the global heritage businesses of Pioneer, DuPont Crop Protection, and Dow AgroSciences)

~~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~~
~~KCP XX~~	~~Author~~	~~YYYY~~	~~Title~~ ~~Company Report N~~ ~~Source~~ ~~GLP/non GLP/GEP/non GEP~~ ~~Published/Unpublished~~	~~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~~
~~KCP XX~~	~~Author~~	~~YYYY~~	~~Title~~ ~~Company Report N~~ ~~Source~~ ~~GLP/non GLP/GEP/non GEP~~ ~~Published/Unpublished~~	~~Y/N~~	~~Owner~~

Appendix 2 Detailed evaluation of the studies relied upon

Comments of zRMS:	Studies for assessment of florpyrauxifen-benzyl groundwater metabolite X12483137 should be evaluated at the EU level.

A 2.1 Other/Special Studies: Studies for assessment of florpyrauxifen-benzyl ground water metabolite X12483137

A 2.1.1 Study 1

CITATION:
Xie, H.; 2021; X12483137: In Vitro Mammalian Cell Micronucleus Assay in Human Peripheral Blood Lymphocytes (HPBL); BioReliance Corporation, Rockville, Maryland, USA; Lab Study No. AG27GT.348.BTL; Sponsor Study No. 201936 ; 10 March 2021; Published: No

COMPLIANCE

Guideline(s):	OECD 487 (2016)
US EPA Guideline(s):	None
Deviations:	None
Dates of work:	16 August 2020 to 25 September 2020
GLP status:	Yes
Number of pages in final report:	53

MATERIALS AND METHODS

Test Item(s)

Test item (Common name):	X12483137
Purity:	99%
Description (physical state):	Whitish yellow-orange powder
Lot/batch no.:	YM3-149305-026 (TSN309064)
Compound stability:	Not conducted

Negative (Untreated control), Vehicle (DMSO) and Positive Control

The vehicle used to deliver the test substance to the test system was DMSO.

Vehicle	Manufactured by	CAS number
Dimethyl Sulfoxide (DMSO)	Sigma-Aldrich	67-68-5

The following positive controls were run concurrently with each test:

Positive control	Manufactured by	CAS No.	Purity (%)	Solvent	Concentration	Metabolic activation
Mitomycin C (MMC)	Sigma-Aldrich	50-07-7	Not reported	Sterile water	0.4 and 0.5 µg/mL	-S9

Positive control	Manufactured by	CAS No.	Purity (%)	Solvent	Concentration	Metabolic activation
Cyclophosphamide (CP)	Sigma-Aldrich	6055-19-2	Not reported	Sterile water	2.5, 5, and 7.5 µg/mL	+S9
Vinblastine (VB)	Sigma-Aldrich	143-67-9	Not reported	Sterile water	5, 7.5, and 10 ng/mL	-S9

Tester System

Cells	Human peripheral blood lymphocytes
Source	Healthy non-smoking 25 years old female
Maintenance	RPMI 1640 containing 15% heat inactivated fetal bovine serum, 2 mM L-glutamine, 100 units penicillin and 100 µg/mL streptomycin, incubation at 37 ± 1°C.
Metabolic activation	Aroclor 1254-induced rat liver S9 (MolTox, Boone, North Carolina). Protein content: 33.9 mg/mL and 35.2 mg/mL

Test Item(s)

~~Test item (Common name):~~ ~~X12483137~~
~~Purity:~~ ~~99%~~
~~Description (physical state):~~ ~~Whitish yellow-orange powder~~
~~Lot/batch no.:~~ ~~YM3-149305-026 (TSN309064)~~
~~Compound stability:~~ ~~Not conducted~~

~~**Negative (Untreated control), Vehicle (DMSO) and Positive Control**~~

~~The vehicle used to deliver the test substance to the test system was DMSO.~~

~~Vehicle~~	~~Manufactured by~~	~~CAS number~~
~~Dimethyl Sulfoxide (DMSO)~~	~~Sigma-Aldrich~~	~~67-68-5~~

~~The following positive controls were run concurrently with each test:~~

~~Positive control~~	~~Manufactured by~~	~~CAS No.~~	~~Purity (%)~~	~~Solvent~~	~~Concentration~~	~~Metabolic activation~~
~~Mitomycin C (MMC)~~	~~Sigma-Aldrich~~	~~50-07-7~~	~~Not reported~~	~~Sterile water~~	~~0.4 and 0.5 µg/mL~~	~~-S9~~
~~Cyclophosphamide (CP)~~	~~Sigma-Aldrich~~	~~6055-19-2~~	~~Not reported~~	~~Sterile water~~	~~2.5, 5, and 7.5 µg/mL~~	~~+S9~~
~~Vinblastine (VB)~~	~~Sigma-Aldrich~~	~~143-67-9~~	~~Not reported~~	~~Sterile water~~	~~5, 7.5, and 10 ng/mL~~	~~-S9~~

~~**Tester System**~~

~~Cells~~	~~Human peripheral blood lymphocytes~~
~~Source~~	~~Healthy non-smoking 25 years old female~~
~~Maintenance~~	~~RPMI 1640 containing 15% heat inactivated fetal bovine serum, 2 mM L-glutamine, 100 units penicillin and 100 µg/mL streptomycin, incubation at 37 ± 1°C.~~
~~Metabolic activation~~	~~Aroclor 1254-induced rat liver S9 (MolTox, Boone, North Carolina). Protein content: 33.9 mg/mL and 35.2 mg/mL~~

Preliminary Toxicity Assay

The preliminary toxicity test was performed with the test concentrations of 0.2, 0.6, 2, 6, 20, 60, 200, 600, and 2000 µg/mL of culture medium. The vehicle control substance for each test condition (one culture per concentration level) was maintained.

The test substance was formulated in dimethyl sulfoxide (DMSO) at 500 mg/mL, the highest stock concentration used in the study. Dilutions were prepared to obtain the required concentrations for the study.

The HPBL cultures were treated for approximately 4 and 24 hours in the absence of S9 metabolic activation, and approximately 4 hours in the presence of S9 metabolic activation. The standard incubation conditions were 37 ± 1ºC in a humidified atmosphere of 5 ± 1% CO_2 in air.

