REGISTRATION REPORT

Part B

Section 6

Mammalian Toxicology

Detailed summary of the risk assessment

Product code: GF-3307

Product name(s): Not yet defined

Chemical active substance(s):

Fenpicoxamid (XDE-777), 50 g/L

Prothioconazole, 100 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorization)

Applicant: Corteva Agriscience

Submission date: July 2021, updated January 2022, May 2022

MS Finalisation date: August 2022 (initial Core Assessment)

January 2023 (final Core Assessment)

Version history

When	What
July 2021	New submission of GF-3307 in the Central Zone
January 2022	Genotoxicity study summaries on processing metabolites moved from dRR B7 to dRR B6 in A 2.13
May 2022	Austria removed from cMS, GAP table updated with 1 use = 1 crop + 1 disease Updates on formulation classification with new in vivo acute tox package. All previous information to assess classification has been moved to Appendix 6.
August 2022	Initial assessment by the zRMS The report in the dRR format has been prepared by the Applicant, therefore all comments, additional evaluations and conclusions of the zRMS are presented in grey commenting boxes. Minor changes are introduced directly in the text and highlighted in grey. Not agreed or not relevant information are ~~struck through and shaded for transparency~~.
January 2023	Final report (Core Assessment updated following the commenting period). Additional information/assessments included by the zRMS in the report in response to comments received from the cMS and the Applicant are highlighted in yellow. Information no longer relevant ~~is struck through and shaded~~.

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Reviewer summary:

This part of dossier summarizes data related to the toxicological and NDE assessment for the plant protection product GF-3307 a new formulation (an emulsion concentrate (EC) containing 50 g/L of fenpicoxamid and 100 g/L of prothioconazole) for use as a fungicide in cereals, which has been submitted to support registration according art. 33 of 1107/2009 for the first time in Poland also for zonal registration for which PL was designated zRMS. For the current product registration, Corteva provided an assessment of the toxicological potential based on *in vivo* studies.

Rationale supporting *in vivo* studies: Extrapolation to data of similar formulations was not possible. Approach using "read across" from data on a "similar" formulation GF-3521 was not possible due to the major difference between the two formulations as a presence of a second active ingredient proprioconazole in GF-3521 and a second active ingredient prothioconazole in GF-3307. The same situation was in case of another "similar" formulation GF-3309. Read across approach was not possible due to the major difference between the two formulations as a presence of a second active ingredient pyraclostrobin in GF-3309. Summarizing it cannot be disregarded that this could influence the overall toxicity of the mixtures.

Regarding mentioned above information also fact that GF-3307 is a new plant protection product therefore in order to obtain approval new tests were submitted and the study reports are provided. The testing strategy considered by the Applicant takes into account methods compliant with the 3R concept for refinement, reduction and replacement of animal testing where applicable and acceptable.

Thus, zRMS accept submitted *in vivo* tests. Data obtained from studies is sufficient to indicate the time course and characteristics of the effect with full details of behavioural changes and possible gross pathological findings at post-mortem also allow to identify of effects following a single exposure to the plant protection product can be established. Considering mentioned above information's zRMS decided to conclude hazard assessment for the GF-3307 based on *in vivo* studies.

NDE assessment for operator, workers and B&R has been calculated using the AOEM model (EFSA calculator, version March 2015) and considering the worst-case exposure scenario to cover all the intended uses (highest application rate per application as well as the highest application rate per year with the shorter interval between each application). All NDE calculations provided for operator, workers and B&R resulting from use of PPP, considering all tasks according to the critical use(s), identify safe use of the product GF-3307.

6 Mammalian Toxicology (KCP 7)

This document reviews the toxicology studies and risk calculations for the plant protection product G-3307, a formulation containing fenpicoxamid (XDE-777) (50 g as/L) and prothioconazole (100 g as/L).

6.1 Summary

Table 6.1-1: Information on GF-3307 *

Product name and code	GF-3307
Formulation type	Emulsion concentrate [EC]
Active substance(s) (incl. content)	Fenpicoxamid, 50 g/L Prothioconazole, 100 g/L
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	France (SZ zRMS)

* Information on the detailed composition of GF-3307 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 GF-3307 according to Regulation (EC) No 1272/2008

Hazard class(es), categories	Eye irritation Cat 2 Acute inhalation tox Cat 4 Chronic aquatic Cat 1
Hazard pictograms or Code(s) for hazard pictogram(s)	GHS07, GHS09
Signal word	Warning
Hazard statement(s)	H319 Causes serious eye irritation H332 Harmful if inhaled H410 Very toxic to aqautic life with long lasting effects
Precautionary statement(s)	P261 Avoid breathing mist/vapours/spray P280 Wear protective gloves/~~clothing~~/eye/face protection P304/340 IF INHALED: Remove person to fresh air and keep comfortable for breathing P305/351/338 IF IN EYES: Rinse cautiously with water for several minutes P391 Collect spillage. P501 Dispose of contents/container in accordance with applicable regulations
Additional labelling phrases	To avoid risks to man and the environment, comply with the instructions for use. [EUH401]

Table 6.1-3: Summary of risk assessment for operators, workers, residents and bystanders for GF-3307

	Result	PPE / Risk mitigation measures
Operators	Acceptable	Gloves during mixing/loading
Workers	Acceptable	Working clothing
Residents	Acceptable	None
Bystanders	Acceptable	None

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

A summary of the critical uses and the overall conclusion regarding exposure for operators, workers and residents/bystanders 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 ******	**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**
1-132	Critical Uses: Winter/Spring Cereals (BBCH 30-69)	F	Spraying, LCTM	a) 1 b) 1	a) 0.075 b) 0.150	100 - 300	F	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				

* 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

**** F: PHI is defined by the application stage at last treatment (time elapsing between last treatment and harvest of the crop).

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

Noticed data gaps are: None.

6.2 Toxicological Information on Active Substance(s)

Information regarding classification of the active substances 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)

	Fenpicoxamid	**Prothioconazole**	**Prothioconazole-Desthio**
Common Name	Fenpicoxamid (proposed)	Prothioconazole	Prothioconazole-Desthio
CAS-No.	517875-34-2	178928-70-6	
Classification and proposed labelling			
With regard to toxicological endpoints (according to the criteria in Reg. 1272/2008, as amended)	Hazard classes (s), categories: None Code(s) for hazard pictogram(s):GHS09 Signal word: Warning Hazard statement(s): H410, Precautionary statement(s): P273, P501	Hazard classes (s), categories: None Code(s) for hazard pictogram(s):GHS09 Signal word: Warning Hazard statement(s): H400 Aquatic Acute 1* H410 Aquatic chronic 1 Precautionary statement(s): None	Not applicable

	Fenpicoxamid	Prothioconazole	Prothioconazole-Desthio
		*Commission Delegated Regulation (EU) 2021/849 of 11 March 2021, ATP 17; Official Journal of the European Union; L188; Volume 64; Legislation; 28 May 2021	
Additional C&L proposal	Not applicable	Not applicable	Not applicable
Agreed EU endpoints			
AOEL systemic	0.05 mg/kg bw/d (with a 100-fold assessment factor, corrected for 12 % oral absorption)	0.2 mg/kg bw/day (with a 100-fold assessment factor) (no correction for oral absorption required)	0.01 mg/kd/day (with a 100-fold assessment factor)
AAOEL systemic	0.2 mg/kg bw/day (as ARfD but with corrected for 12% oral absorption)	Not assigned at EU level	Not assigned at EU level
Reference	EFSA Journal 2018;16(1):5146	SANCO/3923 /07 - final 10 December 2007	EFSA Journal (2007); 106, 1-98
Conditions to take into account/critical areas of concern with regard to toxicology			
According to EFSA Conclusion for Fenpicoxamid According to EFSA Conclusion for Prothioconazole	None	The operator safety in spray applications. Conditions of use should include adequate protective measures.	

6.3 Toxicological Evaluation of Plant Protection Product

A summary of the toxicological evaluation for GF-3307 is given in the following two tables. *In vivo* toxicology studies have been conducted using GF-3307. 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 GF-3307

Type of test, species, model system (Guideline)	Result	Acceptability	Classification (acc. to the criteria in Reg. 1272/2008)	Reference
LD_{50} oral, rat (OECD 423)	2000–5000 mg/kg bw	Yes	None	xxxxxxxxx
LD_{50} dermal, rat (OECD 402)	>2000 mg/kg bw	Yes	None	xxxxxxxxxx
LC_{50} inhalation, rat (OECD 436)	>2.9 mg/L air	Yes	Category 4 H332- Harmful if inhaled	xxxxxxxxxxxx
Skin irritation, Dermal, Rabbit (OECD 404)	~~Mean Erythema Score:1.00, 1.00, 1.00 Mean Oedema Score: 0.67, 0.67, 0.67 Recovery completed by 72 hours~~ Non irritant	Yes	None	xxxxxxxx
Eye irritation, Eye, Rabbit (OECD 405)	~~Mean Redness Score: 2.00, 2.00,~~	Yes	Category 2 H319 Causes serious eye irritation	xxxxxxxx

	~~2.00 Mean Chemosis Score: 1.00, 1.00, 1.00 Mean Corneal Score: 0.00, 0.00, 0.00 Mean Iris Score: 0.00, 0.00, 0.00 Recovery completed by 14 days~~ Irritant			
Skin sensitization	Dermal non sensitiser ~~SI = 1.10, 1.63 and 2.51 at 10%, 25% and 50% (v/v) respectively.~~	Yes	None	xxxxxxxxx
Supplementary studies for combinations of plant protection products	No data – not required	Yes	--	--

Table 6.3-2: Additional toxicological information relevant for classification/labelling of GF-3307

	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)	Fenpicoxamid (50 g/L)	None	Fenpicoxamid: EFSA Journal 2018;16(1):5146	Hazard statement(s): Not applicable
Toxicological properties of active substance(s) (relevant for classification of product)	Prothioconazole	None	SANCO/3923 /07 - final 10 December 2007	Hazard statement(s): Not applicable
Toxicological properties of non-active substance(s) (relevant for classification of product)	See part C, point 1.3.2	See part C, point 1.3.2	See part C, point 1.3.2	See part C, point 1.3.2
Further toxicological information	No data – not required			

Based on the results from the *in vivo* acute toxicity studies outlined above, it can be concluded that GF-3307 has low concern for acute oral, dermal and inhalation toxicity and is not a skin irritant or dermal sensitizer. Based on the results from the acute eye irritation study in the rabbit, there was evidence of eye irritation which resolved by day 14 in all rabbits. Therefore, proposed classification regarding acute toxicity endpoints is:

- Eye irritation: Cat 2 – H319
- Acute inhalation toxicity: Cat 4 – H332

6.4 Toxicological Evaluation of Groundwater Metabolites

All metabolite concentrations are predicted to stay below 0.1 µg/L – no groundwater assessment is required.

6.5 Dermal Absorption (KCP 7.3)

A summary of the dermal absorption rates for the active substances in GF-3307 are presented in the following table.

Table 6.5-1: Dermal absorption rates for the active substances fenpicoxamid and prothioconzole in GF-3307 as well as the metabolite prothioconazole-desthio

	Fenpicoxamid		Prothioconazole		Prothioconazole-desthio	
	Value	**Reference**	**Value**	**Reference**	**Value**	**Reference**
Concentrate	0.33 %	Study ID: 200109 GF-3307: In Vitro Percutaneous Absorption of Fenpicoxamid in Human Skin (Whitfield, C, 2021)	25%	Default value for an undiluted EC formulation as stated in the EFSA guidance document on dermal absorption (EFSA, 2017)	25%	Default value for an undiluted EC formulation as stated in the EFSA guidance document on dermal absorption (EFSA, 2017)
Dilution	12 %	Study ID: 200109 GF-3307: In Vitro Percutaneous Absorption of Fenpicoxamid in Human Skin (Whitfield, C, 2021)	70 %	Default value for a diluted EC formulation as stated in the EFSA guidance document on dermal absorption (EFSA, 2017)	14%	Study ID: 200102 GF-3307: In Vitro Percutaneous Absorption of Prothioconazole-desthio in Human Skin (Whitfield, C, 2020)

6.5.1 Justification for proposed values – Fenpicoxamid

A new dermal absorption study through human split thickness skin using [^{14}C]Fenpicoxamid formulated as GF-3307 (Whitfield, C., 2021) has been generated. The summary of this study is detailed in Appendix 2.

The test preparation was applied at two target concentrations: 50 g.L^{-1} (as concentrate) and 0.2 g.L^{-1} (aqueous spray dilution; 1:250). The contact time was 8 h (normal working hours/day) and the post exposure time was 16 h (i.e., the total study duration was 24 h). The amount of [^{14}C]Fenpicoxamid absorbed was measured and the rates of penetration and test material distribution were determined.

The total absorbed dose using the data from Whitfield (2021), when assessed in accordance with EFSA Guidance on Dermal Absorption (2017) is 0.33% for the 50g fenpicoxamid.L^{-1} concentrate, and 12% for the 0.2 g fenpicoxamid /L aqueous spray dilution. Further details can be found in Appendix 2 (output from the EFSA Guidance Excel calculator). Therefore, these values of 0.33% and 12% have been used in the calculations for estimating potential exposure of fenpicoxamid to operators, bystanders, residents and workers.

Table 6.5-2: Default dermal absorption rates for fenpicoxamid

	Value	**Justification for value**	**Acceptability of justification**
Concentrate	0.33 %	Whitfield, C, 2021	Study accepted. DA values obtained from the study can be used for current product
Dilution	12 %	Whitfield, C, 2021	Study accepted. DA values obtained from the study can be used for current product

6.5.2 Justification for proposed values - Prothioconazole

No data on dermal absorption for prothioconazole in GF-3307 is available. Justifications for default values according to Guidance on Dermal Absorption (EFSA Journal EFSA Journal 2017; 15(6):4873) are presented in the following table.

Table 6.5-3: Default dermal absorption rates for prothioconazole

	Value	Justification for value	Acceptability of justification
Concentrate	25 %	> 5% (50 g/L for liquids) in undiluted product. Default value for a diluted EC formulation as stated in the EFSA guidance document on dermal absorption (EFSA, 2017)	Justification accepted. Endpoint can be used for current product
Dilution	70 %	≤ 5% active substance in diluted product. Default value for a diluted EC formulation as stated in the EFSA guidance document on dermal absorption (EFSA, 2017)	Justification accepted. Endpoint can be used for current product

6.5.3 Justification for proposed values – Prothioconazole-desthio

Prothioconazole-desthio is not part of the formulation. Rather it is a metabolite of prothioconazole which is formed at different rates during the drying process of aqueous diluted solutions of the active substance on surfaces.

A new dermal absorption study through human split thickness skin using [^{14}C] PTZ-desthio formulated as GF-3307 (Whitfield, C., 2020) has been generated. The summary of this study is detailed in Appendix 2.

The test preparation was applied at a target concentration of 0.363 g L 1 (as aqueous spray dilution; 1:250) assuming that all prothioconazole in the diluted formulation formed prothioconazole-desthio. The spray dilution used in the study is representative of the most dilute in-use spray concentration in the GAP assuming 100% conversion of prothioconazole to PTZ-desthio. The contact time was 8 h (normal working hours/day) and the post exposure time was 16 h (i.e., the total study duration was 24 h). The amount of [^{14}C] prothioconazole-desthio absorbed was measured and the rates of penetration and test material distribution were determined.

The total absorbed dose using the data from Whitfield (2020), when assessed in accordance with EFSA Guidance on Dermal Absorption (2017) is 14% for the 0.363 g PTZ-desthio/L aqueous spray dilution. Further details can be found in Appendix 2 (output from the EFSA Guidance Excel calculator). Therefore, this value of 14% has been used in the calculations for estimating potential exposure of diluted PTZ-desthio to operators, bystanders, residents and workers. It is worthy of note that this value is consistent with dermal absorption values in studies used in the Multi-to-One approach.

The dermal absorption value used for the concentrate of PTZ-desthio is the default value of 25% as stated in the EFSA Guidance on Dermal Absorption (2017).

Table 6.5-3: Default dermal absorption rates for prothioconazole-desthio

	Value	Justification for value	Acceptability of justification
Concentrate	25 %	EFSA Journal 2017	Justification accepted. Endpoint can be used for current product
Dilution	14 %	Whitfield, C., 2020	Study accepted. DA values obtained from the study can be used for current product

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	GF-3307		
Formulation type	EC		
Category	Fungicide		
Active substance(s)	**Fenpicoxamid**	**Prothiconazole**	**Prothioconazole-Desthio**

(incl. content)	50 g/L	100 g/L	**(PTZ-Desthio)**
AOEL systemic	0.05 mg/kg bw/day	0.2 mg/kg bw/day	0.01 mg/kg bw/day
Inhalation absorption	100 %	100 %	100 %
Oral absorption	12 %	100 %	100 %
Dermal absorption	Concentrate: 0.33 % (Whitfield, C., 2021) Dilution: 12 % (Whitfield, C., 2021) *For more information please refer to chapter 6.5*	Concentrate: 25 % (EFSA Default) Dilution: 70 % (EFSA Default) *For more information please refer to chapter 6.5*	Concentrate: 25% (EFSA 2017) Dilution: 14% (Whitfield, C., 2020) *For more information please refer to chapter 6.5*

The metabolite prothioconazole-desthio (PTZ-desthio) is not part of the formulation per se. However, it has been found that **prothioconazole (PTZ) can convert to PTZ-desthio in diluted solutions** during the drying process on clothing, skin or on certain plant surfaces. Although PTZ-desthio is not an active substance and not a component of the formulation per se non-dietary **risk assessments are always performed for PTZ-desthio** due to its toxicological properties.

6.6.1 Selection of critical use(s) and justification

The critical GAP 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 is given in Part B, Section 0.

Justification

The critical uses selected represent the worst-case exposure scenarios based on the proposed uses of GF-3307.

The following risk assessments have been conducted for one product rate, 1.5 L Product/ha using a water volume of 100 L/ha.

A worst case scenario of 100% conversion from PTZ to PTZ-desthio has been assumed for the risk assessments for PTZ-desthio. Taking into account the different molar weights of PTZ (344.3 g/mol) and PTZ-desthio (312.2 g/mol) the maximum application rate has been corrected by the molar ratio (i.e. 1.103) to give 0.136 kg a.s./ha (equivalent to 0.15 kg a.s./ha PTZ).

6.6.2 Operator exposure (KCP 7.2.1)

Reviewer comment:

The NDE calculations performed by the applicant using EFSA Operator Model (75th quantile regression) are acceptable and zRMS agrees to the conclusions.
The risk assessment/calculated exposure for operators, workers and B&R are acceptable under conditions of intended uses.

On 24 January 2017 the European Commission Standing Committee had published an update on their guidance on the implementation of EFSA's non-dietary exposure guidance document[1]. It notes that the derivation of the toxicological reference value (AAOEL) for the corresponding acute risk assessments is still outstanding. However, the Standing Committee developed an outline to set AAOELs.

Consideration of acute operator exposure as well as bystander exposure should only be made where an AAOEL has been established during an approval, review or renewal evaluation of an active substance.

[1] COMMISSION GUIDANCE DOCUMENT, Guidance on the assessment of exposure of operators, workers, residents and bystanders in risk assessment for plant protection products, SANTE-10832-2015 rev. 1.7
24 January 2017

Rev. 1.7 of the GD applies to applications for the approval or renewal of approval of active substances and the applications to authorise or renew authorisations for plant protection products submitted since 1st March 2017 as follows: Where necessary, an AAOEL should be proposed during the EU peer-review taking into account the Annex to the Commission guidance document.

For fenpicoxamid an AAOEL has been established during an EU-approval evaluation. Thus an acute non-dietary risk assessment is included in this submission.

For prothioconazole and prothioconazole-desthio no AAOEL has been established during an EU-approval or EU renewal evaluation. Thus, no acute non-dietary risk assessment is included in this submission for prothioconazole and its metabolite prothioconazole-desthio.

As previously stated two scenarios have been presented in the following Operator exposure estimations carried out using the EFSA Model. The data indicated that the acceptable operator exposure level (AOEL) for fenpicoxamid, prothioconazole and the metabolite prothioconazole-desthio will not be exceeded under conditions of intended use (max. rate of 1.5 L product/ha) and with the operator wearing appropriate work-wear and PPE (gloves) for both mixing/loading and application.

Using the EFSA Model, the estimated exposure to fenpicoxamid with the use of PPE (gloves) was 4.3% of the AAOEL at an application rate of 0.075 kg a.s./ha. The estimated longer term exposure to fenpicoxamid with PPE (gloves) was 0.7% of the AOEL at an application rate of 0.075 kg a.s./ha.

The estimated exposure to prothioconazole with the use of PPE (gloves) was 2.5% the AOEL at an application rate of 0.15 kg a.s./ha.

The estimated exposure to desthio-prothioconazole with the use of PPE (gloves) was 30.4% of the AOEL at an application rates of 0.136 kg a.s./ha.

6.6.2.1 Estimation of operator exposure

A summary of the exposure models used for estimation of operator exposure to the active substances during application of GF-3307 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 for fenpicoxamid (acute exposure) and Table 6.6-4 for (longer term exposure). Detailed calculations are in Appendix 3.

Table 6.6-2: Exposure models for intended uses

Critical use(s)	Winter and Spring Cereals (max. 1.5 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 2014;12(10):3874 calculator version: 30/03/2015

Table 6.6-3: Estimated operator exposure, Fenpicoxamid Cereals (acute exposure)

Model data	Level of PPE	Total absorbed dose (mg/kg/day)	% of systemic AAOEL[1] (RVNAS)
Outdoor, Downward spraying, Vehicle-mounted Application rate: 0.075 kg a.s./ha			
EFSA Operator Model (75th quantile regression) Body weight: 60 kg	no PPE[2]	0.0156	7.81
	with PPE[3]	0.0086	4.32

[1] AAOEL (RVNAS) of fenpicoxamid: 0.2 mg/kg bw/day
[2] no PPE: Work wear - arms, body and legs covered
[3] with PPE: Work wear - arms, body and legs covered. In addtion gloves during mixing and loading and when handling contaminated surfaces during application.

Table 6.6-4: Estimated operator exposure, Fenpicoxamid, Cereals (longer term exposure)

Model data	Level of PPE	Total absorbed dose (mg/kg/day)	% of systemic AOEL[1] (RVNAS)
Outdoor, Downward spraying, Vehicle-mounted Application rate: 0.075 kg a.s./ha			
EFSA Operator Model (75^{th} quantile regression) Body weight: 60 kg	no PPE[2]	0.002	4.08
	with PPE[3]	0.0004	0.73

[1]AOEL (RVNAS) of fenpicoxamid: 0.05 mg/kg bw/day
[2]no PPE: Work wear - arms, body and legs covered
[3]with PPE: Work wear - arms, body and legs covered. In addtion gloves during mixing and loading and when handling contaminated surfaces during application.

Table 6.6-5: Estimated operator exposure, Prothioconazole, Cereals (longer term exposure)

Model data	Level of PPE	Total absorbed dose (mg/kg/day)	% of systemic AOEL[1] (RVNAS)
Outdoor, Downward spraying, Vehicle-mounted Application rate: 0.15 kg a.s./ha			
EFSA Operator Model (75^{th} quantile regression) Body weight: 60 kg	no PPE[2]	0.1113	55.67
	with PPE[3]	0.0049	2.46

[1]AOEL (RVNAS) of prothioconazole: 0.2 mg/kg bw/day
[2]no PPE: Work wear - arms, body and legs covered
[3]with PPE: Work wear - arms, body and legs covered. In addtion gloves during mixing and loading and when handling contaminated surfaces during application.

Table 6.6-6: Estimated operator exposure, PTZ-desthio, Cereals (longer term exposure)

Model data	Level of PPE	Total absorbed dose (mg/kg/day)	% of systemic AOEL[1] (RVNAS)
Outdoor, Downward spraying, Vehicle-mounted Application rate: 0.136 kg a.s./ha[2]			
EFSA Operator Model (75^{th} quantile regression) Body weight: 60 kg	no PPE[3]	0.0931	931.37
	with PPE[4]	0.003	30.44

[1]AOEL (RVNAS) of PTZ-desthio: 0.01 mg/kg bw/day
[2]worst case scenario: 100% conversion from PTZ to PTZ-desthio. Taking into account the different molar weights of PTZ (344.3 g/mol) and PTZ-desthio (312.2 g/mol) the maximum application rate has been corrected by the molar ratio (i.e. 1.103) to give 0.159 kg a.s./ha.
[3]no PPE: Work wear - arms, body and legs covered
[4]with PPE: Work wear - arms, body and legs covered. In addtion gloves during mixing and loading and when handling contaminated surfaces during application.

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 for fenpicoxamid, prothioconazole and PTZ-desthio under conditions of intended uses and consideration of the above mentioned personal protective equipment (PPE), a study to provide measurements of operator exposure was not necessary and was therefore not performed.

6.6.3 Worker exposure (KCP 7.2.3)

Worker exposure estimations carried out using the EFSA Model indicated that the acceptable exposure level for fenpicoxamid, prothioconazole and PTZ-desthio will not be exceeded under conditions of intended use and with the worker wearing appropriate workwear.
Using the EFSA Model, the estimated exposures without PPE at an application rate of 1.5 L product/ha was

3% (AOEL) for fenpicoxamid, 7% (AOEL) for prothioconazole and 10% (AOEL) for PTZ-desthio.

6.6.3.1 Estimation of worker exposure

Table 6.6-7 shows the exposure model(s) used for estimation of worker exposure after entry into a previously treated area or handling a crop treated with GF-3307 according to the critical use(s). Relevant parameters used for the worker exposure assessment are presented in Table 6.6-8 and outcome of the estimation is presented in Table 6.6-9. Detailed calculations are in Appendix 3.

Table 6.6-7: Exposure models for intended uses

Critical use(s)	Winter and spring cereals (max.1.5 L product/ha)
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

Table 6.6-8: Relevant parameters used for the worker exposure assessment

Crop / Crop Group	Active substance	Application rate (kg a.s./ha)	Nº of applications	Interval (Days)	TC[1] (cm²/hour)	Task Duration (hours)
Cereals	Fenpicoxamid	0.075	1	N/A	1400	2
	PTZ	0.15				
	PTZ-desthio	0.136[2]				

[1] TC = transfer coefficients assuming arms, body and legs covered (workwear; bare hands)
[2] worst case: 100% conversion from PTZ to PTZ-desthio. MW= 312.2 g/mol, so 0.136 kg/ha (rate corrected by the molar ratio)

The outcome of the estimation is presented in the following table.

Table 6.6-9: Estimated worker exposure for re-entry in Cereals

Model data		Active substance	Application rate (kg a.s./ha)	Total absorbed dose[2] (mg/kg/day)	% of systemic AOEL[1] (RVNAS)
EFSA Worker Model Body weight: 60 kg	DFR: 3 µg/cm²/kg a.s./ha	Fenpicoxamid	0.075	0.0013	2.52
		Prothioconazole	0.150	0.0147	7.35
	DFR: 0.63 µg/cm²/kg a.s./ha	PTZ-desthio	0.136[3]	0.0010	10

[1]AOEL (RVNAS) of fenpicoxamid: 0.05 mg/kg bw/day, PTZ: 0.2 mg/kg bw/day, PTZ-desthio: 0.01 mg/kg bw/day
[2]Assuming arms, body and legs covered (workwear; bare hands)
[3]worst case scenario: 100% conversion from PTZ to PTZ-desthio. Taking into account the different molar weights of PTZ (344.3 g/mol) and PTZ-desthio (312.2 g/mol) the maximum application rate has been corrected by the molar ratio (i.e. 1.103) to give 0.136 kg a.s./ha.

6.6.3.2 Refinement of generic DFR value (KCP 7.2)

For PTZ-desthio two DFR studies have been conducted by BCS (Stuke 2013 and Stuke 2015)[2]. Crop and

[2] Under evaluation in PTZ renewal process

substance specific DFR values (0.63 µg/cm²/kg a.s./ha) have been used from the studies to refine the exposure due to re-entry scenarios.
The outcome of the estimation has been presented in table 6.6-9. For details please refer to Appendix 3.2 (Calculations) or Appendix 4.2 (Details on the DFR study).

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 for fenpicoxamid, prothioconazole and PTZ-desthio under conditions of intended uses and considering above mention PPE, a study to provide measurements of worker exposure was not necessary and was therefore not performed.

6.6.4 Resident and bystander exposure (KCP 7.2.2)

Resident exposure estimations carried out using the EFSA Model indicated that the acceptable exposure level will not be exceeded under conditions of intended use. Using the EFSA Model, the highest estimated all pathways exposure for residents for fenpicoxamid and prothioconazole were 7% and 16% of the AOEL respectively.

The EFSA model was used in conjunction with experimental DFR data in the assessment of PTZ-desthio. The highest estimated all pathways exposure was 53% of the AOEL.

Bystander exposure estimations carried out using the EFSA Model indicated that the acceptable exposure level for fenpicoxamid, will not be exceeded under conditions of intended use.
For fenpicoxamid the highest predicted bystander exposure using the EFSA Model was 2.77% of the AAOEL (spray drift, 95th percentile).

The acute exposure assessment for bystanders covers the exposure that a resident could reasonably be expected to incur in a single day. Therefore, there is no need for a separate acute risk assessment for residents.

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.

On 24 January 2017 the European Commission Standing Committee has published an update on their guidance on the implementation of EFSA's non-dietary exposure guidance document[3]. It notes that the derivation of the toxicological reference value (AAOEL) for the corresponding acute risk assessments is still outstanding. However, the Standing Committee developed an outline to set AAOELs.

Consideration of acute operator exposure as well as bystander exposure should only be made where an AAOEL has been established during an approval, review or renewal evaluation of an active substance.

Rev. 1.7 of the GD applies to applications for the approval or renewal of approval of active substances and the applications to authorise or renew authorisations for plant protection products submitted since 1st March 2017 as follows: Where necessary, an AAOEL should be proposed during the EU peer-review taking into account the Annex to the Commission guidance document.

For prothioconazole and prothioconazole-desthio no AAOEL has been established during an EU-approval or EU renewal evaluation. Thus, no acute non-dietary risk assessment is included in this submission.

[3] COMMISSION GUIDANCE DOCUMENT, Guidance on the assessment of exposure of operators, workers, residents and bystanders in risk assessment for plant protection products, SANTE-10832-2015 rev. 1.7
24 January 2017

Table 6.6-10 shows the exposure model(s) used for estimation of resident exposure to fenpicoxamid, prothioconazole and prothioconazole desthio. The outcome of the estimations are presented in Table 6.6-11, 6.6-12 and Table 6.6-13 (longer term resident exposure) respectively. Table 6.6-13 summarizes the outcome of the resident exposure estimation to PTZ-desthio using a combination of EFSA default data, DFR study data (further details in Appendix 4) and dermal absorption study data (further details in Appendix 2 & 5).

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
-	-	-	-	-	-

The outcome of the acute bystander assessment for fenpicoxamid is presented in table 6.6-14.

Detailed calculations for exposure estimations to fenpicoxamid, prothioconazole & PTZ-desthio are in Appendix 3.

6.6.4.1 Estimation of resident and bystander exposure

A summary of the exposure models used for the estimation of resident exposure to the active substance(s) during application of GF-3307 according to the critical use(s) is presented in the following table. Detailed calculations are presented in Appendix 3.

Table 6.6-10: Exposure models for intended uses

Critical use(s)	Winter and spring cereal (max. 1.5 L product/ha)
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

Regarding the resident exposure to direct drift, exposure calculations are performed for ground boom sprayer (for low crops). The outcome of the estimation is presented in the following tables.

As already mentioned in previous chapters it is known that after foliar spray application of PTZ-containing products diluted PTZ can degrade to prothioconazole-desthio (PTZ-desthio) on plant surfaces, clothing or skin. Accordingly, although PTZ-desthio is not part of the formulation per se non-dietary risk assessments are always performed for PTZ-desthio due to its toxicological properties. No model is available to estimate the conversion of PTZ to PTZ-desthio in a realistic manner. Therefore, risk assessments should always consider measured data whenever such data are available. Thus, resident exposure to PTZ-desthio is presented in chapter 6.6.5.2 using measured DFR values for PTZ-desthio on cereals.

Four pathways of residential exposure have to be considered according to the new EFSA guidance[4]:

- spray drift
- vapour
- surface deposits
- entry into treated crops

Consideration on residential exposure due to <u>spray drift</u>

Exposure to fenpicoxamid prothioconazole and PTZ-desthio is calculated according to EFSA (Tier 1).

Consideration on residential exposure due to <u>vapour</u>

In a tier 1 approach exposure to fenpicoxamid, PTZ and PTZ-desthio is calculated according to EFSA. A worst case conversion of 100% from PTZ to PTZ-desthio is assumed.

[4] EFSA (European Food Safety Authority), 2014. 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, 55 pp., doi:10.2903/j.efsa.2014.3874

Consideration on residential exposure due to surface deposits

Exposure to fenpicoxamid, PTZ and PTZ-desthio is calculated according to EFSA.
A conversion from PTZ to PTZ-desthio of 100% is assumed for the exposure to surface deposits. Taking into account the different molar weights of PTZ (344.3 g/mol) and PTZ-desthio (312.2 g/mol) the maximum application rates in mg a.s./cm² are continuously corrected by the molar ratio (i.e. 1.103).

Consideration on residential exposure due to entry into treated crops

In a tier 1 approach exposure to fenpicoxamid and PTZ is calculated according to EFSA using a default DFR of 3 µg/cm²/kg a.s./ha. As indicated exposure to PTZ-desthio is solely assessed considering measured DFR values (0.63 µg/cm²/kg a.s./ha).

Results of the exposure calculations are summarised in following tables.

Table 6.6-11: Estimated resident exposure, Fenpicoxamid

		Fenpicoxamid	
Model data		**Total absorbed dose (mg/kg bw/day)**	**% of systemic AOEL**
Tractor mounted boom spray application outdoors to low crops Buffer zone 2-3(m) Drift reduction technology: no DT_{50}: 30 days DFR: 3 µg/cm²/kg a.s./ha Nb of applications: 1 Interval between treatments:365 days			
Application rate		0.075 kg a.s./ha	
Resident child Body weight: 10 kg	Drift (75th perc.)	0.0024	4.86
	Vapour (75th perc.)	0.0011	2.14
	Deposits (75th perc.)	0.0001	0.28
	Re-entry (75th perc.)	0.0015	3.04
	Sum (mean)	0.0037	7.45
Resident adult Body weight: 60 kg	Drift (75th perc.)	0.0006	1.16
	Vapour (75th perc.)	0.0002	0.46
	Deposits (75th perc.)	0.0001	0.12
	Re-entry (75th perc.)	0.0008	1.69
	Sum (mean)	0.0012	2.45

Table 6.6-12: Estimated resident exposure, Prothioconazole

		prothioconazole	
Model data		**Total absorbed dose (mg/kg bw/day)**	**% of systemic AOEL**
Tractor mounted boom spray application outdoors to low crops Buffer zone 2-3(m) Drift reduction technology: no DT_{50}: 30 days DFR: 3 µg/cm²/kg a.s./ha Nb of applications: 1 Interval between treatments:365 days			
Application rate		0.15 kg a.s./ha	
Resident child	Drift (75th perc.)	0.0282	14.09

Body weight: 10 kg	Vapour (75th perc.)	0.0011	0.54
	Deposits (75th perc.)	0.0017	0.83
	Re-entry (75th perc.)	0.0177	8.86
	Sum (mean)	0.0319	15.96
Resident adult Body weight: 60 kg	Drift (75th perc.)	0.0067	3.37
	Vapour (75th perc.)	0.0002	0.12
	Deposits (75th perc.)	0.0007	0.36
	Re-entry (75th perc.)	0.0098	4.92
	Sum (mean)	0.0118	5.90

Table 6.6-13: Estimated resident exposure, PTZ-desthio

		PTZ-desthio			
Model data		**Total absorbed dose (mg/kg bw/day)**	**% of systemic AOEL**	**Total absorbed dose (mg/kg bw/day)**	**% of systemic AOEL**
Tractor mounted boom spray application outdoors to low crops Buffer zone 2-3(m) Drift reduction technology: no DT_{50}: 30 days DFR: 0.63 µg/cm²/kg a.s./ha Nb of applications: 1 Interval between treatments:365 days					
Application rate		0.136 kg a.s./ha			
Resident child Body weight: 10 kg	Drift (75th perc.)	0.0051		51.35	
	Vapour (75th perc.)	0.0011		10.70	
	Deposits (75th perc.)	0.0006		6.05	
	Re-entry (75th perc.)	0.0012		12.05	
	Sum (mean)	0.0053		53.07	
Resident adult Body weight: 60 kg	Drift (75th perc.)	0.0012		12.25	
	Vapour (75th perc.)	0.0002		2.30	
	Deposits (75th perc.)	0.0002		2.32	
	Re-entry (75th perc.)	0.0007		6.69	
	Sum (mean)	0.0015		15.16	

Table 6.6-14: Estimated bystander exposure (acute exposure), Fenpicoxamid

		Fenpicoxamid			
Model data		**Total absorbed dose (mg/kg bw/day)**	**% of systemic AAOEL**	**Total absorbed dose (mg/kg bw/day)**	**% of systemic AAOEL**
Tractor mounted boom spray application outdoors to low crops Buffer zone: 2-3 (m) Drift reduction technology: No DFR: 3 µg/cm²/kg a.s./ha					
Application rate		0.075 kg a.s./ha			
Bystander child Body weight: 10 kg	Drift (95th perc.)	0.0055		2.77	
	Vapour (95th perc.)	0.0011		0.54	
	Deposits (95th perc.)	0.0004		0.21	

	Re-entry (95th perc.)	0.0015	0.76
Bystander adult Body weight: 60 kg	Drift (95th perc.)	0.0015	0.75
	Vapour (95th perc.)	0.0002	0.12
	Deposits (95th perc.)	0.0002	0.09
	Re-entry (95th perc.)	0.0008	0.42

6.6.4.2 Measurement of resident and/or bystander exposure

As indicated in the worker exposure assessment, exposure to PTZ-desthio is assessed considering a measured DFR value of 0.63 µg/cm²/kg a.s./ha, (Stuke 2013 and Stuke 2015).

6.6.5 Combined exposure

GF-3307 is a mixture of two active substances, fenpicoxamid and prothioconazole, so combined exposure risk assessment is requested.

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."

6.6.5.1 Exposure assessment of Fenpicoxamid, Prothioconazole and PTZ-desthio in GF-3307

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

At the first tier, combined exposure is calculated as the sum of the component exposures without regard to the mode of action or mechanism/target of toxicity. Initially, the individual Hazard Quotients (HQ) are calculated for all active substances in the PPP 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 from
Table 6.6-3 converted to decimal. The Hazard Index (HI) is the sum of the individual HQs.

GF-3307 is a mixture of two active substances, fenpicoxamid and prothioconazole. However, prothioconazole can be converted to its metabolite (PTZ-desthio) when diluted in aqueous solution. Exposure resulting from use of the formulation is therefore to a mixture containing both the parent compound (PTZ) and the metabolite (PTZ-desthio). In order to ensure the risk assessment is suitably conservative, risk assessments have been conducted for both the parent and the metabolite separately, of which the more conservative has been presented for each exposure scenario. It is not appropriate to combine the risk assessments for both PTZ and PTZ-desthio as the active substance can only be in one of the possible forms – combined assessment for both forms would artificially double the amount of active. Therefore, for the evaluation of combined risk from co-exposure to mixture partner active substances, it is the worst-case assessment for whichever molecule produces the highest HQ for each exposure population (PTZ or PTZ-desthio) which has been used in combination with the risk assessment for fenpicoxamid.

$$HI = HQ_{Fenpicoxamid} + Max\ [HQ_{PTZ}\ or\ HQ_{PTZ\text{-}desthio}]$$

A cumulative assessment was conducted based on a combination of data derived from the EFSA model using default parameters, PPE and study data (DFR studies).

A cumulative assessment for acute exposures is not required as it is only fenpicoxamid that currently has an assigned AAOEL.

Table 6.6-16: Risk assessment from combined exposure (longer term exposure)

Application scenario	Active ingredient	Estimated exposure / AOEL (HQ) (1.5 L product/ha)
Operators	Fenpicoxamid	0.007
	Prothioconazole	0.02
	PTZ-Desthio	0.30
	Cumulative risk operators (HI)	0.33 **(Fenpicoxamid + PTZ-desthio)**
Workers	Fenpicoxamid	0.03
	Prothioconazole	0.07
	PTZ-Desthio	0.10
	Cumulative risk workers (HI)	**0.13 (Fenpicoxamid + PTZ-desthio)**
Resident - child	Fenpicoxamid	
	Drift	0.05
	Vapour	0.02
	Deposits	0.003
	Re-entry	0.03
	Sum of all pathways	0.07
	Prothioconazole	
	Drift	0.14
	Vapour	0.005
	Deposits	0.008
	Re-entry	0.09
	Sum of all pathways	0.16
	PTZ-desthio	
	Drift	0.5
	Vapour	0.1
	Deposits	0.06
	Re-entry	0.12
	Sum of all pathways	0.5
	Cumulative risk resident – child (HI)	
	Drift (fenpicoxamid + PTZ-desthio)	0.05 + 0.5 = 0.55
	Vapour (Fenpicoxamid + PTZ-desthio)	0.02 + 0.1 = 0.12
	Deposits (Fenpicoxamid + PTZ-desthio)	0.003 + 0.06 = 0.063
	Re-entry (Fenpicoxamid + PTZ-desthio)	0.03 + 0.12 = 0.15
	Sum of all pathways (Fenpicoxamid + PTZ-desthio)	**(0.07+0.5) = 0.57**
Resident - adult	Fenpicoxamid	
	Drift	0.01
	Vapour	0.005
	Deposits	0.001
	Re-entry	0.02
	Sum of all pathways	0.02
	Prothioconazole	

Application scenario	Active ingredient	Estimated exposure / AOEL (HQ) (1.5 L product/ha)
	Drift	0.03
	Vapour	0.001
	Deposits	0.004
	Re-entry	0.05
	Sum of all pathways	0.06
	PTZ-desthio	
	Drift	0.12
	Vapour	0.02
	Deposits	0.02
	Re-entry	0.07
	Sum of all pathways	0.15
	Cumulative risk resident – adult (HI)	
	Drift (Fenpicoxmid + PTZ-desthio)	0.01 + 0.12 = 0.13
	Vapour (Fenpicoxamid + PTZ-desthio)	0.005 + 0.02 = 0.025
	Deposits (Fenpicoxamid + PTZ-desthio)	0.001 + 0.02 = 0.021
	Re-entry (Fenpicoxamid + PTZ-desthio)	0.02 + 0.07 = 0.1
	Sum of all pathways (Fenpicoxamid + PTZ-desthio)	**(0.02+0.15) = 0.2**

The Hazard Index is < 1. Thus, combined exposure to all active substances in GF-3307 is not expected to present a risk for operators, workers, residents and bystanders. No further refinement of the assessment is required.

Appendix 1 Lists of data considered in support of the evaluation

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 7.1.1/1	Prajapati, J	2021a	Acute Oral Toxicity Study of GF-3307 in Rats xxxxxxxxxx	Y	Corteva Agriscience
KCP.7.1.2/1	Prajapati, J	2021b	Acute Dermal Toxicity Study of GF-3307 in Rats xxxxxxxxxx Unpublished	Y	Corteva Agriscience
KCP 7.1.3/1	Kegelman, T. A	2021	GF-3307: Inhalation Median Lethal Concentration (LC50) Study in Rats xxxxxxxxxx GLP Unpublished	Y	Corteva Agriscience
KCP 7.1.4/1	Prajapati, J	2021c	Acute Dermal Irritation Study of GF-3307 in Rabbits xxxxxxxxxx GLP Unpublished	Y	Corteva Agriscience
KCP 7.1.5/1	Prajapati, J	2021d	Acute Eye Irritation Study of GF-3307 in Rabbits xxxxxxxxxx GLP Unpublished	Y	Corteva Agriscience
KCP 7.1.6/1	Prajapati, J	2021e	Skin Sensitisation Study of GF-3307 by Local Lymph Node Assay in Mice xxxxxxxxxx GLP Unpublished	Y	Corteva Agriscience
~~KCP 7.1.1/1~~	~~Patel, M.P.~~	~~2017a~~	~~Acute Oral Toxicity Study of GF-3521 in Rats~~ xxxxxxxxxx ~~Unpublished~~	~~Y~~	~~Dow AgroSciences~~
~~KCP 7.1.1/2~~	~~Verma, R.~~	~~2018a~~	~~Acute Oral Toxicity Study of GF-3309 in Rats~~ ~~xxxxxxxxxx~~ ~~Unpublished~~	~~Y~~	~~Dow AgroSciences~~
~~KCP.7.1.2/1~~	~~Patel, M.P.~~	~~2017b~~	~~Acute Dermal Toxicity Study of GF-3521 in Rats~~ ~~xxxxxxxxxxxxxx~~ ~~Unpublished~~	~~Y~~	~~Dow AgroSciences~~
~~KCP.7.1.2/2~~	~~Verma, R.~~	~~2018b~~	~~Acute Dermal Toxicity Study of GF-3309 in Rats~~ ~~xxxxxxxxxxxxxxxx~~ ~~GLP~~	~~Y~~	~~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
KCP 7.1.1/1	Prajapati, J	2021a	Acute Oral Toxicity Study of GF-3307 in Rats xxxxxxxxxxx	Y	Corteva Agriscience
KCP.7.1.2/1	Prajapati, J	2021b	Acute Dermal Toxicity Study of GF-3307 in Rats xxxxxxxxxxx Unpublished	Y	Corteva Agriscience
KCP 7.1.3/1	Kegelman, T. A	2021	GF-3307: Inhalation Median Lethal Concentration (LC50) Study in Rats xxxxxxxxxxx GLP Unpublished	Y	Corteva Agriscience
KCP 7.1.4/1	Prajapati, J	2021c	Acute Dermal Irritation Study of GF-3307 in Rabbits xxxxxxxxxxx GLP Unpublished	Y	Corteva Agriscience
KCP 7.1.5/1	Prajapati, J	2021d	Acute Eye Irritation Study of GF-3307 in Rabbits xxxxxxxxxxx GLP Unpublished	Y	Corteva Agriscience
KCP 7.1.6/1	Prajapati, J	2021e	Skin Sensitisation Study of GF-3307 by Local Lymph Node Assay in Mice xxxxxxxxxxx GLP Unpublished	Y	Corteva Agriscience
			~~Unpublished~~		
~~KCP 7.1.3/1~~	~~Patel, M.P.~~	~~2017c~~	~~Acute Inhalation Toxicity Study of GF-3521 in Rats~~ ~~xxxxxxxxxxxx~~ ~~Unpublished~~	~~Y~~	~~Dow AgroSciences~~
~~KCP.7.1.3/2~~	~~Verma, R.~~	~~2018c~~	~~Acute Inhalation Toxicity Study of GF-3309 in Rats~~ ~~xxxxxxxxxxxxxx~~ ~~Unpublished~~	~~Y~~	~~Dow AgroSciences~~
~~KCP 7.1.4/1~~	~~Settivari, R. S., and Sosinski, L. K.~~	~~2016~~	~~GF-3307: Evaluation of the Skin Irritation Potential Using the In Vitro EpiDerm Skin Model~~ ~~Report No: 140101~~ ~~Source Toxicology and Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan, USA~~ ~~non GLP~~ ~~Unpublished~~	~~N~~	~~Dow AgroSciences~~
~~KCP 7.1.4/2~~	~~Patel, M.P.~~	~~2017d~~	~~Acute Dermal Irritation Study of GF-3521 in Rabbits~~ ~~xxxxxxxxxxxxxx~~ ~~GLP~~	~~Y~~	~~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
KCP 7.1.1/1	Prajapati, J	2021a	Acute Oral Toxicity Study of GF-3307 in Rats xxxxxxxxxxx	Y	Corteva Agriscience
KCP.7.1.2/1	Prajapati, J	2021b	Acute Dermal Toxicity Study of GF-3307 in Rats xxxxxxxxxxx Unpublished	Y	Corteva Agriscience
KCP 7.1.3/1	Kegelman, T. A	2021	GF-3307: Inhalation Median Lethal Concentration (LC50) Study in Rats xxxxxxxxxxx GLP Unpublished	Y	Corteva Agriscience
KCP 7.1.4/1	Prajapati, J	2021c	Acute Dermal Irritation Study of GF-3307 in Rabbits xxxxxxxxxxx GLP Unpublished	Y	Corteva Agriscience
KCP 7.1.5/1	Prajapati, J	2021d	Acute Eye Irritation Study of GF-3307 in Rabbits xxxxxxxxxxx GLP Unpublished	Y	Corteva Agriscience
KCP 7.1.6/1	Prajapati, J	2021e	Skin Sensitisation Study of GF-3307 by Local Lymph Node Assay in Mice xxxxxxxxxxx GLP Unpublished	Y	Corteva Agriscience
			~~Unpublished~~		
~~KCP.7.1.4/3~~	~~Verma, R.~~	~~2018d~~	~~Acute Dermal Irritation Study of GF-3309 in Rabbits~~ ~~xxxxxxxxxxxx~~ ~~GLP~~ ~~Unpublished~~	~~Y~~	~~Dow AgroSciences~~
~~KCP 7.1.5/1~~	~~Settivari, R. S., and Visconti, N. R.~~	~~2016~~	~~Evaluation of the Eye Irritation Potential of GF-3307 Using the *In Vitro* Neutral Red Release Assay~~ ~~Company Report No: 140098~~ ~~Source: Toxicology and Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan, USA~~ ~~non GLP~~ ~~Unpublished~~	~~N~~	~~Dow AgroSciences~~
~~KCP 7.1.5/2~~	~~Patel, M.P.~~	~~2017e~~	~~Acute Eye Irritation Study of GF-3521 in Rabbits~~ ~~xxxxxxxxxxxxxxxxxxxx~~ ~~GLP~~ ~~Unpublished~~	~~Y~~	~~Dow AgroSciences~~
~~KCP.7.1.5/3~~	~~Verma, R.~~	~~2018e~~	~~Acute Eye Irritation Study of GF-3309 in Rabbits~~	~~Y~~	~~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**
KCP 7.1.1/1	Prajapati, J	2021a	Acute Oral Toxicity Study of GF-3307 in Rats xxxxxxxxxxx	Y	Corteva Agriscience
KCP.7.1.2/1	Prajapati, J	2021b	Acute Dermal Toxicity Study of GF-3307 in Rats xxxxxxxxxxx Unpublished	Y	Corteva Agriscience
KCP 7.1.3/1	Kegelman, T. A	2021	GF-3307: Inhalation Median Lethal Concentration (LC50) Study in Rats xxxxxxxxxxx GLP Unpublished	Y	Corteva Agriscience
KCP 7.1.4/1	Prajapati, J	2021c	Acute Dermal Irritation Study of GF-3307 in Rabbits xxxxxxxxxxx GLP Unpublished	Y	Corteva Agriscience
KCP 7.1.5/1	Prajapati, J	2021d	Acute Eye Irritation Study of GF-3307 in Rabbits xxxxxxxxxxx GLP Unpublished	Y	Corteva Agriscience
KCP 7.1.6/1	Prajapati, J	2021e	Skin Sensitisation Study of GF-3307 by Local Lymph Node Assay in Mice xxxxxxxxxxx GLP Unpublished	Y	Corteva Agriscience
			~~Company Report No: 180204~~ ~~Source: Jai Research Foundation, Valvada, Gujarat, India~~ ~~GLP~~ ~~Unpublished~~		
~~KCP 7.1.6/1~~	~~Patel, M.P.~~	~~2017f~~	~~Skin Sensitisation Study of GF-3521 in Rats~~ ~~xxxxxxxxxxxxx~~ ~~Unpublished~~	~~Y~~	~~Dow AgroSciences~~
~~KCP.7.1.6/2~~	~~Verma, R.~~	~~2018f~~	~~Skin Sensitisation Study of GF-3309 by Local Lymph Node Assay in Mice~~ ~~xxxxxxxxxxxxxxxxx~~ ~~Unpublished~~	~~Y~~	~~Dow AgroSciences~~
KCP 7.3/1	Whitfield, C.	2020	GF-3307: In Vitro Percutaneous Absorption of Prothioconazole-desthio in Human Skin Company Report No: 200102 Source: Dow AgroSciences LLC GLP Unpublished	N	~~Dow AgroSciences~~ Corteva Agriscience