At least 500 cells were evaluated to determine the CBPI at each dose level and the control.

Micronucleus Assay

Based on the results of the preliminary toxicity assay, the concentrations chosen for the micronucleus assay were 125, 250, 500, 1000, and 2000 µg/mL for the 4-hour S9-activated test condition, 4-hour non-activated test condition, and 24-hour non-activated test condition. The standard incubation conditions were 37 ± 1ºC in a humidified atmosphere of 5 ± 1% CO_2 in air.

At least 1000 cells (500 cells per culture), were evaluated to determine the CBPI at each dose level and the control.

The slides from at least three test substance treatment groups were coded using random numbers by an individual not involved with the scoring process and scored for the presence of micronuclei based on cytotoxicity. A minimum of 2000 binucleated cells from each concentration (1000 binucleated cells from each culture) were examined and scored for the presence of micronuclei.

Data analysis and interpretation

The CBPI was determined using the following formula:

$$\text{CBPI} = \frac{1 \times \text{Mononucleated cells} + 2 \times \text{Binucleated cells} + 3 \times \text{Multinucleated cells}}{\text{Total number of cells scored}}$$

% Cytostasis (cytotoxicity) = 100 -100 {(CBPIt-1) /(CBPIc-1)}

t = test substance treatment culture

c = vehicle control culture

Acceptance Criteria

The following acceptance criteria were used to determine a valid assay:

a. Vehicle controls: The frequency of cells with micronuclei should ideally be within the 95% control limits of the distribution of the historical negative control database. If the concurrent negative control data fall outside the 95% control limits, they may be acceptable as long as these data are not extreme outliers (indicative of experimental or human error).

b. Positive controls: The percentage of micronucleated cells must be significantly greater than the concurrent vehicle control ($p \leq 0.05$). In addition, the cytotoxicity response must not exceed the upper limit for the assay (60%).

c. Cell proliferation: The CBPI of the vehicle control at harvest must be ≥1.4.

d. Test conditions: The test substance must be tested using a 4-hour treatment with and without S9, as well as a 24-hour treatment without S9.

e. Analyzable concentrations: At least 2000 binucleated cells from at least three appropriate test substance concentrations.

f. Maximum concentration evaluation: The maximum concentration evaluated for micronucleus induction must

- produce cytotoxicity in the target range of 55 ± 5%, or
- produce turbidity or a precipitate visible by eye at the end of the treatment with the test substance or
- if no precipitate or limiting cytotoxicity was observed, be 10 mM, 2 mg/mL or 2 µL/mL, whichever is the lowest.

Evaluation Criteria

The test substance was considered to have clastogenic activity in this study if the following criteria were met:

a. At least one of the test concentrations exhibited a statistically significant increase when compared with the concurrent negative control ($p \leq 0.05$).

b. The increase was concentration-related ($p \leq 0.05$).

c. Results were outside the 95% control limit of the historical negative control data.

The test substance was considered to have induced a clear negative response if none of the criteria for a positive response were met.

Statistics

Statistical analysis was performed using the Fisher's exact test ($p \leq 0.05$) for a pairwise comparison of the percentage of micronucleated cells in each treatment group with that of the vehicle control. The Cochran-Armitage trend test was used to assess dose-responsiveness.

RESULTS AND DISCUSSION

Negative, Vehicle and Positive Controls

The number of micronuclei containing binucleated cells found in the negative (untreated) and vehicle control (DMSO) cultures was within the historical control data range. Positive controls, Mitomycin C, vinblastine and cyclophosphamide both produced statistically significant increases in the incidence of micronuclei containing binucleated cells, indicating that the test conditions were adequate and that the metabolic activation system (S9-mix) functioned properly.

Preliminary Toxicity Assay

Cytotoxicity [lower target of 50% CBPI relative to the vehicle control] was not observed at any dose in any of the three exposure groups.

The test substance was soluble in the treatment medium at all doses tested at the beginning and conclusion of the treatment period. The osmolality of the test substance doses in treatment medium was considered acceptable (<120% of vehicle). The pH of the highest dose of test substance in treatment medium was 7.0.

Mutagenicity Test

The test substance was soluble in the treatment medium at all doses tested at the beginning and conclusion of the treatment period. The pH of the highest dose of test substance in treatment medium was 7.0.

Cytotoxicity [lower target of 50% CBPI relative to the vehicle control] was not observed at any dose in any of the three exposure groups.

Neither statistically significant nor dose-dependent increases in micronuclei induction were observed at any dose in treatment groups with or without S9 ($p > 0.05$; Fisher’s Exact and Cochran-Armitage tests). The results were within the 95% control limit of the historical negative control data.

The summary results are given in the following tables.

~~Table 6.6-4~~ A 1: Results of Micronucleus Test

Treatment Time: 4 hours (-S9)					
	Vehicle	500 µg/mL	1000 µg/mL	2000 µg/mL	Positive control (0.5 µg/mL MMC)
Cytotoxicity (%)	NA	16	10	29	38
Precipitate	No	No	No	No	No
CBPI	1.739	1.618	1.667	1.522	1.456
	Mean				
Total number of binucleated cells scored	2000	2000	2000	2000	2000
% Micronucleated binucleated cells per dose	0.20	0.20	0.15	0.30	5.60[a]
Treatment Time: 4 hours (+S9)					
	Vehicle	500 µg/mL	1000 µg/mL	2000 µg/mL	Positive control (7.5 µg/mL CP)
Cytotoxicity	NA	10	10	26	51
Precipitate	No	No	No	No	No
CBPI	1.626	1.564	1.561	1.465	1.309
	Mean				
Total number of binucleated cells scored	2000	2000	2000	2000	2000
% Micronucleated binucleated cells per dose	0.20	0.20	0.15	0.25	1.65[a]
Treatment Time: 24 hours (-S9)					
	Vehicle	500 µg/mL	1000 µg/mL	2000 µg/mL	Positive control (10 ng/mL VB)
Cytotoxicity	16	16	7	2	60
Precipitate	No	No	No	No	No
CBPI	1.614	1.516	1.574	1.604	1.245
	Mean				
Total number of binucleated cells scored	2000	2000	2000	2000	2000
% Micronucleated binucleated cells per dose	0.25	0.40	0.25	0.25	3.80[a]

a $p \leq 0.01$, Fisher's exact test, relative to the solvent control

Table ~~6.6-5~~ A 2: Historical Control Values, 2017-2019 (female)