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 7.1.1/1	Prajapati, J	2021a	Acute Oral Toxicity Study of GF-3307 in Rats xxxxxxxxxxx	Y	Corteva Agriscience
KCP.7.1.2/1	Prajapati, J	2021b	Acute Dermal Toxicity Study of GF-3307 in Rats xxxxxxxxxxx Unpublished	Y	Corteva Agriscience
KCP 7.1.3/1	Kegelman, T. A	2021	GF-3307: Inhalation Median Lethal Concentration (LC50) Study in Rats xxxxxxxxxxx GLP Unpublished	Y	Corteva Agriscience
KCP 7.1.4/1	Prajapati, J	2021c	Acute Dermal Irritation Study of GF-3307 in Rabbits xxxxxxxxxxx GLP Unpublished	Y	Corteva Agriscience
KCP 7.1.5/1	Prajapati, J	2021d	Acute Eye Irritation Study of GF-3307 in Rabbits xxxxxxxxxxx GLP Unpublished	Y	Corteva Agriscience
KCP 7.1.6/1	Prajapati, J	2021e	Skin Sensitisation Study of GF-3307 by Local Lymph Node Assay in Mice xxxxxxxxxxx GLP Unpublished	Y	Corteva Agriscience
KCP 7.3/2	Whitfield, C.	2021	GF-3307: In Vitro Percutaneous Absorption of Fenpicoxamid in Human Skin Company Report No: 200109 Source: Dow AgroSciences LLC GLP Unpublished	N	~~Dow AgroSciences~~ Corteva Agriscience
KCA 6.10/01	Stuke, S.	2013	Determination of the dislodgeable foliar residues (DFR) of prothioconazole in/on wheat after spray application of JAU 6476 & KWG 4168 EC 460 in the field in Germany Company Report No. M-455270-01-1 Source: Bayer Crop Science GLP Unpublished	N	BCS*
KCA 6.10/02	Stuke, S.	2015	Determination of the dislodgeable foliar residues (DFR) of prothioconazole and BYF 00587 in/on wheat after spraying of Bixafen & Prothioconazole EC 225 in the field in France (North) and Portugal Company Report No. M-507834-01-1 Source: Bayer Crop Science GLP	N	BCS*

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 7.1.1/1	Prajapati, J	2021a	Acute Oral Toxicity Study of GF-3307 in Rats xxxxxxxxxxx	Y	Corteva Agriscience
KCP.7.1.2/1	Prajapati, J	2021b	Acute Dermal Toxicity Study of GF-3307 in Rats xxxxxxxxxxx Unpublished	Y	Corteva Agriscience
KCP 7.1.3/1	Kegelman, T. A	2021	GF-3307: Inhalation Median Lethal Concentration (LC50) Study in Rats xxxxxxxxxxx GLP Unpublished	Y	Corteva Agriscience
KCP 7.1.4/1	Prajapati, J	2021c	Acute Dermal Irritation Study of GF-3307 in Rabbits xxxxxxxxxxx GLP Unpublished	Y	Corteva Agriscience
KCP 7.1.5/1	Prajapati, J	2021d	Acute Eye Irritation Study of GF-3307 in Rabbits xxxxxxxxxxx GLP Unpublished	Y	Corteva Agriscience
KCP 7.1.6/1	Prajapati, J	2021e	Skin Sensitisation Study of GF-3307 by Local Lymph Node Assay in Mice xxxxxxxxxxx GLP Unpublished	Y	Corteva Agriscience
			Unpublished		
KCP 7.2.2.2/01	Anft, T.; Kuester, C.	2015	Exposure of bystanders / residents to spiroxamine and prothioconazole from spray applications with Input in cereals using standard spray nozzles, Company Report No. M-510333-01-1 Source: Bayer Crop Science GLP Unpublished	N	BCS*
KCP 7.2.2.2/02	Kuester, C.; Anft, T	2015	Amendment no.1 to final report of study ID: P-666-15-1700 - Dermal exposure of bystanders / residents to prothioconazole and its main metabolite prothioconazole-desthio from tractor mounted/trailed boom sprayers with Aviator XPRO EC 225 in cereals Company Report No. M-536654-02-1 Source: Bayer Crop Science GLP Unpublished	N	BCS*
	Shipp, E	2019	*In silico* Evaluation of Genotoxic Potential of Fenpicoxamid and its Metabolites X12335723, X12264475, X12314005, and X12019520	N	~~DAS~~ Corteva Agriscience

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 7.1.1/1	Prajapati, J	2021a	Acute Oral Toxicity Study of GF-3307 in Rats xxxxxxxxxxx	Y	Corteva Agriscience
KCP.7.1.2/1	Prajapati, J	2021b	Acute Dermal Toxicity Study of GF-3307 in Rats xxxxxxxxxxx Unpublished	Y	Corteva Agriscience
KCP 7.1.3/1	Kegelman, T. A	2021	GF-3307: Inhalation Median Lethal Concentration (LC50) Study in Rats xxxxxxxxxxx GLP Unpublished	Y	Corteva Agriscience
KCP 7.1.4/1	Prajapati, J	2021c	Acute Dermal Irritation Study of GF-3307 in Rabbits xxxxxxxxxxx GLP Unpublished	Y	Corteva Agriscience
KCP 7.1.5/1	Prajapati, J	2021d	Acute Eye Irritation Study of GF-3307 in Rabbits xxxxxxxxxxx GLP Unpublished	Y	Corteva Agriscience
KCP 7.1.6/1	Prajapati, J	2021e	Skin Sensitisation Study of GF-3307 by Local Lymph Node Assay in Mice xxxxxxxxxxx GLP Unpublished	Y	Corteva Agriscience
			Dupont Solutions SA, Paris, France GLP/GEP (Y/N): N Published (Y/N): N		
KCA 5.8.1/01	Myhre, A.	2020	X12019520: Bacterial Reverse Mutation Test Report Number: 201068 / 22441-500 Haskell R&D Center, Newark, USA GLP/GEP (Y/N): Y Published (Y/N): N	N	~~DAS~~ Corteva Agriscience
KCA 5.8.1/02	Kellum, S.	2021	X12019520: In Vitro Mammalian Cell Micronucleus Test in Human Peripheral Blood Lymphocytes Report Number: 201074 / 22441-523 Haskell R&D Center, Newark, USA GLP/GEP (Y/N): Y Published (Y/N): N	N	~~DAS~~ Corteva Agriscience
KCA 5.8.1/03	Myhre, A.	2020	X12264475: Bacterial Reverse Mutation Test Report Number: 201067 / 22440-500 Haskell R&D Center, Newark, USA	N	~~DAS~~ Corteva Agriscience

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 7.1.1/1	Prajapati, J	2021a	Acute Oral Toxicity Study of GF-3307 in Rats xxxxxxxxxxx	Y	Corteva Agriscience
KCP.7.1.2/1	Prajapati, J	2021b	Acute Dermal Toxicity Study of GF-3307 in Rats xxxxxxxxxxx Unpublished	Y	Corteva Agriscience
KCP 7.1.3/1	Kegelman, T. A	2021	GF-3307: Inhalation Median Lethal Concentration (LC50) Study in Rats xxxxxxxxxxx GLP Unpublished	Y	Corteva Agriscience
KCP 7.1.4/1	Prajapati, J	2021c	Acute Dermal Irritation Study of GF-3307 in Rabbits xxxxxxxxxxx GLP Unpublished	Y	Corteva Agriscience
KCP 7.1.5/1	Prajapati, J	2021d	Acute Eye Irritation Study of GF-3307 in Rabbits xxxxxxxxxxx GLP Unpublished	Y	Corteva Agriscience
KCP 7.1.6/1	Prajapati, J	2021e	Skin Sensitisation Study of GF-3307 by Local Lymph Node Assay in Mice xxxxxxxxxxx GLP Unpublished	Y	Corteva Agriscience
			GLP/GEP (Y/N): Y Published (Y/N): N		
KCA 5.8.1/04	Kellum, S.	2021	X12264475: In Vitro Mammalian Cell Micronucleus Test in Human Peripheral Blood Lymphocytes Report Number: 201073 / 22440-523 Haskell R&D Center, Newark, USA GLP/GEP (Y/N): Y Published (Y/N): N	N	~~DAS~~ Corteva Agriscience
KCA 5.8.1/05	Myhre, A.	2021	X12314005: Bacterial Reverse Mutation Test Report Number: 201065 / 22439-500 Haskell R&D Center, Newark, USA GLP/GEP (Y/N): Y Published (Y/N): N	N	~~DAS~~ Corteva Agriscience
KCA 5.8.1/06	Kellum, S.	2021	X12314005: *In Vitro* Mammalian Cell Micronucleus Test in Human Peripheral Blood Lymphocytes Report Number: 201072 / 22439-523 Haskell R&D Center, Newark, USA GLP/GEP (Y/N): Y	N	~~DAS~~ Corteva Agriscience

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 7.1.1/1	Prajapati, J	2021a	Acute Oral Toxicity Study of GF-3307 in Rats xxxxxxxxxxx	Y	Corteva Agriscience
KCP.7.1.2/1	Prajapati, J	2021b	Acute Dermal Toxicity Study of GF-3307 in Rats xxxxxxxxxxx Unpublished	Y	Corteva Agriscience
KCP 7.1.3/1	Kegelman, T. A	2021	GF-3307: Inhalation Median Lethal Concentration (LC50) Study in Rats xxxxxxxxxxx GLP Unpublished	Y	Corteva Agriscience
KCP 7.1.4/1	Prajapati, J	2021c	Acute Dermal Irritation Study of GF-3307 in Rabbits xxxxxxxxxxx GLP Unpublished	Y	Corteva Agriscience
KCP 7.1.5/1	Prajapati, J	2021d	Acute Eye Irritation Study of GF-3307 in Rabbits xxxxxxxxxxx GLP Unpublished	Y	Corteva Agriscience
KCP 7.1.6/1	Prajapati, J	2021e	Skin Sensitisation Study of GF-3307 by Local Lymph Node Assay in Mice xxxxxxxxxxx GLP Unpublished	Y	Corteva Agriscience
			Published (Y/N): N		

*Letter of Access is provided in Part A for Bayer CropScience data

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
CA 5.1.1/1	Hansen SC Clark AJ Markham DA Staley JL	2012a	XDE-777: PROBE STUDY TO DETERMINE ABSORPTION, METABOLISM AND ELIMINATION IN F344NTac RATS, Crl:CD1(ICR) MICE AND NEW ZEALAND WHITE RABBITS (Revision) xxxx xxxxxxxxxxx Published (Y/N): No	Yes	~~DAS~~ Corteva Agriscience
CA 5.1.1/2	Thomas JA	2012	A PROBE STUDY TO INVESTIGATE THE METABOLISM AND EXCRETION OF 14C-LABELED XDE-777 IN BEAGLE DOGS FOLLOWING A SINGLE ORAL (GAVAGE) ADMINISTRATION	Yes	~~DAS~~ Corteva Agriscience

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**
			xxxxxxxxxxxx GLP/GEP (Y/N): Yes Published (Y/N): No		
CA 5.1.1/3	Hansen SC Clark AJ Staley JL	2012b	XDE-777: TISSUE DISTRIBUTION IN F344DuCrl RATS xxxxxxxxxxxxxxxx Published (Y/N): No	Yes	~~DAS~~ Corteva Agriscience
CA 5.1.1/4	Press R Reynolds I	2013	ELIMINATION OF RADIOACTIVITY IN BILE, URINE, AND FECES FOLLOWING ORAL ADMINISTRATION OF [14C]-LABELED XDE-777 TO RATS xxxxxxxxxxxxx Published (Y/N): No	Yes	~~DAS~~ Corteva Agriscience
CA 5.1.1/5	Hansen SC Clark AJ Douglass L Markham DA Staley JL	2013	XDE-777: PHARMACOKINETICS AND METABOLISM IN F344DuCrl RATS xxxxxxxxxxx GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	~~DAS~~ Corteva Agriscience
CA 5.1.1/6	Zhang F McClymont EL Fiting JA Erskine TC Clark AJ	2014	XDE-777: *In Vitro* Comparative Metabolism Study Toxicology & Environmental Research and Consulting, The Dow Chemical Company DAS Report No.: 130798 GLP/GEP (Y/N): Yes Published (Y/N): No	No	~~DAS~~ Corteva Agriscience
CA 5.2.1/1	Durando J	2011 a	Acute Oral Toxicity Up And Down Procedure In Rats xxxxxxxxxxx GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	~~DAS~~ Corteva Agriscience
CA 5.2.2/1	Durando J	2011 b	Acute Dermal Toxicity Study in Rats xxxxxxxxxxx GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	~~DAS~~ Corteva Agriscience
CA 5.2.3/1	Krieger SM Garlinghouse CR	2012	XR-777: ACUTE DUST AEROSOL INHALATION TOXICITY STUDY IN F344DuCrl RATS xxxxxxxxxxx GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	~~DAS~~ Corteva Agriscience
CA 5.2.4/1	Durando J	2011 c	Primary Skin Irritation Study In Rabbits xxxxxxxxxxx GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	~~DAS~~ Corteva Agriscience

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 5.2.5/1	Durando J	2011 d	Primary Eye Irritation Study in Rabbits Eurofins PSL DAS Report No.: 101666 GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	~~DAS~~ Corteva Agriscience
CA 5.2.6/1	Boverhof DR Sosinski LK	2012	XR-777: LOCAL LYMPH NODE ASSAY IN CBAJ MICE xxxxxxxxxx GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	~~DAS~~ Corteva Agriscience
CA 5.2.7/1	Roth M	2015	XDE-777: Cytotoxicity Assay in vitro with Balb/c 3T3 Cells: Neutral Red (NR) Test during Simultaneous Irradiation with Artificial Sunlight Harlan Cytotest Cell Research GmbH DAS Report No.: 150039 GLP/GEP (Y/N): Yes Published (Y/N): No	No	~~DAS~~ Corteva Agriscience
CA 5.3.1/1	Sura R Murray JA	2010	XR-777: PALATABILITY PROBE STUDY IN F344DuCrl RATS xxxxxxxxxx GLP/GEP (Y/N): No Published (Y/N): No	Yes	~~DAS~~ Corteva Agriscience
CA 5.3.1/2	Stebbins KE Murray JA McCoy AT	2012a	XR-777: 28-DAY DIETARY TOXICITY STUDY IN F344DuCrl RATS xxxxxxxxxx GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	~~DAS~~ Corteva Agriscience
CA 5.3.1/3	Thomas J Murray JA Sura R	2010	XR-777: PALATABILITY PROBE STUDY IN Crl:CD1(ICR) MICE xxxxxxxxxx GLP/GEP (Y/N): No Published (Y/N): No	Yes	~~DAS~~ Corteva Agriscience
CA 5.3.1/4	Thomas J Murray JA McCoy AT	2012	XR-777: 28-DAY DIETARY TOXICITY STUDY IN Crl:CD1(ICR) MICE xxxxxxxxxx GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	~~DAS~~ Corteva Agriscience
CA 5.3.1/5	Heward J	2012	XDE-777: A PRELIMINARY PALATABILITY STUDY IN BEAGLE DOGS xxxxxxxxxx GLP/GEP (Y/N): No Published (Y/N): No	Yes	~~DAS~~ Corteva Agriscience
CA 5.3.1/6	Heward J	2013a	XDE-777: A 28-DAY DIETARY TOXICITY STUDY IN BEAGLE DOGS xxxxxxxxxx GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	~~DAS~~ Corteva Agriscience

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 5.3.2/1	Stebbins K E Brooks K J Andrus AK Clark AJ Hukkanen RR Markham DA McCoy AT Rick DL	2012 b	XR-777: 90 DAY DIETARY TOXICITY STUDY IN F344DuCrl RATS xxxxxxxxxxx GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	~~DAS~~ Corteva Agriscience
CA 5.3.2/2	Thomas J Murray JA McCoy AT	2014	XR777: 90-DAY DIETARY TOXICITY STUDY WITH A 28-DAY RECOVERY IN Crl:CD1(ICR) MICE (Revision) xxxxxxxxxxx GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	~~DAS~~ Corteva Agriscience
CA 5.3.2/3	Heward J	2013 b	XDE-777: A 90-DAY DIETARY TOXICITY STUDY IN BEAGLE DOGS xxxxxxxxxxx GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	~~DAS~~ Corteva Agriscience
CA 5.3.2/4	Heward J	2014	XDE-777: A One-Year Dietary Toxicity Study in Beagle Dog xxxxxxxxxxx GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	~~DAS~~ Corteva Agriscience
CA 5.4.1/1	Dakoulas EW Divi K	2010	Salmonella - Escherichia coli/Mammalian-Microsome Reverse Mutation Assay Preincubation Method with a Confirmatory Assay with XR-777 BioReliance DAS Report No.: 100088 GLP/GEP (Y/N): Yes Published (Y/N): No	No	~~DAS~~ Corteva Agriscience
CA 5.4.1/2	Schisler MR	2011 a	EVALUATION OF XR-777 IN AN IN VITRO CHROMOSOMAL ABERRATION ASSAY UTILIZING RAT LYMPHOCYTES Toxicology & Environmental Research and Consulting, The Dow Chemical Company DAS Report No.: 101069 GLP/GEP (Y/N): Yes Published (Y/N): No	No	~~DAS~~ Corteva Agriscience

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 5.4.1/3	Schisler MR	2011 b	EVALUATION OF XR-777 IN THE CHINESE HAMSTER OVARY CELLHYPOXANTHINE-GUANINE-PHOS-PHORIBOSYL TRANSFERASE (CHOHGPRT) FORWARD MUTATION ASSAY Toxicology & Environmental Research and Consulting, The Dow Chemical Company DAS Report No.: 101089 GLP/GEP (Y/N): Yes Published (Y/N): No	No	~~DAS~~ Corteva Agriscience
CA 5.4.2/1	Schisler MR	2011 c	EVALUATION OF XR-777 IN THE MOUSE PERIPHERAL BLOOD MICRONUCLEUS TEST xxxxxxxxxxx GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	~~DAS~~ Corteva Agriscience
CA 5.4.2/2	Pant K	2014	XDE-777: In Vivo Unscheduled DNA Synthesis (UDS) Test in Mouse Liver Cells xxxxxxxxxxx GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	~~DAS~~ Corteva Agriscience
CA 5.5/1	Thomas J Murray JA McCoy AT	2013	XR-777: 18-MONTH DIETARY ONCOGENICITY STUDY IN Crl:CD1(ICR) MICE xxxxxxxxxxx GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	~~DAS~~ Corteva Agriscience
CA 5.5/2	Stebbins KE Golden RM Hukkanen RR McCoy AT	2014	XDE-777: Two-Year Dietary Chronic Toxicity/Oncogenicity Study in F344/DuCrl Rats xxxxxxxxxxx GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	~~DAS~~ Corteva Agriscience
CA 5.6.1/1	Rasoulpour RJ Zablotny CL McCoy AT Murray JA Thomas J	2012 a	XR-777: DIETARY REPRODUCTION/DEVELOPMENTAL TOXICITY SCREENING TEST IN Crl:CD(SD) RATS xxxxxxxxxxx GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	~~DAS~~ Corteva Agriscience
CA 5.6.1/2	Ellis-Hutchings RG Zablotny CL Hukkanen RR Yano BL	2013a	XDE-777: TWO GENERATION DIETARY REPRODUCTION TOXICITY STUDY IN Crl:CD(SD) RATS xxxxxxxxxxx GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	~~DAS~~ Corteva Agriscience
CA 5.6.2/1	Rasoulpour RJ Brooks KJ Zablotny CL Clark AJ McCoy AT Stebbins KE	2012b	XR-777: DIETARY DEVELOPMENTAL TOXICITY PROBE STUDY IN Crl:CD(SD) RATS xxxxxxxxxxx GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	~~DAS~~ Corteva Agriscience

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 5.6.2/2	Rasoulpour RJ Marshall VA McCoy AT	2012c	XDE-777: DIETARY DEVELOPMENTAL TOXICITY STUDY IN Crl:CD(SD) RATS xxxxxxxxxxx GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	~~DAS~~ Corteva Agriscience
CA 5.6.2/3	Rasoulpour RJ Bell MP Hukkanen RR McCoy AT	2012d	XDE-777: DIETARY DEVELOPMENTAL TOXICITY PROBE STUDY IN NEW ZEALAND WHITE RABBITS xxxxxxxxxxx GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	~~DAS~~ Corteva Agriscience
CA 5.6.2/4	Ellis-Hutchings RG Bell MP McCoy AT	2013b	XDE-777: DIETARY DEVELOPMENTAL TOXICITY STUDY IN NEW ZEALAND WHITE RABBITS xxxxxxxxxxx GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	~~DAS~~ Corteva Agriscience
CA 5.8.1/1	Patel NN	2012	BACTERIAL REVERSE MUTATION TEST OF X642188 USING SALMONELLA TYPHIMURIUM xxxxxxxxxxx Published (Y/N): No	No	~~DAS~~ Corteva Agriscience
CA 5.8.1/2	Dalal V	2013	ACUTE ORAL TOXICITY STUDY OF X642188 IN RATS xxxxxxxxxxx Published (Y/N): No	Yes	~~DAS~~ Corteva Agriscience
CA 5.8.2/3	Scherzer MK Passage JK	2014	XDE-777: Solubility in New Zealand White Rabbit Plasma xxxxxxxxxxx GLP/GEP (Y/N): Yes Published (Y/N): No	No	~~DAS~~ Corteva Agriscience
K-CP 7.1.1/01	Dalal V	2012a	Acute Oral Toxicity Study of GF-2925 in Rats xxxxxxxxxxx GLP/GEP (Y/N): Y Published (Y/N): N	Y	~~DAS~~ Corteva Agriscience
K-CP 7.2.1/01	Dalal V	2012b	Acute Dermal Toxicity Study of GF-2925 in Rats xxxxxxxxxxx GLP/GEP (Y/N): Y Published (Y/N): N	Y	~~DAS~~ Corteva Agriscience
K-CP 7.1.3/01	Verma, R.	2016	ACUTE INHALATION TOXICITY STUDY OF GF-2925 IN RATS xxxxxxxxxxx GLP/GEP (Y/N): Yes Published (Y/N): No	Y	~~DAS~~ Corteva Agriscience
K-CP 7.1.4/01	Dalal V	2012c	Acute Dermal Irritation Study of GF-2925 in Rabbits xxxxxxxxxxx GLP/GEP (Y/N): Y Published (Y/N): N	Y	~~DAS~~ Corteva Agriscience
K-CP 7.1.5/01	Dalal V	2012d	Acute Eye Irritation Study of GF-2925 in Rabbits xxxxxxxxxxx GLP/GEP (Y/N): Y Published (Y/N): N	Y	~~DAS~~ Corteva Agriscience

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
K-CP 7.1.6/01	Dalal V	2012e	Skin Sensitisation Study of GF-2925 by Local Lymph Node Assay in Mice xxxxxxxxxxx Published (Y/N): N	Y	~~DAS~~ Corteva Agriscience
K-CP 7.3/01	Maas WJM	2013	In Vitro Dermal Absorption of XDE-777, Formulated in GF-2925 and Two Dilutions, Through Human Split-Thickness Skin Using Flow-Through Diffusion Cells TNO Triskelion BV DAS Report No.: 120518 GLP/GEP (Y/N): Y Published (Y/N): N	N	~~DAS~~ Corteva Agriscience

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

Not required

Comments of zRMS:	*In vivo* studies submitted by the applicant to support registration of the product GF-3307 has been conducted on the same formulation thus bridging approach is not applicable for this registration process.