Non-activated test system				
Historical values	Micronucleated binucleated cells (%)			
	Negative control[a]		Positive control	
	4-hour	24-hour	4-hour[b]	24-hour[c]
Number of	132	138	41	124

Studies				
Mean	0.42	0.39	3.79	2.03
Standard deviation	0.15	0.15	1.03	0.91
95% Control limits	0.11-0.72	0.10-0.69	1.74-5.84	0.21-3.85
Range[e]	0.15-0.80	0.10-0.95	2.20-5.95	0.28-5.70

S9-activated test system		
Historical values	Micronucleated binucleated cells (%)	
	Negative control[a]	Positive control[d]
Number of Studies	140	136
Mean	0.39	1.40
Standard deviation	0.15	0.33
95% Control limits	0.10-0.68	0.73-2.06
Range[e]	0.15-0.85	0.30-2.50

a Solvents include water, saline, DMSO, ethanol, acetone, and other non-standard and Sponsor supplied vehicles
b Positive control for non-activated 4-hour studies, Mitomycin C (MMC)
c Positive control for non-activated 24-hour studies, Vinblastine (VB)
d Positive control for S9-activated studies, Cyclophosphamide (CP)
e Range from minimum to maximum

CONCLUSION

All criteria for a valid study were met as described in the protocol. From the results of this study, it is concluded that X12483137 did not show any potential to induce micronuclei in cultured human peripheral blood lymphocytes, both in the absence and presence (2% v/v S9 mix) of metabolic activation system under the present experimental conditions.

Test item	Test	Test object	Concentration	Result
X12483137	*In vitro* micronucleus test	Human peripheral blood lymphocytes	500, 1000, 2000 µg/mL	Negative

A 2.1.2 Study 2

Comments of zRMS:	1. The study should be evaluated at the EU level. Some deviations were identified: only duplicates were used instead of triplicates, as required by the OECD TG 471. Furthermore, no parameters were changed in the confirmatory experiment, although this should be taken into account according to the OECD TG.

CITATION
Davis, X. F.; 2020; X12483137: Bacterial Reverse Mutation Test; Haskell R&D Center, E.I. du pont de Nemours and Company, Member of Corteva Agriscience Group of Companies, Newark, Delaware, USA; Lab Study No. 22442-500; Sponsor Study No. 201938; 28 October 2020; No

COMPLIANCE
Guideline(s): OECD 471 (2020); OPPTS 870.5100 (1998); EC B.13/14 (2008)
US EPA Guideline(s): OPPTS 870.5100 (1998)
Deviations: None

Dates of work: 7 August 2020 to 19 August 2020
GLP status: Yes
Number of pages in final report: 51

COMPLIANCE

MATERIALS AND METHODS

Test Item(s)

Test item (Common name):	X12483137
Purity:	99%
Description (physical state):	Not reported
Lot/batch no.:	YM3-149305-034 (TSN309232)
Compound stability:	An analytical verification of the test substance stability was not conducted

Negative (Vehicle) and Positive Control

The vehicle, DMSO, was used as the negative control for each tester strain with and without S9 activation. The test substance vehicle was selected based on solubility testing.

The following positive controls were run concurrently with each test:

Positive control	Manufactured by	Purity (%)	Solvent	Bacterial strains	Concentration	Metabolic activation
Sodium azide	Moltox Inc.	Not reported	Sterile distilled water	TA1535	2.0 µg/plate	Absence of S9 mix
				TA100		
Acridine mutagen ICR-191	Moltox Inc.	Not reported		TA1537	2.0 µg/plate	
4-Nitroquinoline N-oxide	Moltox Inc.	Not reported	DMSO	WP2 *uvr*A	1.0 µg/plate	
2-Nitrofluorene	Moltox Inc.	Not reported		TA98	1.0 µg/plate	
2-Aminoanthracene	Moltox Inc.	Not reported		TA100, TA1535, TA1537	2.5 µg/plate	Presence of S9 mix
				WP2 *uvr*A	25 µg/plate	
Benzo[a]pyrene	Moltox Inc.	Not reported		TA98	2.5 µg/plate	

Tester Strain

Bacterium:	*Salmonella typhimurium* and *Escherichia coli*
Strains:	TA1537, TA1535, TA98, TA100 and WP2 *uvr*A
Source:	Moltox, Inc, Boone, North Carolina, USA

Maintenance:	The minimal glucose agar plates were supplemented with either histidine and biotin or tryptophan, and for strains containing the pKM101 plasmid, ampicillin. Tester strain master plates were stored at 5 ± 3°C.
Confirmation:	All test cultures were tested for acceptable viability and genotype confirmation (including histidine, biotin requirement, rfa, uvrB mutation, pKM101 plasmid presence) concurrently with the test.
Metabolic activation:	S9 fraction from Aroclor 1254 treated rats.

Toxicity-Mutation Test

Toxicity-mutation test established the range of test substance concentrations for the mutagenicity test and provided a preliminary mutagenicity evaluation. the maximum concentration evaluated was 5000 µg/plate for tester strains TA98, TA100, TA1535, TA1537, and WP2 *uvr*A in the absence and presence of S9 metabolic activation. This was achieved by plating a 100 µL aliquot of a 50 mg/mL stock solution. All tester strains and test conditions were evaluated at 8 concentrations along with the negative (vehicle) and positive controls. The concentrations used in this test were 33.3, 66.7, 100, 333, 667, 1000, 3333, and 5000 µg/plate. The plates were incubated at 37 ± 2°C approximately for 66-70 hours and then examined to assess the state of background bacterial growth inhibition, precipitation, and number of revertant colonies.

Mutagenicity Test

The mutagenicity test was conducted to evaluate the mutagenic potential of the test substance. The treatment was performed both in the absence and presence of the metabolic activation. The treatments were performed by the plate incorporation technique. Plates were maintained in triplicate for each test concentration of X12483137, negative and positive controls.

Based on the toxicity-mutation test, the maximum concentration evaluated in the mutagenicity test was 5000 µg/plate. This was achieved by plating a 100 µL aliquot of a 50 mg/mL stock solution. All tester strains and test conditions were evaluated with at least 5 concentrations along with the negative (vehicle) and positive controls. The concentrations used in this test were 333, 667, 1000, 3333, and 5000 µg/plate for all tester strains in the presence and absence of S9 activation.

In the non-activated assays, 0.5 mL of sham mix and 100 µL of vehicle, test substance dilution, or positive control were added to pre-heated (45–48°C) glass culture tubes containing 2 mL of selective top agar, followed by 100 µL of tester strain. In the S9-activated assays, 100 µL of the vehicle, test substance dilution, or positive control were added to pre-heated (45–48°C) glass culture tubes containing 2 mL of selective top agar, followed by 100 µL of tester strain and 0.5 mL of S9 mix. All mixtures were vortexed and overlaid onto the surface of minimum glucose agar plates. After the overlay solidified, the plates were inverted and incubated for approximately 66-70 hours at 37 ± 2°C.

Acceptance Criteria

The following is the criteria to confirm the validity of the assay:

- To demonstrate the presence of the *rfa* mutation, all S. typhimurium tester strain cultures must exhibit sensitivity to crystal violet. To demonstrate the presence of the *uvr*A and *uvr*B mutations, all tester strains cultures must exhibit sensitivity to ultraviolet light. To demonstrate the presence of the pKM101 plasmid, tester strain cultures of TA98 and TA100 must exhibit resistance to ampicillin.

- To ensure that appropriate numbers of bacteria were plated, all tester strain culture densities must be approximately 10^9 cells per milliliter.
- The tester strain cultures must exhibit a characteristic mean number of spontaneous revertants per plate when plated along with the negative (vehicle) control under selective conditions. The acceptable ranges for the mean values of negative controls are TA98 (8-60), TA100 (60-240), TA1535 (4-45), TA1537 (2-25), WP2 *uvr*A (5-60).
- Each mean positive control value must exhibit at least a 3.0-fold increase over the respective mean negative (vehicle) control value for each tester strain.
- A minimum of 3 non-toxic scorable test substance concentrations were required to validate the study. A test substance concentration was considered toxic if it caused:

• A >50% reduction in the mean number of revertants per plate relative to the mean negative control value and exhibited a concentration-dependent drop in the revertant count, or

• A reduction in the background lawn.

In the event that less than 3 non-toxic test substance concentrations were achieved, the affected portion of the test was repeated with an appropriate change in test substance concentrations.

- Data Point Rejection:

• A single data point may have been rejected if contamination or excessive toxicity was seen on a treatment plate. A single data point may also have been rejected if excessive precipitate on the plate prevented accurate colony counting.

• A negative control data point may have been rejected if it fell outside the acceptable spontaneous mutation range.

• A positive control data point may have been rejected if it had a low mutagenic response compared to the other positive control plates in that data set.

Evaluation Criteria

The conditions necessary for determining a positive result were that there should be a dose-related increase in the mean revertants per plate of at least one tester strain over a minimum of two increasing doses of the test article either in the absence or presence of the metabolic activation system.

For strains TA98, TA100 and WP2 *uvr*A datasets were judged positive if the increase in mean revertants at the peak of the dose response was equal to or greater than 2 times the mean negative control value.

For strain TA1535 and TA1537 datasets were judged positive if the increase in mean revertants at the peak of the dose response was equal to or greater than 3 times the mean negative control value.

A data set was judged equivocal if there was a biologically relevant increased response that only partially met criteria for a positive response. A response was evaluated as negative if it was neither positive nor equivocal.

RESULTS AND DISCUSSION

Negative and Positive Controls

The number of revertant colonies for the negative control was within the limits of the historical control of this laboratory for all the strains. All positive controls demonstrated an increase in the number of revertants demonstrating the efficiency of the test system.

Toxicity-Mutation Test

No positive test-substance related mutagenic responses were observed at any concentration in any tester strain in the absence or presence of S9 metabolic activation. A microscopic background lawn reduction was observed with WP2 *uvr*A at 5000 µg/plate in the presence of S9 activation; no other appreciable toxicity was observed at any concentration with any tester strain in either the absence or presence of S9 activation. No test substance precipitation was observed. All negative and positive controls performed as expected.

Mutagenicity Test

No positive test substance-related mutagenic responses were observed at any concentration or with any tester strain in either the absence or presence of S9 metabolic activation. No toxicity was observed at any concentration with any tester strain in either the absence or presence of S9 activation. No test substance precipitation was observed. All negative and positive controls performed as expected. The summary results are given in the following tables.

Table ~~6~~ A 3: Number of revertants per plate (mean of 2 plates); Toxicity-Mutation Test

Test strains	±S9	Concentrations (µg/plate)									
		DMSO	33.3	66.7	100	333	667	1000	3333	5000	PC
TA1537	-S9	11	10	12	11	10	10	12	11	14	1070
	+S9	14	9	15	13	13	19	12	15	14	133
TA1535	-S9	12	11	11	11	10	14	12	16	11	591
	+S9	20	13	12	13	12	14	12	11	12	179
TA98	-S9	26	29	24	27	29	31	37	28	35	104
	+S9	28	32	40	37	41	40	35	37	31	174
TA100	-S9	117	117	126	141	142	136	132	127	127	573
	+S9	150	154	142	136	124	143	149	130	122	1799
WP2 *uvr*A	-S9	31	30	26	34	38	32	38	31	42	432
	+S9	52	51	41	49	39	53	43	43	39	169

PC: Positive control

Table ~~7~~ A 4: Number of revertants per plate (mean of 3 plates); Mutagenicity Test

Test strains	±S9	Concentrations (µg/plate)						
		DMSO	333	667	1000	3333	5000	PC
TA1537	-S9	12	11	10	10	13	9	1108
	+S9	16	12	13	13	13	8	152
TA1535	-S9	13	15	11	15	17	16	556
	+S9	14	17	14	14	12	14	183
TA98	-S9	23	28	24	25	32	32	125
	+S9	33	36	38	39	28	33	234
TA100	-S9	160	145	146	151	154	132	575
	+S9	158	171	179	175	151	148	2048
WP2 *uvr*A	-S9	26	38	33	31	39	34	408
	+S9	55	56	43	46	44	46	213