A 2.2 Acute oral toxicity (KCP 7.1.1)

Comments of zRMS:	Study has been reviewed for compliance with the current guidelines, resulting from scientific progress. OECD 423 recommends the dose level to be used as the starting point selected from one of four fixed levels, 5, 50, 300 and 2000 mg/kg body weight. In the study Prajapati, J.; 2021 additional upper dose level 5000 mg/kg bw has been used. Regarding OECD recommendation this dose level is only justified by specific regulatory needs, in the zRMS opinion this is not a case for current application. However, this is not to be considered as deviation from study protocol. When testing dose level of 5000 mg/kg, only one step (i.e. three animals) is required. If the first animal dosed dies, then dosing procedes at 2000mg/kg in accordance with the flow charts in Annex 2. If the first animal survives, two further animals are dosed. If only one of the three animal dies , the LD_{50} value is expected to exceed 5000mg/kg. If both animals die, then dosing proceeds at 2000mg/kg In the discussed study clinical sign of lethargy, abdominal breathing and dyspnoea were observed in the one rat treated with 5000 mg GF-3307/kg body weight. No signs of toxicity were observed in rats treated with the dose level of 2000 mg GF-3307/kg body weight. Summarizing GF-3307 should not be classified for acute, oral toxicity according to Regulation 1272/2008. Results of the study and conclusions are adequate for risk assessment and classification purpose. Study accepted.

CITATION

xxxxxxxxxxx Acute Oral Toxicity Study of GF-3307 in Rats; xxxxxxxxxxx; 15 November 2021; Published: No

COMPLIANCE

Guideline(s):	OECD 423 (2001), OPPTS 870.1100 (2002), EC B.1 (2008), JMAFF 2-1-1 (2000)
US EPA Guideline(s):	OPPTS 870.1100 (2002)
Guideline Deviations:	None
Dates of work:	18 August 2021 to 11 September 2021
GLP status:	Yes
Number of pages in final report:	46

MATERIALS AND METHODS

Test item(s)

Test item (Common name):	GF-3307
Purity:	4.7 wt% (49 g/L) Fenpicoxamid 9.7 wt% (101 g/L) Prothioconazole
Description (physical state):	Orange liquid
Lot/batch no.:	MAR19CE01Q (TSN400550)

Vehicle: N/A

Test System

Species:	Rat (*Rattus norvegicus*)
Strain:	Wistar (RCCHan:WIST)
Age and weight at dosing:	10 to 12 weeks Weight (g): Minimum 176.2, Maximum 207.3
Source:	xxxxxxxxxxx
Housing:	1 to 3 rats/cage
Feed and water:	Feed: Teklad certified Global 14% Protein Rodent Maintenance Diet (sterilizable) manufactured by Envigo, USA. *ad libitum* with the exception of overnight fasting and three hours post dosing Water: UV sterilized water *ad libitum*
Environmental conditions:	Temperature: 20 to 23°C Humidity: 56 to 66% relative humidity Air changes: Minimum 15 air changes/hour Photoperiod: 12 hours dark/12 hours light
Acclimation period:	6 to 10 days

Study Design

In-life dates

Start: 18 August 2021 End: 11 September 2021

Animal assignment and treatment

Animal assignment is shown in Table 1.

Table 1:Animal assignment

Dose (mg/kg body weight)	No. of Animals
5000	1
2000	6

Following an overnight fast, rats were given a single oral dose of GF-3307 by gavage. The test item was a liquid end-use product and was tested undiluted (at a constant concentration) and dose volume was adjusted according to the dose and body weight to permit constant dose administration.

Animals were then observed daily and weighed weekly for 14 days. Survivors were sacrificed and a necropsy was performed in all animals.

RESULTS AND DISCUSSION

Mortality

Mortality data are presented in the table below:

Table 2: Dose, mortality/animals treated

Dose (mg/kg body weight)	Mortality (# affected /total)	Time range of deaths (hours or days)
5000	1/1	Day 1
2000	0/6	N/A

N/A: not applicable

One animal found died following treatment at 5000 mg GF-3307/kg body weight. No mortality was observed following treatment at 2000 mg GF-3307/kg body weight.

Clinical Observations

Clinical sign of lethargy, abdominal breathing and dyspnoea were observed in the one rat treated with 5000 mg GF-3307/kg body weight. No signs of toxicity were observed in rats treated with the dose level of 2000 mg GF-3307/kg body weight.

Body Weight

Changes in body weight were considered within the expected range for this strain and age of animals and not influenced by the treatment.

Necropsy Observations

External

An external examination of the terminally sacrificed female rats and found dead rat did not reveal any gross lesion of pathological significance.

Internal

Internal examination of found dead rat revealed red discoloration of lungs (rat N° 1), whereas other terminally sacrificed rats did not reveal any abnormality.

CONCLUSION

Mortality was observed in the one rat treated with 5000 mg GF-3307/kg body weight. No mortality was observed in rats treated with 2000 mg GF-3307/kg body weight. The acute oral LD50 of GF-3307 in female Wistar rats was found to be between 2000 and 5000 mg/kg body weight.

Test item	Species	Strain	Sex	Route	Method	Result
GF-3307	Rat	Wistar (RccHan:WIST)	F	Oral	Gavage (undiluted)	LD50 = 2000 - 5000 mg/kg body weight

GHS classification

Globally Harmonized System of Classification and Labelling of Chemicals (rev. 8, GHS 2019)	Category 5

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

Comments of zRMS:	Study has been reviewed for compliance with the current guidelines, resulting from scientific progress. In the study Prajapati, J.; 2021, additional pre dose range level has been considered. Initially, one rat was dermally exposed to the 1:10 diluted GF-3307 at 200 mg/kg body weight for 24 hours. As no irritation was observed in the first treated rat, further testing was performed using undiluted test item. This is not requirement of OECD 402 TG however this is not considered by the zRMS as deviation from study protocol. In the main study no mortality or clinical signs were observed in any rat, throughout the study period. Further, no erythema or oedema was evident in any rat at 24, 48, and 72 hours, post patch removal. All rats treated with GF-3307 at 200 (1:10 dilution), 200, 1000, and

	2000 mg/kg body weight showed no effect on body weight. All animals gained weight during the course of the study. No external or internal gross abnormality was observed at gross necropsy. Results of the study and conclusions are adequate for risk assessment and classification purpose. Study accepted.

CITATION

xxxxxxxxxxx; Acute Dermal Toxicity Study of GF-3307 in Rats; xxxxxxxxxxx; 11 November 2021; Published: No

COMPLIANCE

Guideline(s):	OECD 402 (2017)
US EPA Guideline(s):	N/A
Guideline Deviations:	None
Dates of work:	05 August 2021 to 06 September 2021
GLP status:	Yes
Number of pages in final report:	45

MATERIALS AND METHODS

Test item(s)

Test item (Common name):	GF-3307
Purity:	4.7 wt% (49 g/L) Fenpicoxamid 9.7 wt% (101 g/L) Prothioconazole
Description (physical state):	Orange liquid
Lot/batch no.:	MAR19CE01Q (TSN400550)
Vehicle:	Reverse osmosis (RO) water (only for rat N° 1 dosed at 200 mg/kg body weight)

Test System

Species:	Rat (*Rattus norvegicus*)
Strain:	Wistar (RccHan:WIST)
Age and weight at dosing:	13 to 17 weeks Weight (g): Female: Minimum 227.5, Maximum 254.4
Source:	Animal Breeding Facility, Jai Research Foundation
Housing:	Group-housed during acclimatization; individually caged during the 24-hour exposure period; 1 to 3 rats/cage after patch removal
Feed and water:	Feed: Teklad Certified Global 14% Protein Rodent Maintenance Diet (sterilizable) manufactured by Envigo, USA. *ad libitum* Water: UV sterilized water *ad libitum*
Environmental conditions:	Temperature: 19 to 23°C Humidity: 56 to 66% relative humidity Air changes: Minimum 15 air changes/hour Photoperiod: 12 hours dark/12 hours light
Acclimation period:	6 to 18 days

Study Design

In-life dates

Start: 05 August 2021 End: 06 September 2021

Animal assignment and treatment

Animal assignment is shown in Table 1

Table 1: Animal assignment

Dose (mg/kg body weight)	Number of Animals
200*	1
200	1
1000	1
2000	3

* = diluted in the ratio of 1:10 in RO water

A calculated dose volume/amount (0.05 to 0.50 mL/mg) of GF-3307 was applied over the clipped area (approximately 7 × 5 cm area, corresponding to 10% of the body surface) of the rats. The test item was held in contact with the skin using porous gauze dressing (not more than 8 ply) and a non-irritating tape (Medi tape 330 hypo-allergenic surgical tape) throughout the 24-hour exposure period to prevent any loss of the test item and also to ensure that the rats did not lick or ingest it. At the end of the exposure period, the residual test item was removed using cotton soaked in RO water.
Animals were then observed daily and weighed weekly for 14 days. Survivors were sacrificed and a necropsy was performed in all animals.

RESULTS AND DISCUSSION

Mortality

Mortality data are presented in the table below:

Table 2: Dose, mortality/animals treated

Dose (mg/kg body weight)	Mortality (# affected /total)	Time range of deaths (hours or days)
200*	0/1	N/A
200	0/1	N/A
1000	0/1	N/A
2000	0/3	N/A

N/A: not applicable, * = diluted in the ratio of 1:10 in RO water

No mortality occurred following treatment at 200, 1000, and 2000 mg GF-3307/kg body weight.

Clinical Observations

No clinical signs were observed in any rat treated with 200 (1:10 dilution), 200, 1000, and 2000 mg GF-3307/kg body weight.

No erythema or oedema was observed in any rat at 24, 48, and 72 hours post patch removal.

Body Weight

All rats treated with GF-3307 at 200 (1:10 dilution), 200, 1000, and 2000 mg/kg body weight showed no effect on body weight. All rats gained weight during the course of the study.

Necropsy

External

An external examination of the terminally sacrificed animals did not reveal any gross abnormality of pathological significance.

Internal

The visceral examination of animals sacrificed at the termination did not reveal any gross lesion.

CONCLUSION

No mortality, clinical observation, effect on body weight and macroscopic external or internal gross abnormality at necropsy were observed in any rat treated with 200 (1:10 dilution), 200, 1000, and 2000 mg GF-3307/kg body weight.

Test item	Species	Strain	Sex	Route	Method	Result
GF-3307	Rat	Wistar RccHan: WIST	F	Dermal	Topical (24-hour semi-occlusive exposure)	LD50 = >2000 mg/kg body weight

GHS classification

Globally Harmonized System of Classification and Labelling of Chemicals (rev. 8, GHS 2019)	Unclassified

A 2.4 Acute inhalation toxicity (KCP 7.1.3)

Comments of zRMS:	Study has been reviewed for compliance with the current guidelines, resulting from scientific progress. There is no deviation from studies protocol, the OECD 436 procedure. However concentration of the aerosol failed to reach the upper limit for classification with acute inhalation toxicity cat. 4 (5 mg/L), considering a co-formulant properties to maintain a chamber concentration of components below the Lower Explosive Level, a design concentration of >2.0 mg/L was selected for this study. In the event no deaths occur among animals exposed to a mean concentration of 2.0 mg/L or greater, the LC_{50} is considered equal to or greater than 2.0 mg/L, and no further testing is pursued. Thus, results of the study and conclusions are adequate for risk assessment and classification purpose. Study accepted.

CITATION

xxxxxxxxxx GF-3307: Inhalation Median Lethal Concentration (LC50) Study in Rats; Haskell R& xxxxxxxxxx ; 10 November 2021; Published: No

COMPLIANCE

Guideline(s):	OPPTS 870.1300 (1998); OECD 436 (2009); EC B.2 (2014); JMAFF 12 Nousan 8147 and 13 Seisan 1739 (2000 and 2001)
US EPA Guideline(s):	OPPTS 870.1300 (1998)
Guideline Deviations:	None
Dates of work:	24 August 2021 to 7 September 2021
GLP status:	Yes
Number of pages in final report:	64

MATERIALS AND METHODS

Test item(s)

Test item (Common name):	GF-3307
Purity:	4.7 wt% (49 g/L) fenpicoxamid, 9.7 wt% (101 g/L) prothioconazole
Description (physical state):	Transparent amber liquid
Lot/batch no.:	MAR19CE01Q (TSN400550)
Vehicle:	Air

Test System

Species:	Rat (Rattus norvegicus)
Strain:	Crl:CD(SD)
Age and weight at dosing:	~8 weeks Weight (g): Male: Minimum 260, Maximum 270; Female: Minimum 151, Maximum 181
Source:	Charles River Laboratories International, Inc., Raleigh, North Carolina, U.S.A
Housing:	Except during exposure and during the restrainer acclimation period, animals were housed individually in solid-bottom caging with bedding and appropriate species-specific enrichment.
Feed and water:	Feed: PMI® Nutrition International, LLC Certified Rodent LabDiet® 5002 ad libitum (except during exposure) Water: ad libitum (except during exposure)
Environmental conditions:	Temperature: 20 to 25°C Humidity: 30 to 70% relative humidity Air changes: Not reported Photoperiod: 12 hours dark/12 hours light
Acclimation period:	6 days

Study Design

In-life dates

Start: 24 August 2021 End: 07 September 2021

Animal assignment and treatment

Animal assignment is shown in Table 1.

Table 1: Animal assignment

Dose (mg/L air)	Males	Females	Combined
2.9	3	3	6

The rats were exposed for 4 h (nose only) followed by a 14 day post-exposure observation period during which body weight and clinical observations were recorded. Survivors were sacrificed and a necropsy was performed in all animals.

Each animal was weighed and observed prior to exposure. Animals were observed 3 times during the exposure. After the exposure, animals were individually observed for clinical signs before they were returned to their cages. Animals were weighed and observed on the day of the exposure (test day 1), and on test days 2, 3, 4, 8 and 15. Rats were checked daily for mortality or signs of illness, injury, and abnormal behaviour.

Atmosphere Generation

The chamber atmosphere was generated by aerosolization of the test substance in air with a Spraying Systems Company® nebulizer. The test substance mixture was metered into the nebulizer using a Harvard Apparatus model 22 Infusion pump. High-pressure air, metered into the nebulizer by a Brooks model 5850E mass flow controller (MFC), carried the resulting atmosphere into the exposure chamber. Chamber concentrations of the aerosol test substance mixture were controlled by varying the Infusion pump's feed rate to the nebulizer.

Chamber Construction and Design

The exposure chamber was constructed of glass (cylindrical) with a nominal internal volume of 14 L. A polymethylmethacrylate baffle inside the chamber promoted uniform chamber distribution of the test atmosphere.

Chamber Distribution of Test Substance

Prior to the start of the exposure, the distribution of the test substance aerosol was determined in the exposure chamber using gravimetric chamber samples. Air samples were collected from 3 separate locations in the faceplate and at the sampling port of the exposure chamber 4 times. An overall average of the 7 samples taken was determined and individual samples from the faceplate compared to the overall average. All samples taken from the faceplate were within 10% of the overall mean of the chamber samples collected.

RESULTS AND DISCUSSION

Concentration Details in the Inhalation Chamber

All aerosol (gravimetric) samples taken from the faceplate demonstrated differences that were less than 10% from the overall mean aerosol concentration. The test substance atmosphere was considered to be homogenously distributed in the breathing zone of the animals and the use of the sampling port for air sampling was considered adequate.

During the exposure, rats were exposed to a total atmospheric chamber concentration of 2.9 ± 0.16 mg/L GF-3307 (mean ± standard deviation). The means and standard deviations for aerosol (gravimetric) concentration and vapor (Gas Chromatography) were 1.8 ± 0.13 mg/L and 1.1 ± 0.044, respectively. Two samples were taken to determine mass median aerodynamic diameters (MMADs) during the exposures. The MMADs were 2.6 and 2.5 μm and geometric standard deviations were both 2.3.

The chamber concentrations and aerosol size were considered adequate for the conduct of this study.

Mortality

Mortality data are presented in the following table.

Table 2: Dose, mortality/animals treated

Concentration (mg/L air)	Mortality (# affected/total)			Time range of deaths (hours)	Number with evident toxicity (# affected/total)		
	Male	Female	Combined		Male	Female	Combined
2.9	0/3	0/3	0/6	N/A	0/3	1/3	1/6

N/A: Not applicable

No mortality occurred following exposure to a total mean concentration of 2.9 mg GF-3307/L air.

Clinical Observations

Rats displayed normal startle response throughout the exposure. One female rat was lethargic immediately after the exposure and continued to display lethargy the next day. There were no other toxicologically significant clinical signs observed in any rats during the remainder of the recovery period.

Body Weight

On the day after the exposure, 1 male rat displayed a bodyweight loss 21 grams and 2 females lost 8.2 and 10 grams. After test day 2, all rats displayed weight gains throughout the remainder of the recovery period.

Necropsy Observations

No gross lesions were present in the rats at necropsy.

CONCLUSION

Under the conditions of this study, the 4-hour inhalation median lethal concentration (LC50) for GF-3307 in male and female rats was greater than 2.9 mg/L.

Additional comments

In the acute inhalation toxicity study with GF-3307 the maximum attainable exposure atmosphere concentration was limited by the Lower Explosive Limit (LEL) of benzyl acetate, a co-formulant. Maintaining an exposure chamber atmospheric concentration that does not exceed 50% of the Lower Explosive Limit (LEL) for any tested component is critical for occupational safety of the workers performing the exposure, and to minimize risk of adverse events that could compromise animal welfare. This strategy is also consistent with the OECD guidance and OECD test guidelines that were followed during the conduct of the study. In the Limit Test section of OECD Guidance Document No. 39 on Acute Inhalation Toxicity Testing, page 37 section 50, the following is stated: "In the case of potentially explosive test articles, care should be taken to avoid conditions favorable for an explosion. For safety reasons it is generally advisable to not exceed 50% of the published Lower Explosive Limit (LEL)." Additionally, from the Test Guideline OECD 436, Acute Inhalation Toxicity – Acute Toxic Class Method, page 3 section 13 it is stated: "Care should be taken not to generate explosive atmospheres."

Benzyl acetate comprises approximately 40% of the total GF-3307 liquid formulation and has an LEL of 0.9% (9000 ppm). The vapor pressure of benzyl acetate warranted concentration quantification in both the aerosol and vapor phases of the exposure chamber atmosphere. In attempt to avoid exceeding 50% of the benzyl acetate LEL (4500 ppm) in the exposure chamber atmosphere, the delivery rate of the GF-3307 liquid formulation to the nebulizer used to produce the exposure atmosphere was calculated based on the percentage of benzyl acetate in the liquid formulation. This resulted during exposure, with rats exposed to a total GF-3307 atmospheric chamber concentration of 2.9 ± 0.16 mg/L (mean ± standard deviation). The means and standard deviations for aerosol (gravimetric) and vapor (Gas Chromatography) concentrations were 1.8 ± 0.13 mg/L and 1.1 ± 0.044, respectively. Under the conditions of the study, the 4-hour inhalation median lethal concentration (LC50) for GF-3307 in male and female rats was > 2.9 mg/L.

Test item	Species	Strain	Sex	Route	Method	Result
GF-3307	Rat	Crl:CD(SD)	M/F	Inhalation	Nose only(4-hour)	LC50 >2.9 mg/L air

GHS classification

Globally Harmonized System of Classification and Labelling of Chemicals (rev. 8, GHS 2019)	Category 4

A 2.5 Skin irritation (KCP 7.1.4)

Comments of zRMS:	Study has been reviewed for compliance with the current guidelines, resulting from scientific progress. There is no deviation from studies protocol, the OECD 404 procedure is still valid and acceptable. GF-3307 was applied in a single dose to the skin of an experimental animal; untreated skin areas of the test animal serve as the control. The degree of irritation/corrosion was scored at specified intervals sufficient to evaluate the reversibility or irreversibility of the effects observed. Results of the study and conclusions are adequate for risk assessment and classification purpose. Study accepted.