PC: Positive control

Table ~~8~~ A 5: Historical Control Data

Historical Control Data[a]					
Tester strain	Control (positive control)[b]	Exogenous Metabolic Activation System	Mean	SD[c]	Range
TA98	Negative	Absent	25	9	10-58
	Negative	Present	33	9	11-61
	Positive [2NF-1]	Absent	242	68	123-702
	Positive [BAP-2.5]	Present	392	86	186-615
TA100	Negative	Absent	106	23	51-214
	Negative	Present	124	27	62-291
	Positive [SA-2]	Absent	939	202	458-1558
	Positive [2AA-2.5]	Present	2597	877	523-5889
TA1535	Negative	Absent	13	5	3-30
	Negative	Present	13	5	4-32
	Positive [SA-2]	Absent	839	181	392-1505
	Positive [2AA-2.5]	Present	205	50	78-365
TA1537	Negative	Absent	8	4	1-22
	Negative	Present	12	5	2-31
	Positive [ICR 191-2]	Absent	1133	337	300-2668
	Positive [2AA-2.5]	Present	131	54	48-354
WP2 *uvr*A	Negative	Absent	34	11	13-66
	Negative	Present	41	12	5-68
	Positive [4NQO-1]	Absent	769	252	283-1337
	Positive [2AA-25]	Present	256	79	126-680

[a] Historical data for tester strains used in the reported study. Data are based on studies reported from 2015 through 2019. Data include all control solvents or diluents, and metabolic activation systems based on Aroclor-induced rat liver S9.
[b] Abbreviations for positive controls: 2NF (2-nitrofluorene); BAP (benzo[a]pyrene); SA (sodium azide); 2AA (2-aminoanthracene); ICR 191 (ICR 191 Acridine mutagen); 4NQO (4-nitroquinoline-N oxide). The number following abbreviation is the microgram (µg) amount per plate or vial used for the positive control.
[c] SD = standard deviation

CONCLUSION

All criteria for a valid study were met as described in the protocol. From the results of this study, under the specified experimental conditions, X12483137 is concluded to be non-mutagenic in the Bacterial Reverse Mutation Assay using *Salmonella typhimurium* and *Escherichia coli*.

Test item	Test	Test object	Concentration	Result
X12483137	*In vitro* bacterial reverse mutation assay	*Salmonella typhimurium* (strains TA98, TA100, TA1535, TA1537) and *Escherichia coli* (strain WP2 uvrA)	33.3 to 5000 µ/plate	Non-mutagenic

A 2.1.3 Study 3

Comments of zRMS:	1. The study should be evaluated at the EU level. Some deviations from the current TG were identified: the spontaneous mutant frequency in this test was often less than 5 * 10^{-6}.

CITATION

Miller, A.; 2021; X12483137: In Vitro Mammalian Cell Forward Gene Mutation (CHO/HPRT) Assay with Duplicate Cultures; BioReliance Corporation, Rockville, Maryland, USA; Lab Study No. AG27GT.783.BTL; Sponsor Study No. 201937; 11 March 2021; No

COMPLIANCE

Guideline(s):	OECD 476 (2016)
US EPA Guideline(s):	None
Deviations:	None
Dates of work:	08 September 2020 to 08 October 2020
GLP status:	Yes
Number of pages in final report:	54

MATERIALS AND METHODS

Test Item(s)

Test item (Common name):	X12483137
Purity:	99%
Description (physical state):	Whitish yellow-orange powder
Lot/batch no.:	YM3-149305-026 (TSN309064)
Compound stability:	The test substance is considered stable through 28 Oct 2022 when stored at 5°C to ambient conditions.

Negative (Vehicle) and Positive Control

DMSO was the vehicle of choice based on the solubility of the test substance and compatibility with the target cells. The test substance formed a clear solution in DMSO at a concentration of approximately 500 mg/mL by sonication at 37.0ºC for 10 minutes.

The following positive controls were run concurrently with each test:

Positive control	Manufactured by	CAS No.	Purity (%)	Solvent	Concentration	Metabolic activation
Ethyl methanesulfonate	Sigma-Aldrich	62-50-0	a	DMSO	0.2 µL/mL	Absence of S9 mix
Benzo(a)pyrene	Sigma-Aldrich	50-32-8	99%	DMSO	4.0 µg/mL	Presence of S9 mix

a = Not provided by manufacturer

Tester System

Cell line	Chinese Hamster Ovary-K1 (CHO-K1-BH_4) cell line
Source	Oak Ridge National Laboratories (Oak Ridge, Tennessee)

Cell line	Chinese Hamster Ovary-K1 (CHO-K1-BH_4) cell line
Maintenance	Ham's F12 medium supplemented with 3 mM L glutamine and 5% (v/v) heat-inactivated and dialyzed fetal bovine serum (F12FCM5) under standard conditions (37 ± 1°C in a humidified atmosphere of 5 ± 1% CO2 in air). All media contained antimycotics and antibiotics.
Mycoplasma contamination check	Checked. No contamination
Model chromosome number	20
Metabolic activation	S9 fraction from Aroclor 1254 treated rats (Procured from Moltox, Boone, North Carolina, Lot # 4201).

The final concentrations of the components in the S9 mix are indicated below.

Component	Final concentration in cultures
NADP (sodium salt)	0.8 mM
Glucose-6-phosphate	1 mM
Calcium chloride	2 mM
Potassium chloride	6 mM
Magnesium chloride	2 mM
Sodium Phosphate	10 mM
S9 homogenate	20 µL/mL

Preliminary Toxicity Test

Assessed with CHO-K1-BH_4 cell line up to the limit dose of 2000 µg/mL. The cytotoxicity test was performed both in the absence and presence (2% v/v S9 fraction) of metabolic activation at the concentrations of 3.91, 7.81, 15.6, 31.3, 62.5, 125, 250, 500, 1000 and 2000 µg/mL. A concurrent negative (DMSO) control was also maintained. Cultures were exposed for a period of 5 ± 0.5 hours.

Mutagenicity Test

Dose selection for the gene mutation experiments was based on pre-tests-considering the cytotoxicity, the occurrence of precipitation and changes in the pH or osmolality.

Five test substance concentrations (125, 250, 500, 1000 and 2000 µg/mL), as well as the appropriate positive and vehicle controls, were tested in duplicate cultures with and without S9. The pH of the treatment medium was measured and adjusted at concentration 2000 µg/mL using 1N sodium hydroxide. No pH adjustment was necessary to maintain neutral pH in the treatment medium at the remaining concentrations tested. Precipitation was assessed at the beginning and end of treatment.

Cells were plated (on Day -1) in 225-cm^2 cultures at a density of ~8 x 10^6 in 30 mL Complete Ham's F12. Following an overnight incubation (on Day 0) at standard conditions, the cultures were washed twice with HBSS and re-fed with 22 mL treatment medium (for 2 flasks), or 17.6 mL treatment medium plus 4.4 mL S9 mix (adjusted for the test substance dose volume if >1%, v/v), as appropriate. Following addition of the test or control substance formulations (220 µL) to the flasks, the cultures were incubated under standard conditions for 5 ± 0.5 hours (positive control substances were prepared in DMSO and added to the flasks using a 1% dose volume).

After the 5-hour treatment, the treatment media were removed, the cultures were washed twice with CMF-HBSS and then were trypsinized and counted. Cells were sub-cultured at ~2.4 x 10^6 cells/225-cm^2 flask in 30 mL Complete Ham's F12 in duplicate (or all available into 1 or 2 flasks) for phenotypic expression and incubated under standard conditions.

Hypoxanthine-free Complete Ham's F12 (Complete Ham's F12-Hx) was used for mutant selection and to determine cloning efficiency at the time of selection. At the end of the phenotypic expression period, the cultures were washed twice with CMF-HBSS and 2.4 x 10^6 cells from each culture were plated at a density of 6 x 10^5 cells/150-mm plate (4 plates total) in 30 mL Complete Ham's F12-Hx containing 10 µM TG. Three 60-mm plates also were plated, at 200 cells/plate in 5 mL Complete Ham's F12-Hx in triplicate, to determine the cloning efficiency at the time of selection. The plates were incubated under standard conditions for 7 days.

After the 7-day incubation period, the colonies were fixed with methanol, stained with crystal violet and counted. Mutant frequencies were expressed as the number of TG^r mutants/10^6 clonable cells. The number of clonable cells was determined from the triplicate 60-mm plates.

Assay Acceptance Criteria

The following criteria were fulfilled to confirm the validity of the assay.

a. The average absolute cloning efficiency of the vehicle controls must be >60% (at initial survival and selection). In addition, the average spontaneous mutant frequency of the vehicle controls should ideally be within the 95% control limits of the distribution of the historical negative control database. If the concurrent negative control data fall outside the 95% control limits, they may be acceptable as long as these data are not extreme outliers (indicative of experimental or human error). Spontaneous mutant frequencies will be calculated separately for cultures with and without S9.

b. Positive controls induced a significant increase in the mutant frequency above the concurrent vehicle control ($p \leq 0.01$).

c. The highest concentration evaluated was the limit dose for this assay (2000 µg/mL), or must have induced 10 to 20% adjusted relative survival, or must be the highest concentration able to be prepared in the vehicle and administered (whichever is lowest). If increasing cytotoxicity was observed at precipitating concentrations, precipitation was the determining factor. There was no maximum concentration or toxicity requirement for test substances which clearly showed mutagenic activity.

d. A minimum of four acceptable concentrations was required for a valid assay. Fewer concentrations may be justified for test substances which clearly show mutagenic activity.

Assay Evaluation Criteria

A test item was considered positive in the mutation assay if:

i. The test substance was considered to have produced a positive response if it induced a dose-related increase in average mutation frequency and an increase exceeding 95% historical vehicle control limits in at least one test dose level(s) as compared with concurrent vehicle control ($p<0.01$). If only one criterion was met (a statistically significant or dose-dependent increase exceeding the historical control 95% confidence interval), the results were considered equivocal. If none of these criteria were met, the results were considered to be negative.

ii. Other criteria also may be used in reaching a conclusion about the study results (e.g., comparison to historical control values, biological significance, etc.). In such cases, the Study Director used sound scientific judgment and clearly reported and described any such considerations.

Statistics

Statistical analyses were performed using the method of Snee and Irr (1981).

RESULTS AND DISCUSSION

Negative and Positive Controls

Vehicle control: Both the initial survival and parallel average cloning efficiencies were >60%, and the average mutant frequency was within the 95% historical vehicle control range.

Positive control: A statistically significant increase was observed in the average mutant frequency over the concurrent vehicle control ($p < 0.01$).

Preliminary Toxicity Test

No visible precipitate was observed at the beginning or end of treatment. The test substance in DMSO formed clear solutions from 0.391 to 200 mg/mL. The pH of the cultures was adjusted at concentration 2000 µg/mL to maintain neutral pH or osmolality of the cultures [483 mmol/kg for the vehicle control and 423 for the highest concentration (2000 µg/mL), respectively]. Adjusted relative survival was 70.50 and 71.33% at a concentration of 2000 µg/mL with S9 and without S9, respectively. Based upon these results, the concentrations chosen for the definitive mutagenicity assay were 125, 250, 500, 1000 and 2000 µg/mL with and without S9.

Mutagenicity Test

No visible precipitate was observed at the beginning or end of treatment. The pH of the cultures was adjusted at concentration 2000 µg/mL to maintain neutral pH. The average adjusted relative survival was 70.35 and 84.15% at a concentration of 2000 µg/mL with and without S9, respectively. Cultures treated at all concentrations were chosen for mutant selection.

There was no statistically significant or dose-dependent increase in average mutation frequency at any of the concentrations evaluated. All the test substance treated cultures had average number of mutants within the 95% vehicle control limit.