CITATION

xxxxxxxxxxx; Acute Dermal Irritation Study of GF-3307 in Rabbits; xxxxxxxxxxx. 211322 ; 15 November 2021; Published: No

COMPLIANCE

Guideline(s): OECD 404 (2015), OPPTS 870.2500 (1998), EC B.4 (2008), JMAFF 2-1-4 (2000)

US EPA Guideline(s):	OPPTS 870.2500
Guideline Deviations:	None
Dates of work:	19 August 2021 to 31 August 2021
GLP status:	Yes
Number of pages in final report:	39

MATERIALS AND METHODS

Test item(s)

Test item (Common name):	GF-3307
Purity:	4.7 wt% (49 g/L) Fenpicoxamid 9.7 wt% (101 g/L) Prothioconazole
Description (physical state):	Orange liquid
Lot/batch no.:	MAR19CE01Q (TSN400550)
Vehicle:	N/A

Test System

Species:	Rabbit (Oryctolagus cuniculus)
Strain:	New Zealand White (NZW)
Age and weight at dosing:	4 to 5 months Weight (kg): Minimum 2.0, Maximum 2.1
Source:	Vab Biosciences, Hyderabad, India
Housing:	Individual
Feed and water:	Feed: Teklad certified Global High Fiber Rabbit Feed manufactured by Envigo, U.S.A. ad libitum Water: UV sterilized water ad libitum
Environmental conditions:	Temperature: 18 to 23°C Humidity: 63 to 65% relative humidity Air changes: Minimum 15 air changes/hour Photoperiod: 12 hours dark/12 hours light
Acclimation period:	7 to 9 days

Test item(s)

Species:	Rabbit (*Oryctolagus cuniculus*)
Strain:	New Zealand White (NZW)
Age and weight at dosing:	4 to 5 months Weight (kg): Minimum 2.0, Maximum 2.1
Source:	Vab Biosciences, Hyderabad, India
Housing:	Individual
Feed and water:	Feed: Teklad certified Global High Fiber Rabbit Feed manufactured by Envigo, U.S.A. *ad libitum* Water: UV sterilized water *ad libitum*
Environmental conditions:	Temperature: 18 to 23°C Humidity: 63 to 65% relative humidity Air changes: Minimum 15 air changes/hour Photoperiod: 12 hours dark/12 hours light
Acclimation period:	7 to 9 days

Study Design

In-life dates

Start: 19 August 2021 End: 31 August 2021

Animal assignment and treatment

The pH of GF-3307 was found to be 4.39 (1% aqueous solution in distilled water at room temperature), which is considered acceptable for treatment.

A total of 03 rabbits (females) were assigned to treatment. A sequential testing strategy was adopted. Initially one rabbit was tested. As severe effects were not observed in the first treated rabbit, two additional rabbits were subsequently treated in an identical manner.

A volume of 0.5 mL of GF-3307 (undiluted) was applied evenly to one of the clipped sites of each rabbit. The contralateral site remained untreated and served as control. The treated and the control sites were covered with gauze patches of approximately 6 cm^2 (gauze rolled) with semi-occlusive dressing (not more than 8-ply) and were secured at the margins by non-irritating tape (Medi tape 330 hypo-allergenic surgical tape) to ensure that the rabbits did not ingest the test item. At the end of the 4-hour exposure period (day 0), the residual test item was removed with cotton soaked in distilled water.

Irritation was scored by the method of Draize (as described in OECD Test Guideline 404) at 1, 24, 48, and 72 hours post patch removal. General health condition and body weight were monitored.

RESULTS AND DISCUSSION

Dermal Irritation

Individual animal irritation scores are presented in Table 1.

Table 5: ***Doses, scoring/animals treated***

Rabbit No.	Treatment Site	Control Site	Observation (post patch removal)											
			Erythema						Oedema					
			Hour				Day		Hour				Day	
			1	24	48	72	7	14	1	24	48	72	7	14
1	Left	Right	1	2	1	0	N/A	N/A	1	1	1	0	N/A	N/A
2	Right	Left	1	2	1	0	N/A	N/A	1	1	1	0	N/A	N/A
3	Right	Left	1	2	1	0	N/A	N/A	1	1	1	0	N/A	N/A

Key: N/A: Not applicable

Erythema
0: No erythema
1: Very slight erythema (barely perceptible)
2: Well-defined erythema
3: Moderate to severe erythema
4: Severe erythema (beef redness) to eschar formation preventing grading of erythema
Maximum possible: 4

Oedema
0: No oedema
1: Very slight oedema (barely perceptible)
2: Slight oedema (edges of area well defined by raising)
3: Moderate oedema (raised approximately 1 mm)
4: Severe oedema (raised more than 1 mm and extending beyond area of exposure)
Maximum possible: 4

Systemic toxicity

No signs of toxicity were observed and all animals gained body weight throughout the study.

CONCLUSION

Based on these study results, GF-3307 caused a minimal dermal irritation in all rabbits, fully reversible by 72 hours. No systemic effect was observed. The individual rabbit mean dermal irritation score at 24, 48, and 72 h post patch removal was 1.00, 1.00, 1.00 for erythema, 0.67, 0.67, 0.67 for oedema for rabbit N° 1, 2 and 3 respectively.

Test item	Species	Strain	Sex	Route	Method	Result
GF-3307	Rabbit	NZW	F	Dermal	Topical (4-hour, semi-occlusive)	Mean Erythema Score: 1.00, 1.00, 1.00 Mean Oedema Score: 0.67, 0.67, 0.67 Recovery completed by 72 hours

GHS classification

Globally Harmonized System of Classification and Labelling of Chemicals (rev. 8, GHS 2019)	Unclassified

A 2.6 Eye irritation (KCP 7.1.5)

Comments of zRMS:	Study has been reviewed for compliance with the current guidelines, resulting from scientific progress. There is no deviation from studies protocol OECD 405 (rev. 2017). Following pretreatment, means analgesics and anesthetics without impacting of the final outcome, considering 3R approach approximately 5 minutes prior to GF-3307 application, one to two drops of 0.5% proparacaine hydrochloride was applied to each eye and also subsequently, buprenorphine hydrochloride 0.01 mg/kg body weight SC was administered every 12 (±30 minutes) hours, and in conjunction with meloxicam 0.5 mg/kg body weight SC every 24 (± 30 minutes) hours, until the ocular lesions resolved (Day 14) GF-3307 was applied in a single dose to one of the eyes of the experimental animal. The degree of eye irritation/serious eye damage was evaluated by scoring lesions of conjunctiva, cornea, and iris, at specific intervals. No corneal opacity, iritis, or discharge was observed in any rabbit throughout the experimental period. An examination with fluorescein dye and cobalt blue filter carried out at 24 h post-application revealed no disruption of corneal epithelium in any rabbit. The duration of the study was sufficient to evaluate the reversibility or irreversibility of the effects. Other effects in the eye and adverse systemic effects are also described to provide a complete evaluation of the effects. Results of the study and conclusions are adequate for risk assessment and classification purpose. Study accepted.

CITATION

xxxxxxxxxxx 2021; Acute Eye Irritation Study of GF-3307 in Rabbits; Jai Research Foundation, xxxxxxxxxxx; 15 November 2021; Published: No

COMPLIANCE

Guideline(s):	OECD 405, OPPTS 870.2400, EC B.5, JMAFF 2-1-5
US EPA Guideline(s):	OPPTS 870.2400
Guideline Deviations:	None
Dates of work:	20 August 2021 to 11 September 2021
GLP status:	Yes
Number of pages in final report:	45

MATERIALS AND METHODS

Test item(s)

Test item (Common name):	GF-3307
Purity:	4.7 wt% (49 g/L) Fenpicoxamid, 9.7 wt% (101 g/L) Prothioconazole
Description (physical state):	Orange liquid
Lot/batch no.:	MAR19CE01Q (TSN400550)
Vehicle:	N/A

Test System

Species:	Rabbit (*Oryctolagus cuniculus*)
Strain:	New Zealand White (NZW)
Age and weight at dosing:	4 to 5 months Weight (kg): Minimum 2.143, Maximum 2.285
Source:	Vab Biosciences, Hyderabad, India
Housing:	Individual
Feed and water:	Feed: Teklad certified Global High Fiber Rabbit Feed manufactured by Envigo, U.S.A. *ad libitum* Water: UV sterilized water *ad libitum*
Environmental conditions:	Temperature: 18 to 22 °C Humidity: 63 to 64% relative humidity Air changes: Minimum 15 air changes/hour Photoperiod: 12 hours dark/12 hours light
Acclimation period:	6 to 8 days

Study Design

In-life dates

Start: 20 August 2021 End: 11 September 2021

Animal assignment and treatment

The pH of GF-3307 was found to be 4.39 (1% aqueous solution in distilled water at room temperature), which was considered acceptable for treatment.

A total of 03 rabbits (females) were assigned to treatment. A sequential testing strategy was adopted. Initially one rabbit was tested. Immediately after administration of the test item, assessments of any initial local pain reactions were made. As severe effects were not observed in the first treated rabbit, two additional rabbits were subsequently treated in an identical manner.

0.1 mL of GF-3307 was instilled in the conjunctival sac after gently pulling the lower lid away from the eyeball. Then the lids were gently held together for about one second in order to prevent loss of the test item. The contralateral (untreated) eye served as the control. In all animals, both the eyes were gently washed with 0.9% normal saline at 24 hours post instillation.

On day 0, approximately 60 minutes prior to the test item instillation, buprenorphine 0.01 mg/kg body weight was administered by subcutaneous injection (SC). Approximately 5 minutes prior to the test item instillation, one or two drops of 0.5% proparacaine hydrochloride was applied to each eye.

Approximately 8 hours (± 30 minutes) post instillation, buprenorphine 0.01 mg/kg body weight (SC) and meloxicam 0.5 mg/kg body weight were administered both subcutaneously to provide a continued therapeutic level of systemic analgesia.

Subsequently, buprenorphine 0.01 mg/kg body weight was administered subcutaneously every 12 hours (± 30 minutes), in conjunction with meloxicam 0.5 mg/kg body weight every 24 hours (± 30 minutes), until the ocular lesions resolved.

Irritation was scored by the method of Draize (as described in OECD Test Guideline 405) at 1, 24, 48, and 72 hours and up to 14 days after GF-3307 instillation. Fluorescein staining was used to assess the corneal epithelium damage at 24 hours after the test item instillation in all animals. General health conditions and body weights were monitored.

RESULTS AND DISCUSSION

Eye Irritation

At 1 h post-application, the treated eye of all rabbits revealed conjunctival redness (score of 1) and conjunctival chemosis (score of 1).

At 24, 48 and 72 h post-application, the treated eye of all rabbits revealed conjunctival redness (score of 2) and conjunctival chemosis (score of 1).

On day 7 post-application, the treated eye of all rabbits revealed conjunctival redness (injected) (score of 1) and conjunctival chemosis (score of 1).

On day 14 post-application, the treated eye of all rabbits recovered completely and appeared normal.

No corneal opacity, iritis, or discharge was observed in any rabbit throughout the experimental period. An examination with fluorescein dye and cobalt blue filter carried out at 24 h post-application revealed no disruption of corneal epithelium in any rabbit.

Individual animal irritation scores are presented in Table 1.

Table 6: Grades for ocular lesions (eye treated with the test item)

Rabbit no.	1							2							3						
Site of application	Right							Right							Right						
Reaction post application	Hour				Day			Hour				Day			Hour				Day		
	1	24	48	72	7	14	21	1	24	48	72	7	14	21	1	24	48	72	7	14	21
Conjunctivae (redness)	1	2	2	2	1	0	N/A	1	2	2	2	1	0	N/A	1	2	2	2	1	0	N/A
Conjunctivae (chemosis)	1	1	1	1	1	0	N/A	1	1	1	1	1	0	N/A	1	1	1	1	1	0	N/A
Cornea (degree of opacity)	0	0	0	0	0	0	N/A	0	0	0	0	0	0	N/A	0	0	0	0	0	0	N/A
Iris inflammation	0	0	0	0	0	0	N/A	0	0	0	0	0	0	N/A	0	0	0	0	0	0	N/A

Key: N/A: Not applicable

Conjunctivae - Redness (refers to palpebral and bulbar conjunctivae; excluding cornea and iris)
0: Normal
1: Some blood vessels hyperaemic (injected)
2: Diffuse, crimson colour; individual vessels not easily discernible
3: Diffuse beefy red
Maximum possible: 3

Chemosis – Swelling (refers to lids and/or nictating membranes)
0: Normal
1: Some swelling above normal
2: Obvious swelling, with partial eversion of lids
3: Swelling, with lids about half closed
4: Swelling, with lids more than half closed
Maximum possible: 4

Opacity: degree of density
0: No ulceration or opacity
1: Scattered or diffuse areas of opacity (other than slight dulling of normal lustre); details of iris clearly visible
2: Easily discernible translucent area; details of iris slightly obscured
3: Nacrous area; no details of iris visible; size of pupil barely discernible
4: Opaque cornea; iris not discernible through the opacity
Maximum possible: 4

Iris
0: Normal
1: Markedly deepened rugae, congestion, swelling, moderate circumcorneal hyperaemia; or injection; iris reactive to light (a sluggish reaction is considered to be an effect
2: Hemorrhage, gross destruction, or no reaction to light
Maximum possible: 2

Systemic toxicity

No signs of toxicity was observed and all animals gained body weight throughout the study.

CONCLUSION

GF-3307 caused conjunctival redness (scores of 1 or 2) and conjunctival chemosis (score of 1) at 1, 24, 48 and 72 h and on day 7, which resolved by day 14 in all rabbits.

Test item	Species	Strain	Sex	Route	Method	Result
GF-3307	Rabbit	NZW	F	Eye	Instillation (washing at 24 hours post instilla-tion)	Mean Redness Score: 2.00, 2.00, 2.00 Mean Chemosis Score: 1.00, 1.00, 1.00 Mean Corneal Score: 0.00, 0.00, 0.00 Mean Iris Score: 0.00, 0.00, 0.00 Recov-ery completed by 14 days

GHS classification

Globally Harmonized System of Classification and Labelling of Chemicals (rev. 8, GHS 2019)	Category 2/2A

A 2.7 Skin sensitisation (KCP 7.1.6)

Comments of zRMS:	Study has been reviewed for compliance with the current guidelines, resulting from scientific progress. There is no deviation from studies protocol, the OECD 429 procedure is valid and acceptable. Study is in line with the suggestions of point 5 of Regulation 284/2013 and Annex VII to REACH REG (EC) No 1907/2006. Results of the study and conclusions are adequate for risk assessment and classification purpose. Study accepted.

CITATION

xxxxxxxxxxx Skin Sensitisation Study of GF-3307 by Local Lymph Node Assay in Mice; xxxxxxxxxxx; 15 November 2021; Published: No

COMPLIANCE

Guideline(s):	OECD 429 (2010), OPPTS 870.2600 (2003), EC B.42 (2012)
US EPA Guideline(s):	OPPTS 870.2600
Guideline Deviations:	None
Dates of work:	18 August 2021 to 04 September 2021
GLP status:	Yes
Number of pages in final report:	66

MATERIALS AND METHODS

Test item(s)

Test item (common name):	GF-3307
Purity:	4.7 wt% (49 g/L) Fenpicoxamid 9.7 wt% (101 g/L) Prothioconazole
Description (physical state):	Orange liquid
Lot/batch no.:	MAR19CE01Q (TSN400550)

Vehicle/Control Item(s)

Vehicle/Negative control:	1% Pluronic® L-92 in water (1% L-92)
Positive control:	α-hexylcinnamaldehyde, 25% v/v in 1% Pluronic® L-92

Test System

Species:	Mouse *(Mus musculus)*
Strain:	CBA/J
Age and weight at dosing:	9 to 10 weeks Weight (g): Minimum 17.5, Maximum 23.9
Source:	Animal Breeding Facility, Jai Research Foundation
Housing:	Group-housed during acclimatisation; individually caged on the days of test item application (days 0, 1 and 2); 5 mice/cage from day 3; 5 mice/cage in metabolic cages from day 5 (post injection of radiolabelled material)
Feed and water:	Feed: Teklad Certified Global 14% Protein Rodent Maintenance Diet (sterilizable) manufactured by Envigo, USA. *ad libitum* Water: UV sterilized water *ad libitum*
Environmental conditions:	Temperature: 20 to 23°C Humidity: 57 to 66% relative humidity Air changes: Minimum 15 air changes/hour Photoperiod: 12 hours dark/12 hours light
Acclimation period:	7 days

Study Design

In-life dates

Start:	18 August 2021	End:	05 September 2021

Formulation procedure

Procedure:	The Test Item and the Positive Control Item were freshly dissolved/suspended in the vehicle. An adjustment was not made for the purity of the Test or Positive Control Item.
Stability in the vehicle:	Unknown
Formulation analysis:	Concentration/homogeneity check not performed
Concentrations used:	see description below

Preliminary test and dose selection

In a preliminary test, 6 groups of female mice comprising 2 females per group were treated topically for three consecutive days (days 0, 1 and 2) on the dorsal surface of both ears (25 µL/ear) with GF-3307 at concentrations of 5%, 10%, 25%, 50%, 75% (v/v) and 100% (undiluted) in 1% Pluronic® L-92.

Individual clinical observations (including systemic clinical signs and scoring of irritation) were recorded daily during the experiment. Ear thickness was measured on days 0, 2 and 5. Body weight was recorded on days 0 and 5.

In the preliminary assay, no erythema was observed, and an increase of less than 25% in ear thickness was observed at tested concentrations up to 50% in 1% Pluronic® L-92 whereas an increase of greater than 25% in ear thickness was observed at concentrations 75% in 1% Pluronic® L-92 and 100% (undiluted), erythema (score of 1) was also observed at concentration 100% (undiluted). Therefore, dose concentrations of 10%, 25% and 50% (v/v) in 1% Pluronic® L-92 were evaluated in the main study of LLNA.

Animal assignment and treatment

In the main assay, 3 groups of female mice comprising 5 females per group were treated topically for three consecutive days (days 0, 1 and 2) on the dorsal surface of both ears (25 µL/ear) with GF-3307 at concentrations of 10%, 25% and 50% (v/v) in 1% Pluronic® L-92. Female mice from the vehicle control and positive control groups were maintained in similar conditions with treatment of 1% Pluronic® L-92 and 25% (v/v) of HCA in L-92, respectively.

Individual clinical observations (including systemic clinical signs and scoring of irritation) were recorded daily during the experiment. Body weight was recorded on days 0 and 5. On day 5 of treatment, all mice from each group were injected intravenously (tail vein) with 250 µL of sterile phosphate buffered saline (PBS) containing approximately (20±1) µCi of tritiated methyl thymidine (3H-TdR). On day 5, 5 hours - post injection of 3H-TdR, the animals were euthanized and the draining auricular (local) lymph node from both ears of each animal was excised and collected into PBS. Single cell suspensions of lymph node cells from individual animals were prepared. The uptake of 3H-TdR into the auricular (local) lymph nodes draining the site of chemical application was measured to assess the lymph node proliferative response.

Statistics

All the parameters characterised by continuous data such as body weight and radioactive disintegrations per minute (DPM) were subjected to Bartlett's test to assess the homogeneity of variance before conducting Analysis of Variance (ANOVA). To compare vehicle and positive control data, Student's t-test was performed to calculate significance.

RESULTS AND DISCUSSION

Clinical Observations and Irritation

No erythema was observed at the site of application at 10%, 25% and 50% (v/v) GF-3307 in 1% Pluronic® L-92. A local reaction consisting of very slight erythema (score of 1) was observed in all mice treated with 25% (v/v) HCA from day 1 to 4.

Body Weight

No effect on the body weight was observed in mice treated with GF-3307, positive control, and vehicle control.

Group Mean DPM

Proliferative responses in the draining lymph nodes were monitored by measuring the incorporation of 3H-methyl thymidine. These analyses revealed the group mean DPM/mouse value of 689.60, 756.40, 1121.40, and 1729.80, for the vehicle control (1% Pluronic® L-92), 10%, 25% and 50% (v/v) in 1% Pluronic® L-92 treated groups, and 3947.60 for positive control (25% v/v HCA), respectively.

Stimulation Index (SI Value) and EC_3 Value

Stimulation Index (SI) values calculated for groups treated with GF-3307 were found to be 1.10, 1.63, and 2.51 at 10%, 25% and 50% (v/v) in 1% Pluronic® L-92, respectively, and 5.72 for 25% (v/v) HCA positive control group.

A correlation was observed between the dose and the proliferative response in groups treated with GF-3307 when compared to the control. The SI obtained for GF-3307 at the tested concentrations showed a less than threefold increase over the control value. Therefore, GF-3307 did not demonstrate dermal sensitisation potential in the local lymph node assay.

The SI value of 5.72 obtained for the concurrent positive control α-Hexylcinnamaldehyde, showed a greater than three-fold increase compared to the vehicle control value, indicating a clear positive response for this known weak sensitiser. This response was within the historical control data range of the laboratory, which confirmed the reliability of this test procedure.

The SI obtained for GF-3307 showed a less than threefold increase over the control value at all tested concentrations. Therefore, an EC3 value was not calculated.

Individual and group mean values are reported in Table 1.

Table 7: Dose concentration, group mean DPM value and Stimulation Index

Test Material/ Dose concentration	Animal #	Individual Animal DPM	Group Mean +/- SE (DPM)	Stimulation Index (SI)*
Vehicle (1% Pluronic® L-92)	1	817	689 ± 119.43	1
	2	630		
	3	753		
	4	735		
	5	513		
GF-3307 10% (v/v) in vehicle]	6	909	756.40 ± 209.87	1.10
	7	867		
	8	686		
	9	418		
	10	902		
GF-3307 25% (v/v) in vehicle	11	1486	1121.40 ± 283.23	1.63
	12	1091		
	13	1117		
	14	699		
	15	1214		
GF-3307 50% (v/v) in vehicle	16	1296	1729.80 ± 509.38	2.51
	17	1133		
	18	2080		
	19	1806		
	20	2334		
HCA (Positive control) 25% (v/v) in vehicle	21	2799	3947.60 ± 1287.77	5.72
	22	3007		
	23	3285		
	24	5004		
	25	5643		

CONCLUSION

The SI obtained for GF-3307 at all tested concentrations showed a less than three-fold increase over the control value. Therefore, GF-3307 did not demonstrate dermal sensitisation potential in the local lymph node assay.

Test item	Species	Strain	Sex	Route	Method	Result
GF-3307	Mouse	CBA/J	F	Dermal	Topical - Local lymph node assay	Dermal non sensitiser SI = 1.10, 1.63 and 2.51 at 10%, 25% and 50% (v/v) respectively.

GHS classification

Globally Harmonized System of Classification and Labelling of Chemicals (rev 8, GHS 2019)	Not classified as a skin sensitiser

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

No supplementary studies were conducted.

A 2.9 Data on co-formulants (KCP 7.4)

A 2.10 Material safety data sheet for each co-formulant

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

A 2.11 Available toxicological data for each co-formulant

Available toxicological data for each co-formulant can be found in the confidential dossier of this submission (Registration Report - Part C).

A 2.12 Studies on dermal absorption (KCP 7.3)

Default dermal absorption values as detailed in the EFSA guidance document om dermal absorption (EFSA, 2017) were used for prothioconazole.

Prothioconazole-desthio is not part of the formulation. Rather it is a metabolite of prothioconazole which is formed at different rates during the drying process of aqueous diluted solutions of the active substance on surfaces. One specific *in vitro* dermal absorption study was performed with GF-3307 assessing the absorption of PTZ-desthio through human skin.

zRMS comment A 2.12.1	Dermal absorption study on prothioconazole-desthio has been conducted according to the OECD TG 428 revision 2004. For testing human split thickness skin has been used. There were no deviations from the TG. The absorbed dose of Prothioconazole-desthio from the GF-3307 spray dilution has been calculated based on EFSA GD 2017 also EFSA Calculator. Highest spray dilution according to the GAP is 150g of PTZ/ha in 300 L water/ha (refer dRR B0) which is corresponding to 0.5 g prothioconazol/L. Considering 100% conversion of prothioconazol to prothioconazol-desthio is assumed then the in-use dilution is covered by the dilution tested in the study, and no pro-rata correction is required. DA for the in use-dilution was found to be 14%. Results of the DA study and conclusions are adequate for risk assessment (NDE) Study accepted.

A 2.12.1 Prothioconazole-desthio, dermal absorption study using *in vitro* human skin

Reference	KCP 7.3/1
Report	Whitfield, C.; 2020; GF-3307: In Vitro Percutaneous Absorption of Prothioconazole-desthio in Human Skin; Haskell R&D Center, E.I. du Pont de Nemours and Company, Member of the Corteva Agriscience Group of Companies, Newark, Delaware, U.S.A.; Lab Study No. 22368-1377; DAS Study No. 200102 ; 20 March 2020; Unpublished
Guideline(s)	Yes: OECD 428 (2004)
Deviations	None
GLP	Yes
Acceptability	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

Test Item(s)

Test item #1

Test item (Common name):	Prothioconazole-desthio (active ingredient)
Purity:	99.6%
Description (physical state):	Solid
Lot/batch no.:	1107201601 (TSN312897)

Test item #2

Radiolabelled test item (Common name):	[^{14}C]Prothioconazole-desthio
Radiochemical purity:	100%
Specific activity	33.5 mCi/mmol, 107.3 µCi/mg
Description (physical state):	Solid
Lot/batch no.:	DE3-171310-100

Test item #3

Test item (Common name):	Fenpicoxamid (ancillary active ingredient)

Purity:	82.8 wt%
Description (physical state):	Solid
Lot/batch no.:	XDE-777-01-02 (TSN303159)

Study Design

The study was designed to examine the *in vitro* dermal absorption of [^{14}C]prothioconazole-desthio formulated as GF-3307 (assuming that all prothioconazole in the diluted formulation formed prothioconazole-desthio) in 24 hours through human skin following an 8-hour dermal exposure to a single, finite application. The test preparation was tested at target concentrations: 0.363 g L^{-1} (aqueous spray dilution; 1:250). The spray dilution reflects the concentration recommended for use in the field (*i.e.*, in-use spray dilution). The study design is summarised below.