The summary results are given in the following tables. The percent mean absolute cloning efficiency (ACE) and percent relative cloning efficiency/survival following the treatment both in the absence and presence of metabolic activation in trial I and II are summarised below:

Table ~~9~~ A 6: Cloning efficiency (CE) and relative survival following treatment

Group (µg/mL)	- S9		+ S9 (2% v/v S9)	
	CE (%)	RS (%)	CE (%)	RS (%)
NC (DMSO)	70.6±0.8	100.0 ±6.7	86.8±4.2	99.9±1.4
T1 (125)	70.6±3.9	95.01±1.0	69.8±1.6	76.0±7.3
T2 (250)	69.5±3.3	100.8±8.4	68.8±6.6	83.7±0.1
T3 (500)	69.1±11.0	98.8±8.2	76.2±0.9	95.6±3.1
T4 (1000)	73.7±8.3	102.5±15.7	78.8±1.3	97.4±5.0
T5 (2000)	62.3±2.9	84.1±4.2	56.7±5.2	70.4±3.5
PC	42.7±1.4	53.5±4.8	16.6±2.2	19.4±2.6

* Negative Control (Solvent used - Dimethyl sulfoxide)
Note: PC = Positive control [0.2 µL Ethyl methanesulfonate/mL in absence of metabolic activation and 4.0 µg of Benzo(a)pyrene/mL in presence of metabolic activation].

Table ~~10~~ A 7: Mean cloning efficiency at selection and mean mutant frequency

Group (µg/mL)				
	- S9		+ S9 (2% v/v S9)	
	Mean Cloning Efficiency (CE)	Mean Mutant Frequency (MF)	Mean CE	Mean AF
NC (DMSO)	72.5±2.6	4.3±0.3	95.9±4.1	3.5±0.1
T1 (125)	70.3±11.4	3.8±0.6	82.0±4.5	2.6±3.0
T2 (250)	74.2±7.3	5.0±0.3	73.4±5.3	4.6±0.3
T3 (500)	69.4±4.4	4.6±1.6	82.9±12.8	6.1±0.9
T4 (1000)	80.0±3.5	9.0±5.1	86.8±10.1	7.1±2.6
T5 (2000)	66.8±3.8	4.7±2.5	81.7±3.8	4.5±2.7
PC	51.6±7.7	321.23±46.0[a]	81.2±11.8	150.8±27.0[a]

* Negative Control (Solvent used - Dimethyl sulfoxide)
a P <0.01 compared to the vehicle control (T-test)
Note: PC = Positive control [0.2 µL Ethyl methanesulfonate/mL in absence of metabolic activation and 4.0 µg of Benzo(a)pyrene/mL in presence of metabolic activation].

Table ~~11~~ A 8: Historical Control Data (2015-2019)

	Mutation Frequency (per million cells)			
	Non-activated test system		S9-activation test system	
	Vehicle control[a]	EMS[b] (0.2 µL/mL)	Vehicle control[a]	B(a)P[c] (4.0 µg/mL)
Mean	5.0	300.8	5.1	169.3
Standard deviation	3.7	78.1	3.8	55.4
95% control limits	0-12	145-457	0-13	59-280
Range	0-16	140-534	0-19	6-323

a Solvents include: Distilled water, saline, DMSO, ethanol, DMF:EtOH, EtOH: diH2O or vehicle supplied by Sponsor
b EMS: Ethyl methanesulfonate
c B(a)P: Benzo(a)pyrene

CONCLUSION

Based on these results, it is concluded that X12483137 does not have potential to induce gene mutations at the *hgprt* locus of CHO-K1cells both in the absence and presence of the metabolic activation system under the present experimental conditions.

Test item	Test	Test object	Concentration	Result
X12483137	*In Vitro* mammalian cell forward gene mutation assay	CHO/HPRT	125 to 2000 µg/mL	Negative

Appendix 3 Exposure calculations

A 3.1 Operator exposure calculations (KCP 7.2.1.1)

A 3.1.1 Calculations for florpyrauxifen-benzyl

Table A1: Input parameters considered for the estimation of operator exposure (Overall, Broadcast foliar spray)

Formulation type	EC		Crop type	Low Vegetables
Application rate (AR)	0.002	kg a.s./ha	Application method	Downward spraying
Area treated per day (A)	50	ha	Application equipment	Vehicle-mounted
Dermal absorption (DA)	0.45	% (concentr.)	Indoor/outdoor	Outdoor
	41	% (dilution)	Closed cabin	No
Inhalation absorption (IA)	100	%	Drift reduction	No
Body weight (BW)	60	kg/person	Cultivation	Normal
AOEL	0.13	mg/kg bw/d	Water soluble bag	No
AAOEL	NA	mg/kg bw/d		

Table A2: Estimation of operator exposure for mixing and loading, tractor mounted/trailed sprayer towards florpyrauxifen-benzyl according to EFSA guidance

Model data	Level of PPE	Total absorbed dose [mg/kg bw per day]	% of systemic AOEL
Low vegetables/Outdoor/Downward spraying/Vehicle-mounted/Drift reduction: 0 %/75th percentile Crop density: Normal			
Florpyrauxifen-benzyl	Number of applications and application rate: 1 x 0.002 kg a.s./ha Dermal absorption (concentrate): 0.45 % Dermal absorption (in-use dilution): 41.044776119403 %		
	M/L: Workwear App: Workwear	0.0003	0.2

A 3.2 Worker exposure calculations (KCP 7.2.3.1)

A 3.2.1 Calculations for florpyrauxifen-benzyl

Table A5: Input parameters considered for the estimation of worker exposure Crop inspection and irrigation activities in Low Vegetables

Intended use(s)	Low Vegetables		Dislodgeable foliar residue (DFR)	3	µg/cm²/kg a.s./ha
Application rate (AR)	0.0005	kg a.s./ha	Dermal absorption (DA)	70	% (worst case)
Number of applications (NA)	4		Inhalation absorption (IA)	100	%
Interval between applications	5	days	Work rate per day (WR)	2	h/d
Half-life of active substance	30	days	TC dermal (potential)	12500	cm²/h
Multiple application factor (MAF)	3.39		TC dermal (work wear)	1400	cm²/h
Body weight (BW)	60	kg/person	TC dermal (work wear, gloves)	1250	cm²/h
AOEL	0.13	mg/kg bw/d	Task specific factor inhalation	NA	ha/h x 10^{-3}
AAOEL	NA	mg/kg bw/d			

Table A6: Estimation of acute worker exposure towards active substance according to EFSA guidance

Level of PPE	**Total absorbed dose [mg/kg bw per day]**	**% of systemic AOEL**	**Re-entry restriction [days]**
Inspection, irrigation (All) / Outdoor Work rate: 2 hours/day Interval: 5 days Body weight: 60 kg TC (potential): 12500 cm²/h TC (workwear (arms, body and legs covered)): 1400 cm²/h TC (workwear (arms, body and legs covered) and gloves): 1250 cm²/h TC (gloves): NA cm²/h			
Florpyrauxifen-benzyl	Number of applications & application rate: 4 x 5e-04 kg a.s./ha Dermal absorption: 70 % DFR: 3 µg/cm² foliage per kg a.s./ha DT50: 30 days		
Potential	0.001	1.1	0
Workwear	0.0002	0.1	0
Workwear and gloves	0.0001	0.1	0
Hands covered, no workwear	NA	NA	NA

A 3.3 Resident and bystander exposure calculations (KCP 7.2.2.1)

A 3.3.1 Calculations for florpyrauxifen-benzyl

Table A7: Input parameters considered for the estimation of longer-term resident exposure tractor mounted/trailed sprayer

Intended use(s)	Low Vegetables		Drift reduction (DR)	NA	%
Application rate (AR)	0.0005	kg a.s./ha	Transfer coefficient surface deposits (TC)	7300	cm^2/h (adult)
				2600	cm^2/h (child)
Minimum water volume (V)	100	L/ha	Drift on surface (D) - 75th perc.	5.6	%
Buffer strip	2-3	m	Drift on surface (D) - mean	4.1	%
Number of applications (NA)	4		Turf Transferable Residues (TTR)	5	%
Interval between applications	5	days	Exposure duration dermal (H_D)	2	h
Half-life of active substance	30	days	Exposure duration inhal. (H_I)	24	h
Multiple application factor (MAF)	3.39*		Exposure duration entry into treated crops (H_E)	0.25	h
Body weight (BW)	60	kg/person (adults)	Airborne Concentration of Vapour (VC)	0.001	mg/m^3
	10	kg/person (children)			
Dermal absorption (DA)	70	% ('worst case')	Dislodgeable foliar residue (DFR)	3	$\mu g/cm^2/kg$ a.s.
Inhalation absorption (IA)	100	%	Light clothing adjustment factor (CF)	18	%
Oral absorption (OA)	~~25~~ 100	%	Saliva Extraction Factor (SE)	50	%
AOEL	0.13	mg/kg bw/d	Surface Area of Hands (SA)	20	cm^2
Spray drift dermal (SD) - 75th perc.	0.47	mL spray dilution (adult)	Frequency of Hand to Mouth (Freq)	20	events/h
	0.33	mL spray dilution (child)			
Spray drift inhal. (SI) - 75th perc.	0.00012	mL spray dilution (adult)	Dislodgeable residues object to mouth (DR_{OM})	20	%
	0.00016	mL spray dilution (child)			
Spray drift dermal (SD) - mean	0.22	mL spray dilution (adult)	Ingestion Rate for Mouthing of Grass (IgR)	25	cm^2/d
	0.18	mL spray dilution (child)			
Spray drift inhal. (SD) - mean	0.00011	mL spray dilution (adult)	TC entry into treated crops - 75th perc.	7500	cm^2/h (adult)
	0.00012	mL spray dilution (child)		2250	cm^2/h (child)
Inhalation rate (IR)	0.27	m^3/day/kg (adult)	TC entry into treated crops - mean:	5980	cm^2/h (adult)
	0.8	m^3/day/kg (child)		1794	cm^2/h (child)

*This parameter was reported by the new on-line EFSA OPEX calculator version 1.0.0, in the worker Appendix. The whole Resident/Bystander assessment are missing in the report. Additionally, several of the parameters/inputs presented in the above table are described in the guidance document and its not available on the Report.

Table A8: Estimation of longer-term resident exposure towards florpyrauxifen-benzylaccording to EFSA guidance (max. single application scenario, 1 x 0.002 kg a.s./ha)

Model data	Level of PPE	Total absorbed dose [mg/kg bw per day]	% of systemic AOEL
Season: Not relevant Buffer zone: 2-3 m Drift reduction technology: 0 % Interval between treatments: NA Minimum volume of water: 100 l			
Florpyrauxifen-benzyl		Number of applications and application rate: **1 x 0.002 kg a.s./ha** Dermal absorption: 41.0447761194 % DFR: 3 µg/cm² foliage per kg a.s./ha DT50: 30 days	
Resident child Body weight: 10 kg	Drift (75th perc.)	0.0002	0.2
	Vapour (75th perc.)	0.0008	0.6
	Deposits (75th perc.)	1e-05	0.01
	Re-entry (75th perc.)	0.0001	0.1
	Sum (mean)	**0.001**	**0.8**
Resident adult Body weight: 60 kg	Drift (75th perc.)	5e-05	0.04
	Vapour (75th perc.)	0.0003	0.2
	Deposits (75th perc.)	6e-06	0.004
	Re-entry (75th perc.)	8e-05	0.06
	Sum (mean)	0.0004	0.3

Table A9: Estimation of longer-term resident exposure towards florpyrauxifen-benzyl according to EFSA guidance (multiple application scenario, 4 x 0.0005 kg a.s./ha))

Model data	Level of PPE	Total absorbed dose [mg/kg bw per day]	% of systemic AOEL
Season: Not relevant Buffer zone: 2-3 m Drift reduction technology: 0 % Interval between treatments: 5 days Minimum volume of water: 100 l			
Florpyrauxifen-benzyl	Number of applications and application rate: **4 x 5e-04 kg a.s./ha** Dermal absorption: **70 %** DFR: 3 µg/cm² foliage per kg a.s./ha DT50: 30 days		
Resident child Body weight: 10 kg	Drift (75th perc.)	9e-05	0.07
	Vapour (75th perc.)	0.0008	0.6
	Deposits (75th perc.)	2e-05	0.01
	Re-entry (75th perc.)	0.0002	0.2
	Sum (mean)	0.001	**0.8**
Resident adult Body weight: 60 kg	Drift (75th perc.)	2e-05	0.02
	Vapour (75th perc.)	0.0003	0.2
	Deposits (75th perc.)	8e-06	0.006
	Re-entry (75th perc.)	0.0001	0.09
	Sum (mean)	0.0004	0.3

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)

No further evaluation is needed.