Table 8:In vitro dermal absorption from GF-3307: study design

Test group	No. of replicates*	Species	Target concentration of Prothioconazole-desthio (g L^{-1})	Target dose of Prothioconazole-desthio (µg cm^{-2})	Exposure duration	Serial sampling time points
A	8[a]	Human	0.363	3.63	8 h	0-24 h

*2 replicates per donor.
a One replicate excluded due to misapplication of dose.

Preparation of skin membranes

Human surgical skins were prepared in duplicate from four separate donors (n=8).

After thawing the frozen skin samples, the skin was dermatomed using a dermatome to a recorded thickness of *ca* 0.2-0.4 mm measured with a digimatic micrometer.

Integrity of the skin membranes

Skin was hydrated in 0.9% saline for approximately 15 minutes at room temperature. Following hydration, the skin was mounted onto the top of the receptor chamber, which was filled with 0.9% saline. The donor chamber was then clamped in place and filled with 0.9% saline. The skin was then allowed to equilibrate for approximately 30 minutes. Following equilibration, a resistance measurement of each skin membrane was taken using a Tinsley 6401 Databridge set in the resistance (R) and parallel equivalent (PAR) modes at an alternating current (AC) frequency of 1000 Hertz (1 kHz). Skin with a resistance of ≥17 kΩ was considered intact and retained for use on study. Skin not meeting these criteria was replaced, and electrical resistance confirmed following equilibration. This procedure was followed until 8 skin preparations represented by 4 individuals (2 replicates per donor) was achieved. Following the electrical resistance measurement, saline in the donor chamber was removed and discarded. The skin was rinsed with deionized water and dried with a lint-free wipe. Saline in the receptor chamber was removed and replaced with the receptor fluid.

Solubility in the receptor fluid

The solubility of Prothioconazole-desthio in the receptor fluid (≥108 µg mL^{-1}) was observed to be at least 10 fold higher than the maximum concentration achieved in the receptor fluid, ensuring the receptor fluid offered sink conditions to Prothioconazole-desthio and was not rate limiting to passive diffusion. The maximum penetration of Prothioconazole-desthio into the receptor fluid was 0.157 µg equivalents mL^{-1} (Cell 5).

In addition, in the flow through cells used, the volume of the receptor fluid in the receptor chamber beneath the skin was *ca* 0.2 mL. At a flow rate of *ca* 1.5 mL h^{-1}, this volume was replenished continuously such that the rate of diffusion into the receptor fluid did not become a rate-limiting step in the experiment.

Study conduct and sample collection

The study was performed in flow-through diffusion cells (PermeGear Inc., Riegelsville, Pennsylvania, USA). The prepared formulations were applied to each skin surface (0.64 cm^2), via the donor chamber, at a rate of 10 µL cm^{-2}. The dose was distributed evenly over the exposure area using a glass rod. The donor chamber remained unoccluded for the duration of the study. The exposed area of each skin (0.64 cm^2) remained in contact with the test item for a period of 8 h (normal working h day^{-1}) with a post-exposure time of 16 h (*i.e.*, the total study duration was 24 h). Skin washing was performed at the end of both the contact (at 8 h) and post-exposure (at 24 h) periods. Skins were then removed from the cells and tape-stripped up to 15 times each to remove the *stratum corneum*. The tape stripped skin was then placed on an aluminum pan and heated in an oven at approximately 55ºC for approximately one minute and 45 seconds. The epidermis was then peeled away from the dermis using a scalpel and/or forceps. Epidermis and dermis samples were collected in separate vials for analysis. The donor and receptor compartments were rinsed to collect any remaining test material.

In order to determine the recovery, the amount of Prothioconazole-desthio was measured by liquid scintillation counting in the following samples:

a. test concentrations
b. spreader device rinses
c. receptor fluid samples collected as: 0-1 h, 1-2 h followed by 2-h intervals until 24 h after application
d. skin washes at 8 h and 24 h, separately
e. the *stratum corneum* (SC) (*ca* 15 tape strips, individually analyzed)
f. the dermis and epidermis (without SC), separately
g. receptor and donor compartments rinse, separately

RESULTS AND DISCUSSION

The absorbed dose of Prothioconazole-desthio from the GF-3307 spray dilution is calculated based on guidance from different regulatory bodies and presented below:

Table 9:Absorbed doses

Absorbed dose	Aqueous spray dilution ($0.363\ g\ L^{-1}$) (1:250)
Absorbed dose I[1]	9.68 ± 3.22
Absorbed dose II[2]	10.36 ± 3.04
Absorbed dose III[3]	11.53 ± 2.49

[1] Absorbed dose I is calculated from the amount recovered in receptor fluid, the receptor compartment wash, and the dermis.
[2] Absorbed dose II is calculated from the absorbed dose I, plus the epidermis (without *stratum corneum*). The absorbed dose II can be considered conservative.
[3] Absorbed dose III is calculated from the absorbed dose II plus the *stratum corneum* (excluding tape strips 1 and 2). The absorbed dose III can be considered highly conservative.

The mean total recovery of [^{14}C]prothioconazole-desthio for the aqueous spray dilution was 99.18 (±1.34)%.

For the aqueous spray dilution, absorption was 9.68 ± 3.22% (absorbed dose I = the amount recovered in receptor fluid, the receptor compartment wash and the dermis), or 10.36 ± 3.04% (absorbed dose II

= absorbed dose I plus epidermis without *stratum corneum*), or 11.53 ± 2.49% (absorbed dose III = the absorbed dose II plus the *stratum corneum* (excluding tape strips 1 and 2)) of the applied dose.

Dermal absorption and total recovery data for the test group is summarised in the following table.

Table 10: Total recoveries and dermal absorption of prothioconazole-desthio from GF-3307 aqueous spray dilution through human skin

	A – Aqueous spray dilution (1:250)	
Total concentration [g L^{-1}]	0.363	
Dose [µg.cm^{-2}]	3.63	
N	7	
Cumulative penetration into the receptor fluid	% of dose	µg equiv m^{-2}
after 12 h	--	0.0961 ± 0.0556
after 24 h	7.67 ± 3.39	0.247 ± 0.109
Maximal flux [µg equiv. cm^{-2} h^{-1}]	0.0162 ± 0.0073	
Lag time [h]	6.2 ± 1.4	
Recovery of [^{14}C]Prothioconazole-desthio (% of dose, mean ± SD)		
Receptor fluid (0-24 h)	7.67 ± 3.39	
Receptor compartment wash	0.34 ± 0.11	
Dermis	1.66 ± 0.42	
Stratum corneum (SC) Total	2.42 ± 2.03	
Tape strips (1-2)	1.25 ± 1.08	
Tape strips (3 – 15)	1.17 ± 0.98	
Epidermis	0.69 ± 0.39	
Skin wash t = 8 h	79.89 ± 4.33	
Skin wash t = 24 h	6.34 ± 3.39	
Donor compartment wash	0.17 ± 0.11	
Total recovery	99.18 ± 1.34	

CONCLUSION

The percent dermal absorption values for prothioconazole-desthio are:

Total concentration [g L^{-1}]	Aqueous spray dilution (0.363 g L^{-1}) (1:250)
Absorbed dose I[1]	9.68 ± 3.22
Absorbed dose II[2]	10.36 ± 3.04
Absorbed dose III[3]	11.53 ± 2.49

[1] Absorbed dose I is calculated from the amount recovered in receptor fluid, the receptor compartment wash, and the dermis.
[2] Absorbed dose II is calculated from the absorbed dose I, plus the epidermis (without *stratum corneum*). The absorbed dose II can be considered conservative.
[3] Absorbed dose III is calculated from the absorbed dose II plus the *stratum corneum* (excluding tape strips 1 and 2). The absorbed dose III can be considered highly conservative.

EFSA Calculator

Results and discussion					
	Concentrate		Dilution 1 (1:200)		
Target concentration [mg/mL]	0		1.363		
Target dose [µg/cm^2]	0		3.63		
Mean actual applied dose [µg/cm^2]			3.23		
Recovery [%]	Mean	SD	Mean	SD	
Dislodgeable dose					
Skin wash after 8 hours	N/A	N/A	87.71	4.85	
Donor chamber wash	N/A	N/A	0.19	0.12	
Skin associated dose					
Tape strips 1-2	N/A	N/A	1.86	2.00	
Tape strips 3-x	N/A	N/A	1.49	1.29	
Skin preparation	N/A	N/A	2.79	1.45	
Absorbed dose					
Receptor fluid	N/A	N/A	8.16	3.43	
Receptor chamber wash	N/A	N/A	0.41	0.21	
Total recovery	#DIV/0!	#DIV/0!	99.18	1.34	
LLC of t_0.5 absorption	#DIV/0!	N/A	28.75	7.62	
Absorption complete?	#DIV/0!		No		
Measured absorption, if LLC of t_0.5<=75%	#DIV/0!	#DIV/0!	12.85	4.38	
Measured absorption, if LLC of t_0.5>75%	#DIV/0!	#DIV/0!	N/A	N/A	
Measured absorption corrected	#DIV/0!	#DIV/0!	11.53	2.49	
Relevant absorption estimate	#DIV/0!		13.824		
Final estimate (rounded)	#DIV/0!		14		

A 2.12.2 Fenpicoxamid, dermal absorption study using *in vitro* human skin

zRMS comment A 2.12.1	Dermal absorption study on A 2.12.2 fenpicoxamid has been conducted according to the OECD TG 428 revision 2004. For testing human split thickness skin has been used. There were no deviations from the TG. The absorbed dose of fenpicoxamid from the GF-3307 spray dilution has been calculated based on EFSA GD 2017 also EFSA Calculator. DA for the in use-dilution was found to be 12%. Highest spray dilution according to the GAP is 75 g fenpicoxamid/ha in 300 L water/ha (refer dRR B0) which is corresponding to 0.25 g fenpicoxamid/L. Thus, the in-use dilution is covered by the dilution tested in the study 0.2 g/L, and no pro-rata correction is required. Results of the DA study and conclusions are adequate for risk assessment (NDE) Study accepted.

Reference	KCP 7.3/2
Report	Whitfield, C.; 2021; GF-3307: In Vitro Percutaneous Absorption of Fenpicoxamid in Human Skin; Haskell R&D Center, E.I. du Pont de Nemours and Company, Member of the Corteva Agriscience Group of Companies, Newark, Delaware, U.S.A.; Lab Study No. 22369-1377; DAS Study No. 200109 ; 29 January 2021; Unpublished
Guideline(s)	Yes: OECD 428 (2004)
Deviations	None
GLP	Yes
Acceptability	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

Test item(s)

Test item (Common name):	GF-3307 (Formulation)
Purity:	4.7 wt% (49 g/L) fenpicoxamid; 9.7 wt% (101 g/L) prothioconazole
Description (physical state):	Liquid
Lot/batch no.:	MAR19CE01Q (TSN400550)
Test item (Common name):	Fenpicoxamid (Active Ingredient)
Purity:	82.8%
Description (physical state):	Solid
Lot/batch no.:	XDE-777-01-02 (TSN303159)
Test item (Common name):	XDE-777-pyr-2-14C (Radiolabeled Active Ingredient)
Purity:	99.8% (Radiochemical Purity)
Description (physical state):	Solid
Lot/batch no.:	INV-175292-066 (INV403689)

Study Design

The study was designed to examine the *in vitro* dermal absorption of [^{14}C]Fenpicoxamid formulated as GF-3307 formulation through human skin following an 8 hour dermal exposure to a single, finite application. The test preparation was tested at two target concentrations: 50 g.L^{-1}

(as concentrate) and 0.2 $g.L^{-1}$ (aqueous spray dilution; 1:250). The study design is summarised below.

Table 11: In vitro dermal absorption from GF-3307: study design

Test group	No. of replicates*	Species	Target concentration of Fenpicoxamid ($g.L^{-1}$)	Target dose of Fenpicoxamid ($\mu g.cm^{-2}$)	Exposure duration	Serial sampling time points
A	8[a]	Human	50	500	8 h	0-24 h
B	8	Human	0.2	2	8 h	0-24 h

*2 replicates were from each 4 donors.
a One replicate excluded due to expected damage to skin at dosing.

Preparation of skin membranes

Human skin membranes were prepared in duplicate from four separate donors (n=8).

After thawing the frozen skin samples, the skin was dermatomed using a dermatome to a recorded thickness of *ca* 0.2-0.4 mm measured with a digimatic micrometer.

Integrity of the skin membranes

The integrity of each skin was assessed by measurement of electrical resistance prior to application of test substance. Skin was hydrated in 0.9% saline for approximately 15 minutes at room temperature. Following hydration, the skin was mounted onto the top of the receptor chamber, which was filled with 0.9% saline. The donor chamber was then clamped in place and filled with 0.9% saline. The skin was then allowed to equilibrate for approximately 30 minutes. Following equilibration, a resistance measurement of each skin membrane was taken using a Tinsley 6401 Databridge set in the resistance (R) and parallel equivalent (PAR) modes at an alternating current (AC) frequency of 1000 Hertz (1 kHz). Skin with a resistance of ≥17 kΩ was considered intact and retained for use on study. Skin not meeting these criteria was replaced, and electrical resistance confirmed following equilibration. This procedure was followed until 8 skin preparations represented by 4 individuals (2 replicates per donor) per dose concentration was achieved. Following the electrical resistance measurement, saline in the donor chamber was removed and discarded. The skin was rinsed with deionized water and dried with a lint-free wipe. Saline in the receptor chamber was removed and replaced with the receptor fluid.

Solubility in the receptor fluid

The solubility of fenpicoxamid in the receptor fluid (≥31.7 $\mu g.mL^{-1}$) was observed to be at least 10-fold higher than the maximum concentration achieved in the receptor fluid, ensuring the receptor fluid offered sink conditions to fenpicoxamid and was not rate limiting to passive diffusion. The maximum penetration of fenpicoxamid into the receptor fluid was 0.00264 μg equivalents.mL^{-1} (Cell 3, Group A).

In addition, in the flow through cells used, the volume of the receptor fluid in the receptor chamber beneath the skin was ca 0.7 mL. At a flow rate of ca 1.5 $mL.h^{-1}$, this volume was replenished continuously such that the rate of diffusion into the receptor fluid did not become a rate limiting step in the experiment.

Study conduct and sample collection

The study was performed in flow-through diffusion cells (PermeGear Inc., Hellertown, Pennsylvania, USA). The prepared formulations were applied to each skin surface (0.64 cm^2), via the donor chamber, at a rate of 10 $\mu L.cm^{-2}$. The dose was distributed evenly over the exposure area using a glass rod. The donor chamber remained unoccluded for the duration of the study. The exposed area of each skin (0.64 cm^2) remained in contact with the test item for a period of 8 h (normal working $h.day^{-1}$) with a

post-exposure time of 16 h (*i.e.*, the total study duration was 24 h). Skin washing was performed at the end of both the contact (at 8 h) and post-exposure (at 24 h) periods. Skins were then removed from the cells and tape-stripped up to 15 times each to remove the *stratum corneum*. The tape stripped skin was then placed on an aluminum pan and heated in an oven at approximately 55ºC for approximately one minute and 45 seconds. The epidermis was then peeled away from the dermis using a scalpel and/or forceps. Epidermis and dermis samples were collected in separate vials for analysis. The donor and receptor compartments were rinsed to collect any remaining test material.

In order to determine the recovery, the amount of fenpicoxamid was measured by liquid scintillation counting in the following samples:

a. test concentrations
b. spreader device rinses
c. receptor fluid samples collected as: 0-1 h, 1-2 h followed by 2-h intervals until 24 h after application
d. skin washes at 8 h and 24 h, separately
e. the *stratum corneum* (SC) (*ca* 15 tape strips, individually analyzed)
f. the dermis and epidermis (without SC), separately
g. receptor and donor compartments rinse, separately

RESULTS AND DISCUSSION

The absorbed dose of fenpicoxamid from the GF-3307 undiluted concentrate and its aqueous spray dilution are calculated based on guidance from different regulatory bodies and are presented below:

Table 12: Absorbed doses

Absorbed dose	Concentrate ($50\ g.L^{-1}$) (undiluted)	Aqueous spray dilution ($0.2\ g.L^{-1}$) (1:250)
Absorbed dose I[1]	0.022 ± 0.005	0.40 ± 0.12
Absorbed dose II[2]	0.10 ± 0.07	3.35 ± 2.33
Absorbed dose III[3]	0.21 ± 0.13	8.14 ± 4.16

[1] Absorbed dose I = Percentage of applied dose detected in the receptor fluid, receptor chamber wash, and dermis
[2] Absorbed dose II = Percentage of applied dose detected in the receptor fluid, receptor chamber wash, dermis, and epidermis (without *stratum corneum*).
[3] Absorbed dose III = Percentage of applied dose detected in the receptor fluid, receptor chamber wash, dermis, epidermis, and *stratum corneum* (excluding the first 2 tape strips).

The mean total recovery of [^{14}C]Fenpicoxamid in human skin was 99.03 ± 1.78% (concentrate) and 101.04 ± 3.02% (aqueous spray dilution).

For the concentrate, absorption was 0.022 ± 0.005% (absorbed dose I), or 0.10 ± 0.07% (absorbed dose II) or 0.21 ± 0.13% (absorbed dose III) of the applied dose.

For the aqueous spray dilution, absorption was 0.40 ± 0.12% (absorbed dose I), or 3.35 ± 2.33% (absorbed dose II) or 8.14 ± 4.16% (absorbed dose III) of the applied dose.

Dermal absorption and total recovery data for each test group is summarised in the following table.

Table 13: Total recoveries and dermal absorption of [^{14}C]Fenpicoxamid from GF-3307 and its aqueous spray dilution through human skin

	A – Concentrate (undiluted)		B – Aqueous spray dilution (1:250)	
Total concentration [g.L^{-1}]	50		0.2	
Dose [µg.cm^{-2}]	500		2	
N	7[a]		8	
Cumulative penetration into the receptor fluid	% of dose	µg equiv. cm^{-2} h^{-1}	% of dose	µg equiv. cm^{-2} h^{-1}
after 12 h	--	0.0259±0.0067	--	0.00158±0.00132
after 24 h	0.0097±0.0018	0.0481±0.0086	0.18±0.10	0.00300±0.00177
Maximal flux [µg equiv. cm^{-2} h^{-1}]	0.00335±0.00181		0.00018±0.00016	
Lag time [h]	2.8±2.7		2.1±2.1	
Recovery of [14C]Fenpicoxamid (% of dose, mean ± SD)				
Receptor fluid (0-24 h)	0.0097±0.0018		0.18±0.10	
Receptor compartment wash	0.0016±0.0005		0.048±0.013	
Dermis	0.011±0.007		0.16±0.05	
Stratum corneum (SC) Total	0.33±0.15		10.70±3.38	
Tape strips (1-2)	0.23±0.12		5.91±3.39	
Tape strips (3 – 15)	0.12±0.09		4.79±1.90	
Epidermis	0.081±0.068		2.96±2.28	
Skin wash t = 8 h	98.11±1.83		78.17±5.35	
Skin wash t = 24 h	0.45±0.08		8.21±2.96	
Donor compartment wash	0.045±0.033		0.61±0.29	
Total recovery	99.03±1.78		101.04±3.02	

a One replicate excluded due to expected damage to skin at dosing.

CONCLUSION

The percent dermal absorption values for fenpicoxamid are:

Absorbed dose	Concentrate (50 g.L^{-1}) (undiluted)	Aqueous spray dilution (0.2 g.L^{-1}) (1:250)
Absorbed dose I[1]	0.022 ± 0.005	0.40 ± 0.12
Absorbed dose II[2]	0.10 ± 0.07	3.35 ± 2.33
Absorbed dose III[3]	0.21 ± 0.13	8.14 ± 4.16

[1] Absorbed dose I = Percentage of applied dose detected in the receptor fluid, receptor chamber wash, and dermis
[2] Absorbed dose II = Percentage of applied dose detected in the receptor fluid, receptor chamber wash, dermis, and epidermis (without *stratum corneum*).
[3] Absorbed dose III = Percentage of applied dose detected in the receptor fluid, receptor chamber wash, dermis, epidermis, and *stratum corneum* (excluding the first 2 tape strips).

EFSA Calculator

Results and discussion					
	Concentrate		Dilution 1 (1:250)		
Target concentration [mg/mL]	50		0.2		
Target dose [µg/cm^2]	500		2		
Mean actual applied dose [µg/cm^2]	499		1.63		
Recovery [%]	Mean	SD	Mean	SD	
Dislodgeable dose					
Skin wash after 8 and 24 hours	97.54	3.34	85.84	2.72	
Donor chamber wash	0.04	0.03	0.61	0.29	
Skin associated dose					
Tape strips 1-2	0.21	0.11	5.91	3.39	
Tape strips 3-x	0.12	0.08	4.79	1.90	
Skin preparation	0.09	0.07	3.12	2.32	
Absorbed dose					
Receptor fluid	0.73	2.05	0.18	0.10	
Receptor chamber wash	0.03	0.08	0.05	0.01	
Total recovery	99.03	1.78	100.50	3.11	
LLC of t_0.5 absorption	49.11	4.15	40.33	9.23	
Absorption complete?	No		No		
Measured absorption, if LLC of t_0.5<=75%	0.96	2.12	8.14	4.16	
Measured absorption, if LLC of t_0.5>75%	N/A	N/A	N/A	N/A	
Measured absorption corrected	0.21	0.13	8.14	4.16	
Relevant absorption estimate	0.330		11.640		
Final estimate (rounded)	0.33		12		

A 2.13 Other studies

Reviewer comment:

In the beginning it must be noted that **no ground water fenpicoxamid metabolites were identified** (refer *EFSA (European Food Safety Authority), 2018. Conclusion on the peer review of the pesticide risk assessment of the active substance fenpicoxamid (XDE-777). EFSA Journal 2018;16(1):5146, 27 pp*)
The metabolites X12314005, X12019520, X12326349, X12264475 and X12335723 are provisionally included in the residue definition for risk assessment in processed commodities (see Section 3 mentioned above EFSA Conclusions) and their toxicological profiles were discussed in the experts meeting PPR 162.
During PPR 162 has been agreed conclusions and some data gaps were identified by EFSA. To elucidate genotoxic potential of fenpicoxamid and its plant metabolites X12335723, X12264475, X12314005, and X12019520 Applicant provided additional information reflecting genotoxicity assessment, taking into account *in vitro* (Ames test), *in vivo* (micronucleus test) and Q(SAR) *in silico* prediction. **Based on the predictive data all metabolites listed above are not considered to be toxicologically relevant**.
In the zRMS PL opinion all data gaps mentioned in the EFSA conclusion (2018) should be discussed at EU level. Furthermore in the Reviewer opinion, data summarized below are not key studies for current product authorization and has no impact on final conclusion reflecting risk assessment resulting from product application thus has not been reviewed by the zRMS PL.

A 1.1.1.1 In silico evaluation of processing metabolites

Reference: In silico Evaluation of Genotoxic Potential of Fenpicoxamid and its Metabolites X12335723, X12264475, X12314005, and X12019520. Shipp, E. 2019.

The predicted genotoxic potential of Fenpicoxamid and its plant metabolites X12335723, X12264475, X12314005, and X12019520 was evaluated by *in silico* means using Derek Nexus, OASIS Times, Leadscope, and OECD QSAR Toolbox software packages.

All of the predictions for *in vitro* bacterial (Ames) mutagenicity were negative for fenpicoxamid and the four metabolites evaluated. This is supported by a negative Ames study conducted with fenpicoxamid (Dakoulas and Divi, 2010).

Derek Nexus predicted a positive outcome for *in vitro* chromosomal aberrations for fenpicoxamid and X12335723, although both Leadscope and OASIS Times predicted a lack of genotoxicity for these two structures. The predictions for the remaining three metabolites were negative in all packages used. The positive prediction for fenpicoxamid in Derek Nexus is consistent with the positive result from the *in vitro* clastogenicity assay (Schisler, 2011a) in rat lymphocytes, however this positive outcome is negated by a negative *in vivo* mouse peripheral blood micronucleus assay (Schisler, 2011b). As the positive prediction for both fenpicoxamid and the metabolite X12335723 in the *in vitro* clastogenicity model is based on the presence of the same alkyl aldehyde, the lack of *in vivo* clastogenicity with fenpicoxamid can be applied to the metabolite as well.

All substances are predicted to be negative in the *in vivo* micronucleus model.

Overall, the *in silico* predictions for fenpicoxamid and its plant metabolites X12335723, X12264475, X12314005, and X12019520 indicate that there is no likelihood of a genotoxic outcome.

A 1.1.1.2 X12019520 Bacterial Reverse Mutation Test

Reference: KCA 5.8.1/0

CITATION

Myhre, A.; 2020; X12019520: 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. 22441-500; Sponsor Study No. 201068; 02 December 2020; Published: 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:	11 August 2020 to 4 September 2020
GLP status:	Yes
Number of pages in final report:	54

MATERIALS AND METHODS

Test Item(s)

Test item (Common name):	X12019520
Purity:	100%
Description (physical state):	Liquid
Lot/batch no.:	B180472-70-1 (TSN403117)
Compound stability:	Stability 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 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	

Positive control	Manufactured by	Purity (%)	Solvent	Bacterial strains	Concentration	Metabolic activation
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 trypto-phan, and for strains containing the pKM101 plasmid, ampicillin. The cultures were placed in a shaker/incubator overnight at 100 to 200 rpm and 37 ± 2°C.
Confirmation:	All test cultures were tested for acceptable viability and genotype confirmation (including histi-dine, biotin requirement, *rfa*, *uvr*B 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 48-68 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 X12019520, 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 at 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. All concentration levels of test substance, vehicle control and positive controls were plated in triplicate.

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 contain-ing 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 solidi-fied, the plates were inverted and incubated for approximately 48 to 68 hours at 37 ± 2°C.

Acceptance Criteria

The following criteria were fulfilled 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 response that did not meet all three of the above criteria (magnitude, concentration-responsiveness, reproducibility) was not being evaluated as positive.

Negative results obtained in the first trial were confirmed by a second trial, using the same method as specified above, with an alteration in concentration spacing and metabolic activation.

RESULTS AND DISCUSSION

Negative and Positive Controls

The number of revertant colonies for the negative control was within reasonable limits of the historical control of this laboratory for all the strains (Table 16). 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. Toxicity was observed with TA98 and TA1537 in the absence of S9 activation at 5000 µg/plate; and with TA1537 in the presence of S9 activation starting at 3333 µg/plate. No other appreciable toxicity was observed. No test substance precipitation was observed.

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. Toxicity was observed with TA98, TA100, and TA1537 in the absence of S9 activation at 5000 µg/plate; and with TA1535 in the presence of S9 activation starting at 3333 µg/plate. No other appreciable toxicity was observed. No test substance precipitation was observed. The summary results are given in the following tables.

Table 23: 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	8	10	7	7	5	5	8	6	2	1217
	+S9	11	11	13	7	14	7	10	4	5	173
TA1535	-S9	8	5	10	9	9	7	11	10	7	363
	+S9	9	12	8	11	9	10	11	6	8	166
TA98	-S9	19	15	17	21	19	24	19	26	10	159
	+S9	29	23	26	23	32	23	24	23	20	294
TA100	-S9	115	131	103	107	113	99	99	100	81	422
	+S9	141	126	113	120	148	133	150	167	142	1803
WP2 *uvr*A	-S9	19	20	16	19	23	17	16	16	14	561
	+S9	49	52	46	53	31	30	26	29	26	260

PC: Positive control

Table 24: 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	4	5	3	5	5	3	1029
	+S9	7	4	7	7	4	4	163
TA1535	-S9	9	8	9	6	8	5	513
	+S9	11	9	7	10	5	3	145

TA98	-S9	18	17	18	14	24	6	113
	+S9	18	20	18	22	18	14	203
TA100	-S9	90	86	84	77	106	40	396
	+S9	130	127	137	143	153	115	1827
WP2 *uvr*A	-S9	17	21	20	21	17	21	440
	+S9	24	23	24	21	30	15	220

PC: Positive control

Table 16: Historical Control Data

Historical Control Data[a]					
Tester strain	Control (positive control)[b]	Exogenous Metabolic Activation System	Mean	SD[c]	Range
TA98	Negative	-S9	25	9	10-58
	Negative	+S9	33	9	11-61
	Positive [2NF-1]	-S9	242	68	123-702
	Positive [BAP-2.5]	+S9	392	86	186-615
TA100	Negative	-S9	106	23	51-214
	Negative	+S9	124	27	62-291
	Positive [SA-2]	-S9	939	202	458-1558
	Positive [2AA-2.5]	+S9	2597	877	523-5889
TA1535	Negative	-S9	13	5	3-30
	Negative	+S9	13	5	4-32
	Positive [SA-2]	-S9	839	181	392-1505
	Positive [2AA-2.5]	+S9	205	50	78-365
TA1537	Negative	-S9	8	4	1-22
	Negative	+S9	12	5	2-31
	Positive [ICR 191-2]	-S9	1133	337	300-2668
	Positive [2AA-2.5]	+S9	131	54	48-354
WP2 *uvr*A	Negative	-S9	34	11	13-66
	Negative	+S9	41	12	5-68
	Positive [4NQO-1]	-S9	769	252	283-1337
	Positive [2AA-25]	+S9	256	79	126-680

[a] Historical data for tester strains used in the reported study. Data are based on 73 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, X12019520 is concluded to be non-mutagenic in the Bacterial Reverse Mutation Assay using *Salmonella typhimurium*.

Test item	Test	Test object	Concentration	Result
X12019520	*In vitro* bacterial reverse mutation test	TA98, TA100, TA1535, TA1537, WP2 *urv*A	0, 33.3, 66.7, 100, 333, 667, 1000, 3333 & 5000 µg/plate	Negative ±S9

A 1.1.1.3 X12019520 In Vitro Mammalian Cell Micronucleus Test in HPBL

Reference: KCA 5.8.1/02

CITATION

Kellum, S. N.; 2021; X12019520: In Vitro Mammalian Cell Micronucleus Test in Human Peripheral Blood Lymphocytes; 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. 22441-523-12; Sponsor Study No. 201074; 12 March 2021;

Published: No

COMPLIANCE

Guideline(s):	OECD 487 (2016); EU B.49 (2017)
US EPA Guideline(s):	None
Guideline Deviations:	None
Dates of work:	29 June 2020 to 6 February 2021
GLP status:	Yes
Number of pages in final report:	68

MATERIALS AND METHODS

Test Item(s)

Test item (Common name):	X12019520
Purity:	100%
Description (physical state):	Liquid
Lot/batch no.:	B180472-70-1 (TSN403117)
Compound stability:	Not conducted

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

The test substance vehicle was used as the concurrent negative control. The final concentration of DMSO in the treatment medium did not exceed 1%.

Vehicle	Manufactured by	CAS number
Dimethyl Sulfoxide (DMSO)	Sigma-Aldrich	67-68-5
Dimethyl Sulfoxide (DMSO)	BioWorld	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)	Fisher Scientific	50-07-7	Not reported	Sterile water	0.2 and 0.4 µg/mL	4-hour non-activated
Cyclophosphamide (CP)	Santa Cruz Biotechnology	50-18-0	Not reported	Sterile water	5 and 10 µg/mL	4-hour S9-activated
Vinblastine (VB)	MP Biomedicals	143-67-9	Not reported	Sterile water	6.25 and 12.5 ng/mL	24-hour non-activated

Tester System

Cells	Human peripheral blood lymphocytes
Source	Healthy 23/26 year old female
Maintenance	RPMI 1640 containing approximately 15% fetal bovine serum, 2 mM L-glutamine, 100 units penicillin/mL and 100 µg/mL streptomycin, incubation at 37 ± 2°C. Cytochalasin B (6.0 µg/mL) added to media for re-fed of 4-hour cultures and for entire treatment of 24-hour cultures.
Metabolic activation	Liver homogenate (S9), prepared from male Sprague-Dawley rats induced with phenobarbital/5-6 benzoflavone (Moltox, Inc., Boone, North Carolina, U.S.A.). Protein content: 34.6 mg/mL and 35 mg/mL

Methods

Preliminary Toxicity Assay

The preliminary toxicity test was performed with the test concentrations of 10, 50, 100, 250, 500, 750, 1000, 1500, and the limit dose, as per OECD 487 (2016), of 1882 µg/mL (10mM). 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 188.2 mg/mL, the highest stock concentration used in the study, and formed a clear colourless solution. 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 ± 2ºC in a humidified atmosphere of 5 ± 2% CO2 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 25, 50, 100, 250, and 500 µg/mL for the 4-hour non-activated test condition, 25, 50, 100, 250, 275, and 300 µg/mL for the 4-hour S9-activated test conditions, and 5, 10, 25, 50, 75, and 100 µg/mL for the 24-hour non-activated test condition. Due to not obtaining concentrations adequate for micronuclei evaluation, the 24-hour non-activated test condition and 4-hour S9-activated test condition were repeated. The 24-hour non-activated test condition was repeated at 5, 10, 25, 50, 75, 80, 85, 90, and 100 µg/mL. It is noted that the 4-hour S9-activated test condition required two repeats. In the initial repeat, the concentrations selected were 50, 100, 200, 250, 300, 350, 400, 450, and 500 µg/mL, and in the second repeat were 100, 250, 500, 1000, and 1882 µg/mL. The standard incubation conditions were 37 ± 2ºC in a humidified atmosphere of 5 ± 2% 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.

For each test condition, micronuclei evaluation was conducted for at least 3 test substance concentrations, the vehicle control, and a positive control. The maximum concentration evaluated in each test condition was the lowest precipitating concentration. For each test condition, micronucleus frequencies were evaluated in at least 2000 bi-nucleated cells per selected concentration, vehicle control, and selected positive control concentration, equally divided among replicates. It is noted that for the data collected for 4-hour S9-activated second repeat assay, only the A cultures are being used due to the

1000 µg/mL B culture exhibiting toxicity of 93.8%, whereas the A culture was 57.6%, which was within the 55 ± 5% required range, therefore, only the A cultures were used for all micronuclei analysis from the second repeat concentration. Care was taken to not score binucleate cells with irregular shape or where the 2 nuclei differed greatly in size. In addition, binucleate cells were not confused with poorly spread multi-nucleate cells. Cells containing more than 2 main nuclei were not analysed for micronuclei, as the baseline frequency may have been higher in these cells.

Data Analysis and interpretation

Calculations

The CBPI were determined using the following formula:

$$\text{CBPI} = \frac{1 \text{ x mono-nucleated cells} + 2 \text{ x bi-nucleated cells} + 3 \text{ x multi-nucleated cells}}{\text{total number of cells scored}}$$

% cytostasis (cytotoxicity) = $100 - 100\ \{(CBPI_t - 1) / (CBPI_c - 1)\}$

where:

t = test substance treatment culture

c = vehicle control culture

Acceptance Criteria

An assay was considered acceptable for evaluation of test results only if all of the following criteria were satisfied. The metabolically activated and non-activated assays of the test are independent and, if necessary, were repeated separately.

a. Negative Controls: The frequency of cells with micronuclei was within the 95% control limits of the distribution of the historical negative control data. If the concurrent negative control data fell outside the 95% control limits, they were acceptable as long as these data were not extreme outliers (indicative of experimental or human error).

b. Positive Controls: The frequency of cells with micronuclei was significantly greater ($p \leq 0.05$, Fisher's exact test) than the vehicle control response and induced responses compatible with those generated in the historical control database.

c. Cell Proliferation: The CBPI of the vehicle control at harvest was ≥1.4.

Evaluation Criteria

The following conditions were used as a guide to determine a positive response:

- A statistically significant increase ($p \leq 0.05$, Fisher's exact test) in the frequency of cells with micronuclei was seen in one or more treatment groups relative to the vehicle control response.
- The observed increased frequencies were accompanied by a concentration-related increase when evaluated by the trend test ($p \leq 0.05$, Cochran-Armitage test).
- Any of the results were outside the 95% control limit distribution of the historical negative control data.
- Note: Statistically significant values that did not exceed the historical control range for the negative/vehicle control were judged as not being biologically relevant.

The following condition was used as a guide to determine an equivocal response:

- Results observed in any of the assays resulted in statistically significant elevations in micronuclei at more than one test concentration level without demonstrating a dose-responsive trend.

The test substance was judged negative if the following conditions were met:

- There was no statistically significant increase in the frequency of cells with micronuclei in any treatment group relative to the vehicle control group.
- There was no concentration-related increase when evaluated with an appropriate trend test.
- All results were within the 95% control limit of the distribution of the historical negative control database.

Statistics

Statistical analysis was used as a guide to determine whether or not the test substance induced a positive response. Interpretation of the statistical analysis also relied on additional considerations including the magnitude of the observed test substance response relative to the vehicle control response and the presence of a dose-responsive trend. Statistical analysis consisted of a Fisher's exact test (with Bonferroni-Holm Adjustment) to compare the frequency of micronuclei in the test substance-treated groups with the vehicle control response. A Cochran-Armitage test for dose responsiveness was conducted.

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 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

Test substance precipitation was not observed at the beginning or end of treatment in any test condition. There were no observed pH changes at the beginning or end of treatment in any test condition. Cytotoxicity of 55 ± 5%, or greater, based on cytokinesis-block proliferation index (CBPI) was observed at 500 µg/mL in the 4-hour non-activated test condition and at 100 and 250 µg/mL in the 24-hour non-activated test condition. It is noted that concentrations greater than 500 µg/mL in the 4-hour non-activated test condition and 250 µg/mL in the 24-hour non-activated test condition were not indexed due to toxicity observed at lower concentrations. Cytotoxicity of 55 ± 5%, or greater, based CBPI was not observed in the 4-hour S9-activated test condition.

Micronucleus Assay

Test substance precipitation was observed at the beginning at 250, 275, and 300 µg/mL in the 4-hour S9-activated test condition and 250 and 500 µg/mL in the 4-hour non-activated test condition. Test substance precipitation was not observed at the beginning of treatment in the 24-hour non-activated test condition. Test substance precipitation was not observed at the end of treatment in any test condition. There were no observed pH changes at the beginning or end of treatment in any test condition. Cytotoxicity of 55 ± 5%, or greater, based on CBPI was observed at 500 µg/mL in the 4-hour non-activated test condition. Cytotoxicity exceeding 55 ± 5% was observed at 100 µg/mL on the 24-hour non-activated test condition. Cytotoxicity of 55 ± 5%, or greater, based on CBPI was not observed in the 4-hour S9-activated test condition.

In the repeat assay, test substance precipitation was observed at the beginning of treatment starting at 300 µg/mL in the 4-hour S9-activated test condition and from 75 to 100 µg/mL in the 24-hour non-

activated test condition. Cytotoxicity of 55 ± 5%, or greater, was observed starting at 80 µg/mL on the 24-hour non-activated test condition. Cytotoxicity of 55 ± 5%, or greater, based on CBPI was not observed in the 4-hour S9-activated test condition, therefore, an addition repeat was conducted at 100, 250, 500, 1000, and 1882 µg/mL. Test substance precipitation was observed at 1882 µg/mL at the beginning and end of treatment. Cytotoxicity of 55 ± 5% based on CBPI was observed in the A culture of the 1000 µg/mL concentration. Cytotoxicity exceeding 55 ± 5% based on CBPI was observed in the B culture of the 1000 µg/mL concentration. It is noted that the 1882 µg/mL concentration was not indexed due to very few cells on the slides and high amounts of debris.

The observed changes in osmolality were ≤20% and pH changes ≤1 and were, therefore, not considered significant.

For the non-activated 4-hour exposure test condition, the CBPI for the highest test concentration evaluated microscopically for micronuclei, 500 µg/mL, was 1.38, compared with 1.84 for the vehicle control. This represents a 55.5% decrease in cell survival relative to the vehicle control. The concentrations selected for microscopic analysis of micronuclei were 100, 250, and 500 µg/mL and were selected based on cytotoxicity. The occurrence of binucleated cells with micronuclei in the test substance-treated groups were not significantly increased above that of the vehicle control in any test condition ($p > 0.05$, Fisher's exact test). No dose responsive trend was observed in any test condition ($p > 0.05$, Cochran-Armitage test).

The occurrence of binucleated cells with micronuclei in the MMC (positive control) treatment group (n=89) was statistically significant in relation to the occurrence in the vehicle control n=20) ($p \leq 0.05$, Fisher's exact test) and were within expected values based on historical ranges, hence meeting positive control acceptance criteria.

For the S9-activated 4-hour exposure test condition, the CBPI for the highest test concentration evaluated microscopically for micronuclei, 1000 µg/mL, was 1.35, compared with 1.83 for the vehicle control and was assayed in the second repeat. This represents a 57.6% decrease in cell survival relative to the vehicle control. It is noted that for the data collected for the second repeat assay, only the A cultures are being used due to the 1000 µg/mL B culture exhibiting toxicity exceeding 60%, whereas the A culture was within the 55 ± 5% required range, therefore, only the A cultures were used for all micronuclei analysis from the second repeat concentration. The concentrations selected for microscopic analysis of micronuclei were 50, 100, 250, 350, 500, and 1000 µg/mL and were selected based on cytotoxicity. The 50, 100, 250, and 350 µg/mL concentrations were analyzed from the repeat assay, and 500 and 1000 µg/mL were analyzed from the second repeat assay. Each repeat had its own set of vehicle and positive control cultures that were analyzed. The occurrence of binucleated cells with micronuclei in the test substance-treated groups were not significantly increased above that of the vehicle control in any test condition ($p > 0.05$, Fisher's exact test). No dose responsive trend was observed in any test condition ($p > 0.05$, Cochran-Armitage test).

The occurrence of binucleated cells with micronuclei in the CP (positive control) treatment group (repeat n=40, second repeat n=41) was statistically significant in relation to the occurrence in the vehicle control (repeat n=16, second repeat n=10) ($p \leq 0.05$, Fisher's exact test) and were within expected values based on historical ranges, hence meeting positive control acceptance criteria.

For the non-activated 24-hour exposure test condition, The CBPI for the highest test concentration evaluated microscopically for micronuclei, 80 µg/mL, was 1.37, compared with 1.78 for the vehicle control. This represents a 52.6% decrease in cell survival relative to the vehicle control. The concentrations selected for microscopic analysis of micronuclei were 25, 50, and 80 µg/mL and were selected based on cytotoxicity. The occurrence of binucleated cells with micronuclei in the test substance-treated groups were not significantly increased above that of the vehicle control in any test condition ($p > 0.05$, Fisher's exact test). No dose responsive trend was observed in any test condition ($p > 0.05$, Cochran-Armitage test).

The occurrence of binucleated cells with micronuclei in the VB (positive control) treatment group (n=58) was statistically significant in relation to the occurrence in the vehicle control (n=10) ($p \leq 0.05$,

Fisher's exact test) and were within expected values based on historical ranges, hence meeting positive control acceptance criteria.

The summary results are given in the following tables.

Table 26: Results of Micronucleus Test

Treatment Time: 4 hours (-S9)						
	Vehicle[b]	100 µg/mL	250 µg/mL	500 µg/mL	Positive control[c]	
Cytotoxicity	NA	26.2	40.8	55.5	59.7	
Precipitates	No	No	Yes[a]	Yes[a]	No	
CBPI	1.84	1.62	1.50	1.38	1.34	
Total number of binucleated cells scored	2000	2000	2000	2000	2000	
Total number of binucleated cells with micronucleus	20	22	25	20	89	
% of cells with micronucleus	1.0	1.1	1.3	1.0	4.5[d]	
Treatment Time: 4 hours (+S9) (repeat assay)						
	Vehicle[b]	50 µg/mL	100 µg/mL	250 µg/mL	350 µg/mL	Positive control[c]
Cytotoxicity	NA	-2.0	32.6	47.5	31.1	45.4
Precipitates	No	No	No	No	Yes[a]	No
CBPI	1.66	1.68	1.45	1.35	1.46	1.36
Total number of binucleated cells scored	2000	2000	2000	2000	2000	2000
Total number of binucleated cells with micronucleus	16	16	17	17	13	40
% of cells with micronucleus	0.8	0.8	0.9	0.9	0.7	2.0[d]
Treatment Time: 4 hours (+S9) (second repeat assay)						
	Vehicle[b]	500 µg/mL		1000 µg/mL	Positive control[c]	
Cytotoxicity	NA	15.1		57.6	44.2	
Precipitates	No	No		No	No	
CBPI	1.83	1.71		1.35	1.46	
Total number of binucleated cells scored	2000	2000		2000	2000	
Total number of binucleated cells with micronucleus	10	12		12	41	
% of cells with micronucleus	0.5	0.6		0.6	2.1[d]	
Treatment Time: 24 hours (-S9) (repeat assay)						
	Vehicle[b]	25 µg/mL	50 µg/mL	80 µg/mL	Positive control[c]	
Cytotoxicity	NA	0.1	10.9	52.6	42.0	
Precipitates	No	No	No	No	No	
CBPI	1.78	1.78	1.70	1.37	1.45	
Total number of binucleated cells scored	2000	2000	2000	2000	2000	
Total number of binucleated cells with micronucleus	10	13	10	18	58	
% of cells with micronucleus	0.5	0.7	0.5	0.9	2.9[d]	

a Precipitation observed at the beginning of treatment
b DMSO
c 0.4 µg/mL MMC (4-hour -S9); 10 µg/mL CP (4-hour +S9); 6.25 ng/mL VB (24-hour -S9)
d Statistically significant difference from control at $p \leq 0.05$ by Fisher's test
Data taken from pages 30-36, 41 of 68

Table 27 Historical Control Data[a]

Negative Controls			
	4-Hour Non-Activated Test System	24-Hour Non-Activated Test System	4-Hour Activated Test System[b]
Historical Values	% Micronucleated Bi-Nucleate Cells	% Micronucleated Bi-Nucleate Cells	% Micronucleated Bi-Nucleate Cells
95% Control Limits	0.2-1.2	0.3-0.9	0.1-1.2
Mean	0.6	0.6	0.7
Standard Deviation	0.24	0.18	0.17
Range	0.3-1.1	0.3-0.9	0.4-0.9
Positive Controls[c]			
	% Micronucleated Bi-Nucleate Cells		
	4-Hour Non-Activated Test System 0.4 µg/mL Mitomycin C	24-Hour Non-Activated Test System 12.5 ng/mL Vinblastine	4-Hour Activated Test System[b] 10 µg/mL Cyclophosphamide
Historical Values	% Micronucleated Bi-Nucleate Cells	% Micronucleated Bi-Nucleate Cells	% Micronucleated Bi-Nucleate Cells
95% Control Limits	2.8-5.2	1.4-4.0	1.2-2.4
Mean	3.9	2.6	1.8
Standard Deviation	0.65	0.73	0.031
Range	2.8-4.7	1.4-4.0	1.2-2.3

a Data are based on studies reported in 2019. Data include all control vehicles. All values are reported as percent micronucleated bi-nucleate cells.
b Metabolic activation systems based on PB/BNF-induced rat liver S9.
c Values are based on the highest concentration tested in the assay.
Data taken from pages 68 of 68

CONCLUSION

All criteria for a valid study (negative controls; positive controls; proliferation) were met.

Under the conditions of this study, X12019520 was not found to induce micronuclei in the *in vitro* micronucleus test in human peripheral blood lymphocytes. It was concluded that the test substance was negative in this *in vitro* test.

Test item	Test	Test object	Concentration	Result
X12019520	*In vitro* micronucleus	Human peripheral blood lymphocytes	100, 250, 500 µg/mL (4 h -S9); 50, 100, 250, 350 µg/mL (repeat 4 h +S9); 500, 1000 µg/mL (second repeat 4 h +S9); 25, 50, 80 µg/mL (24 h -S9)	Negative

A 1.1.1.4 X12264475 Bacterial Reverse Mutation Test

Reference: KCA 5.8.1/03

CITATION

Myhre, A.; 2020; X12264475: 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. 22440-500; Sponsor Study No. 201067 ; 02 December 2020; Published: 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:	6 August 2020 to 19 August 2020
GLP status:	Yes
Number of pages in final report:	51

MATERIALS AND METHODS

Test Item(s)

Test item (Common name):	X12264475
Purity:	99%
Description (physical state):	Solid
Lot/batch no.:	SYN-FS-SY1400161-6 (TSN308398)
Compound stability:	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 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. The cultures were placed in a shaker/incubator overnight at 100 to 200 rpm and 37 ± 2°C.
Confirmation:	All test cultures were tested for acceptable viability and genotype confirmation (including histidine, biotin requirement, *rfa*, *uvr*B 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 50-67 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 X12264475, 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 50-67 hours at 37 ± 2°C. The condition of the bacterial background lawn and tests substance precipitation were also evaluated. Toxicity and degree of precipitation were scored relative to the vehicle control plate.

Acceptance Criteria

The following criteria were fulfilled 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 response that did not meet all three of the above criteria (magnitude, concentration-responsiveness, reproducibility) was not being evaluated as positive.

Negative results obtained in the first trial were confirmed by a second trial, using the same method as specified above, with an alteration in concentration spacing and metabolic activation.

RESULTS AND DISCUSSION

Negative and Positive Controls

The number of revertant colonies for the negative control was within reasonable 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. No appreciable toxicity was observed at any concentration with any tester strain in either the absence or presence of S9 activation. A >50% reduction in mean number of revertants was observed at 3333 µg/plate with tester strain TA1537 in the presence of S9 activation; however, this reduction occurred at an intermediate concentration with no concentration related correlation and was not considered to be biologically relevant. 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 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. The summary results are given in the following tables.

Table 28: 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	7	8	7	11	10	7	8	7	9	1034
	+S9	13	14	11	12	8	12	15	5	11	173
TA1535	-S9	12	10	16	14	13	13	12	10	10	611
	+S9	16	11	12	13	12	11	12	15	10	201
TA98	-S9	19	25	29	27	24	24	28	26	24	99
	+S9	34	28	25	30	36	28	27	24	24	329
TA100	-S9	113	104	121	99	108	126	111	100	108	541
	+S9	164	174	168	139	176	171	173	170	162	2389
WP2 *uvr*A	-S9	26	36	36	23	22	32	36	27	27	476
	+S9	42	49	48	42	45	49	56	38	45	319

PC: Positive control

Table 29: 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	5	5	5	6	3	5	976
	+S9	7	7	7	6	7	8	132
TA1535	-S9	11	9	8	10	10	9	298
	+S9	10	12	10	11	11	13	150

TA98	-S9	15	18	19	20	16	17	100
	+S9	25	23	23	25	17	24	221
TA100	-S9	86	108	112	112	99	94	540
	+S9	126	135	143	139	144	147	2083
WP2 *uvr*A	-S9	50	38	30	29	35	45	565
	+S9	57	57	46	40	42	32	286

PC: Positive control

Table 30: 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, X12264475 is concluded to be non-mutagenic in the Bacterial Reverse Mutation Assay using *Salmonella typhimurium*.

Test item	Test	Test object	Concentration	Result
X12264475	*In vitro* bacterial reverse mutation test	TA98, TA100, TA1535, TA1537 and WP2 *uvr*A	33.3 to 5000 µg/plate	Negative

A 1.1.1.5 X12264475 In Vitro Mammalian Cell Micronucleus Test in Human

Reference: KCA 5.8.1/04

CITATION

Kellum, S. N.; 2021; X12264475: In Vitro Mammalian Cell Micronucleus Test in Human Peripheral Blood Lymphocytes; 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. 22440-523; Sponsor Study No. 201073; 15 February 2021;

Published: No

COMPLIANCE

Guideline(s):	OECD 487 (2016), EC B.49 (2017)
US EPA Guideline(s):	None
Deviations:	None
Dates of work:	22 July 2020 to 1 December 2020
GLP status:	Yes
Number of pages in final report:	42

MATERIALS AND METHODS

Test Item(s)

Test item (Common name):	X12264475
Purity:	99%
Description (physical state):	Solid
Lot/batch no.:	SYN-FS-SY1400161-6 (TSN308398)
Compound stability:	Not conducted

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

The test substance vehicle was used as the concurrent negative control. The final concentration of DMSO in the treatment medium did not exceed 1%.

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)	Fisher Scientific	50-07-7	Not reported	Sterile water	0.2 and 0.4 µg/mL	4-hour non-activated
Cyclophosphamide (CP)	Santa Cruz Biotechnology	50-18-0	Not reported	Sterile water	5 and 10 µg/mL	4-hour S9-activated

Positive control	Manufactured by	CAS No.	Purity (%)	Solvent	Concentration	Metabolic activation
Vinblastine (VB)	MP Biomedicals	143-67-9	Not reported	Sterile water	6.25 and 12.5 ng/mL	24-hour non-activated

Tester System

Cells	Human peripheral blood lymphocytes
Source	Healthy 25 year old female
Maintenance	RPMI 1640 containing approximately 15% fetal bovine serum, 2 mM L-glutamine, 100 units penicillin/mL and 100 µg/mL streptomycin, incubation at 37 ± 2°C. Cytochalasin B (6.0 µg/mL) added to media for re-fed of 4-hour cultures and for entire treatment of 24-hour cultures.
Metabolic activation	Liver homogenate (S9), prepared from male Sprague-Dawley rats induced with phenobarbital/5-6 benzoflavone (Moltox, Inc., Boone, North Carolina, U.S.A.). Protein content: 34.6 mg/mL and 39.2 mg/mL

Methods

Preliminary Toxicity Assay

The preliminary toxicity test was performed with the test concentrations of 10, 50, 100, 250, 500, 750, 1000, 1500, 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 200 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 ± 2ºC in a humidified atmosphere of 5 ± 2% 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 100, 250, 500, 1000, and 2000 µg/mL for all test conditions. The standard incubation conditions were 37 ± 2ºC in a humidified atmosphere of 5 ± 2% 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.

For each test condition, micronuclei evaluation was conducted for at least 3 test substance concentrations, the vehicle control, and a positive control. The maximum concentration evaluated in each test condition was the highest concentration tested, 2000 µg/mL, based on OECD 487 (2016) recommended limit dose. 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

Calculations

The CBPI were determined using the following formula:

$$\text{CBPI} = \frac{1 \times \text{mono-nucleated cells} + 2 \times \text{bi-nucleated cells} + 3 \times \text{multi-nucleated cells}}{}$$

total number of cells scored

% cytostasis (cytotoxicity) = 100 -100 {($CBPI_t$-1) /($CBPI_c$-1)}

where:

t = test substance treatment culture

c = vehicle control culture

Acceptance Criteria

An assay was considered acceptable for evaluation of test results only if all of the following criteria were satisfied. The metabolically activated and non-activated assays of the test are independent and, if necessary, were repeated separately.

a. Negative Controls: The frequency of cells with micronuclei was within the 95% control limits of the distribution of the historical negative control data. If the concurrent negative control data fell outside the 95% control limits, they were acceptable as long as these data were not extreme outliers (indicative of experimental or human error).
b. Positive Controls: The frequency of cells with micronuclei was significantly greater ($p \leq 0.05$, Fisher's exact test) than the vehicle control response and induced responses compatible with those generated in the historical control database.
c. Cell Proliferation: The CBPI of the vehicle control at harvest was ≥1.4.

Evaluation Criteria

The following conditions were used as a guide to determine a positive response:

- A statistically significant increase ($p \leq 0.05$, Fisher's exact test) in the frequency of cells with micronuclei was seen in one or more treatment groups relative to the vehicle control response.
- The observed increased frequencies were accompanied by a concentration-related increase when evaluated by the trend test ($p \leq 0.05$, Cochran-Armitage test).
- Any of the results were outside the 95% control limit distribution of the historical negative control data.
- Note: Statistically significant values that did not exceed the historical control range for the negative/vehicle control were judged as not being biologically relevant.

The following condition was used as a guide to determine an equivocal response:

- Results observed in any of the assays resulted in statistically significant elevations in micronuclei at more than one test concentration level without demonstrating a dose-responsive trend.

The test substance was judged negative if the following conditions were met:

- There was no statistically significant increase in the frequency of cells with micronuclei in any treatment group relative to the vehicle control group.
- There was no concentration-related increase when evaluated with an appropriate trend test.
- All results were within the 95% control limit of the distribution of the historical negative control database.

Statistics

Statistical analysis was used as a guide to determine whether or not the test substance induced a positive response. Interpretation of the statistical analysis also relied on additional considerations including the magnitude of the observed test substance response relative to the vehicle control response and the presence of a dose-responsive trend. Statistical analysis consisted of a Fisher's exact test (with Bonferroni-Holm Adjustment) to compare the frequency of micronuclei in the test substance-treated groups with the vehicle control response. A Cochran-Armitage test for dose responsiveness was conducted.

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, cyclophosphamide and vinblastine 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

Test substance precipitation was not observed at the beginning or end of treatment for any test condition. There were no pH changes observed at the beginning or end of treatment. Cytotoxicity of 55 ± 5%, or greater, based on CBPI, was not observed in any test condition.

Micronucleus Assay

Test substance precipitation was not observed at the beginning or end of treatment for any test condition. There were no pH changes observed at the beginning or end of treatment. Cytotoxicity of 55 ± 5%, or greater, based on CBPI was not observed in any test condition.

Osmolality and pH measurements were taken from two test substance concentrations and the vehicle control media for all three treatment medias (complete RPMI, CytoB Media, and S9 Media). The observed changes in osmolality were ≤20% and were, therefore, not considered significant.

In the 4-hour non-activated exposure group, the CBPI for the highest test concentration evaluated microscopically for micronuclei, 2000 µg/mL, was 1.88, compared with 1.99 for the vehicle control. This represents a 10.8% decrease in cell survival relative to the vehicle control. The concentrations selected for microscopic analysis of micronuclei were 500, 1000, and 2000 µg/mL and were selected based on the highest concentration tested based on OECD 487 (2016) recommended limit dose. A total of 2000 binucleated cells per concentration, vehicle, and positive control were evaluated. The occurrence of binucleated cells with micronuclei in the test substance-treated groups were not significantly increased above that of the vehicle control in any test condition ($p > 0.05$, Fisher's exact test). No dose responsive trend was observed in any test condition ($p > 0.05$, Cochran-Armitage test).

The occurrence of binucleated cells with micronuclei in the MMC (positive control) treatment group (n=100) was statistically significant in relation to the occurrence in the vehicle control (n=16) ($p \leq 0.05$, Fisher's exact test).

In the S9-activated 4-hour exposure group, the CBPI for the highest test concentration evaluated microscopically for micronuclei, 2000 µg/mL, was 1.85, compared with 1.88 for the vehicle control. This represents an 3.2% decrease in cell survival relative to the vehicle control. The concentrations selected for microscopic analysis of micronuclei were 500, 1000, and 2000 µg/mL and were selected based on the highest concentration tested based on OECD 487 (2016) recommended limit dose. A total of 2000 binucleated cells per concentration, vehicle, and positive control were evaluated. The

occurrence of binucleated cells with micronuclei in the test substance-treated groups were not significantly increased above that of the vehicle control in any test condition (p >0.05, Fisher's exact test). No dose responsive trend was observed in any test condition (p >0.05, Cochran-Armitage test).

The occurrence of binucleated cells with micronuclei in the CP (positive control) treatment group (n=40) was not statistically significant in relation to the occurrence in the vehicle control (n=22) (p =0.0575, Fisher's exact test), however, the results were within the laboratory historical positive control 95% limits and range, therefore considered valid.

In the non-activated 24-hour exposure group, the CBPI for the highest test concentration evaluated microscopically for micronuclei, 2000 µg/mL, was 1.72, compared with 1.78 for the vehicle control. This represents a 7.8% decrease in cell survival relative to the vehicle control. The concentrations selected for microscopic analysis of micronuclei were 500, 1000, and 2000 µg/mL and were selected based on the highest concentration tested based on OECD 487 (2016) recommended limit dose. A total of 2000 binucleated cells per concentration, vehicle, and positive control were evaluated. The occurrence of binucleated cells with micronuclei in the test substance-treated groups were not significantly increased above that of the vehicle control in any test condition (p >0.05, Fisher's exact test). No dose responsive trend was observed in any test condition (p >0.05, Cochran-Armitage test).

The occurrence of binucleated cells with micronuclei in the VB (positive control) treatment group (n=41) was statistically significant in relation to the occurrence in the vehicle control (n=10) (p ≤0.05, Fisher's exact test).

The summary results of Cytotoxicity (CBPI relative to the vehicle control) and % of micronucleated binucleated cells was observed as:

Table 1: Results of Micronucleus Test

Treatment Time: 4 hours (-S9)					
	Vehicle	500 µg/mL	1000 µg/mL	2000 µg/mL	Positive control[a]
Cytotoxicity	NA	7.2	14.7	10.8	54.8
Precipitates	No	No	No	No	No
CBPI	1.99	1.92	1.84	1.88	1.45
Total number of binucleated cells scored	2000	2000	2000	2000	2000
Total number of binucleated cells with micronucleus	16	16	16	15	100
% of cells with micronucleus	0.8	0.8	0.8	0.8	5.0[c]
Treatment Time: 4 hours (+S9)					
	Vehicle	500 µg/mL	1000 µg/mL	2000 µg/mL	Positive control[a]
Cytotoxicity	NA	1.2	2.8	3.2	41.1
Precipitates	No	No	No	No	No
CBPI	1.88	1.87	1.86	1.85	1.52
Total number of binucleated cells scored	2000	2000	2000	2000	2000
Total number of binucleated cells with micronucleus	22	19	11	19	40
% of cells with micronucleus	1.1	1.0	0.6	1.0	2.0 [c]
Treatment Time: 24 hours (-S9)					
	Vehicle	500 µg/mL	1000 µg/mL	2000 µg/mL	Positive control[a]
Cytotoxicity[b]	NA	-1.2	-0.6	7.8	9.2
Precipitates	No	No	No	No	No
CBPI	1.78	1.79	1.79	1.72	1.71
Total number of binucleated cells scored	2000	2000	2000	2000	2000
Total number of binucleated cells with micronucleus	10	16	16	11	41
% of cells with micronucleus	0.5	0.8	0.8	0.6	2.1 [c]

[a] 0.4 µg/mL MMC (4-hour -S9); 10 µg/mL CP (4-hour +S9); 6.25 ng/mL VB (24-hour -S9)
[b] A negative value indicates an increase in cell survival
[c] Statistically significant ($p \leq 0.05$, Fisher's Test)

Table 2: Historical Control Data[a]

Negative Controls			
	4-Hour Non-Activated Test System	24-Hour Non-Activated Test System	4-Hour Activated Test System[b]
Historical Values	% Micronucleated Bi-Nucleate Cells	% Micronucleated Bi-Nucleate Cells	% Micronucleated Bi-Nucleate Cells
95% Control Limits	0.2-1.2	0.3-0.9	0.1-1.2
Mean	0.6	0.6	0.7
Standard Deviation	0.24	0.18	0.17
Range	0.3-1.1	0.3-0.9	0.4-0.9
Positive Controls[c]			
	% Micronucleated Bi-Nucleate Cells		
	4-Hour Non-Activated Test System 0.4 µg/mL Mitomycin C	24-Hour Non-Activated Test System 12.5 ng/mL Vinblastine	4-Hour Activated Test System[b] 10 µg/mL Cyclophosphamide
Historical Values	% Micronucleated Bi-Nucleate Cells	% Micronucleated Bi-Nucleate Cells	% Micronucleated Bi-Nucleate Cells
95% Control Limits	2.8-5.2	1.4-4.0	1.2-2.4
Mean	3.9	2.6	1.8
Standard Deviation	0.65	0.73	0.031
Range	2.8-4.7	1.4-4.0	1.2-2.3

[a] Data are based on studies reported in 2019. Data include all control vehicles. All values are reported as percent micronucleated bi-nucleate cells.
[b] Metabolic activation systems based on PB/BNF-induced rat liver S9.
[c] Values are based on the highest concentration tested in the assay.

CONCLUSION

All criteria for a valid study were met as described in the protocol. From the results of this study, it is concluded that X12264475 did not show any potential to induce micronuclei in cultured human peripheral blood lymphocytes, both in the absence and presence (10% v/v S9 mix) of metabolic activation system under the present experimental conditions.

Test item	Test	Test object	Concentration	Result
X12264475	*In vitro* micronucleus	Human peripheral blood lymphocytes	100, 250, 500*, 1000*, and 2000* µg/mL *Analyzed for MNT	Negative

A 1.1.1.6 X12314005 Bacterial Reverse Mutation Test

Reference: KCA 5.8.1/05

CITATION

Myhre, A., Davis, F. X.; 2020; X12314005: 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. 22439-500; Sponsor Study No. 201065 ; 14 January 2021; Published: 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:	01 September 2020 to 28 September 2020
GLP status:	Yes
Number of pages in final report:	53

MATERIALS AND METHODS

Test Item(s)

Test item (Common name):	X12314005
Purity:	98%
Description (physical state):	Liquid
Lot/batch no.:	B190273-65-1 (TSN403310)
Compound stability:	Stability 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 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	

Positive control	Manufactured by	Purity (%)	Solvent	Bacterial strains	Concentration	Metabolic activation
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. The cultures were placed in a shaker/incubator overnight at 100 to 200 rpm and 37 ± 2°C.
Confirmation:	All test cultures were tested for acceptable viability and genotype confirmation (including histidine, biotin requirement, *rfa*, *uvr*A, *uvr*B 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 49-69 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 X12314005, 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 at 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 49 to 69 hours at 37 ± 2°C.

Acceptance Criteria

The following criteria were fulfilled 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 response that did not meet all three of the above criteria (magnitude, concentration-responsiveness, reproducibility) was not being evaluated as positive.

Negative results obtained in the first trial were confirmed by a second trial, using the same method as specified above, with an alteration in concentration spacing and metabolic activation.

RESULTS AND DISCUSSION

Negative and Positive Controls

The number of revertant colonies for the negative control was within reasonable limits of the historical control of this laboratory for all the strains (Table 33). 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. Toxicity was observed with tester strain TA1537 starting at 3333 µg/plate in both the presence and absence of S9 activation. No other appreciable toxicity was observed at any concentration with any tester strain in either the absence or presence of S9. 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. Toxicity was observed with tester strain WP2 *uvr*A starting at 3333 µg/plate in the presence of S9 activation; and with tester strain TA1537 at 5000 µg/plate in the absence of S9 activation. No other appreciable toxicity was observed at any concentration with any tester strain in either the absence or presence of S9. No test substance precipitation was observed. The summary results are given in the following tables.

Table 31: 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	4	5	6	4	5	6	5	1	1	932
	+S9	9	10	8	10	7	8	8	4	3	127
TA1535	-S9	8	6	8	6	8	10	10	11	9	575
	+S9	11	8	14	10	9	9	8	8	6	128
TA98	-S9	17	19	13	13	18	17	18	18	12	102
	+S9	20	18	24	25	25	28	20	23	20	222

TA100	-S9	130	114	140	122	118	129	132	100	76	580
	+S9	168	175	151	148	158	117	133	122	110	1994
WP2 *uvr*A	-S9	20	23	29	23	25	19	23	23	22	543
	+S9	33	33	29	29	32	25	26	23	18	215

PC: Positive control

Table 32: 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	14	14	12	12	11	6	1136
	+S9	19	14	14	15	15	13	181
TA1535	-S9	14	11	13	14	14	15	639
	+S9	16	18	18	19	12	15	191
TA98	-S9	40	22	31	35	33	33	183
	+S9	40	36	38	43	35	37	371
TA100	-S9	125	114	120	122	107	96	634
	+S9	182	165	152	176	176	156	1829
WP2 *uvr*A	-S9	38	33	37	28	22	26	647
	+S9	29	19	18	18	6	3	194

PC: Positive control

Table 33: Historical Control Data

Historical Control Data[a]					
Tester strain	Control (positive control)[b]	Exogenous Metabolic Activation System	Mean	SD[c]	Range
TA98	Negative	-S9	25	9	10-58
	Negative	+S9	33	9	11-61
	Positive [2NF-1]	-S9	242	68	123-702
	Positive [BAP-2.5]	+S9	392	86	186-615
TA100	Negative	-S9	106	23	51-214
	Negative	+S9	124	27	62-291
	Positive [SA-2]	-S9	939	202	458-1558
	Positive [2AA-2.5]	+S9	2597	877	523-5889
TA1535	Negative	-S9	13	5	3-30
	Negative	+S9	13	5	4-32
	Positive [SA-2]	-S9	839	181	392-1505
	Positive [2AA-2.5]	+S9	205	50	78-365
TA1537	Negative	-S9	8	4	1-22
	Negative	+S9	12	5	2-31
	Positive [ICR 191-2]	-S9	1133	337	300-2668
	Positive [2AA-2.5]	+S9	131	54	48-354
WP2 *uvr*A	Negative	-S9	34	11	13-66
	Negative	+S9	41	12	5-68

	Positive [4NQO-1]	-S9	769	252	283-1337
	Positive [2AA-25]	+S9	256	79	126-680

[a] Historical data for tester strains used in the reported study. Data are based on 73 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. Based on the results generated under the experimental conditions of this study, X12314005 is concluded to be non-mutagenic in the Bacterial Reverse Mutation Assay using *Salmonella typhimurium* and *Escherichia coli* both with and without S9.

Test item	Test	Test object	Concentration	Result
X12314005	*In vitro* bacterial reverse mutation test	*S. typhimurium* TA98, TA100, TA1535, TA1537, *E. Coli* WP2 *urv*A	0, 33.3, 66.7, 100, 333, 667, 1000, 3333 & 5000 µg/plate	Negative ± S9

A 1.1.1.7 X12314005 In Vitro Mammalian Cell Micronucleus Test in Human

Reference: KCA 5.8.1/06

CITATION

Kellum, S. N.; 2021; X12314005: In Vitro Mammalian Cell Micronucleus Test in Human Peripheral Blood Lymphocytes; 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. 22439-523; Sponsor Study No. 201072; 15 February 2021;

Published: No

COMPLIANCE

Guideline(s):	OECD 487 (2016), EC B.49 (2017)
US EPA Guideline(s):	None
Deviations:	None
Dates of work:	7 August 2020 to 25 January 2021
GLP status:	Yes
Number of pages in final report:	46

MATERIALS AND METHODS

Test Item(s)

Test item (Common name):	X12314005
Purity:	98%
Description (physical state):	Liquid
Lot/batch no.:	B190273-65-1 (TSN403310)

Compound stability: Not conducted

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

The test substance vehicle was used as the concurrent negative control. The final concentration of DMSO in the treatment medium did not exceed 1%.

Vehicle	Manufactured by	CAS number
Dimethyl Sulfoxide (DMSO)	Sigma-Aldrich	67-68-5
Dimethyl Sulfoxide (DMSO)	BioWorld	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)	Fisher Scientific	50-07-7	Not reported	Sterile water	0.2 and 0.4 µg/mL	4-hour non-activated
Cyclophosphamide (CP)	Santa Cruz Biotechnology	50-18-0	Not reported	Sterile water	5 and 10 µg/mL	4-hour S9-activated
Vinblastine (VB)	MP Biomedicals	143-67-9	Not reported	Sterile water	6.25 and 12.5 ng/mL	24-hour non-activated

Tester System

Cells	Human peripheral blood lymphocytes
Source	Healthy 25/26 year old female
Maintenance	RPMI 1640 containing approximately 15% heat fetal bovine serum, 2 mM L-glutamine, 100 units penicillin/mL and 100 µg/mL streptomycin, incubation at 37 ± 2°C. Cytochalasin B (6.0 µg/mL) added to media for re-fed of 4-hour cultures and for entire treatment of 24-hour cultures.
Metabolic activation	Liver homogenate (S9), prepared from male Sprague-Dawley rats induced with phenobarbital/5-6 benzoflavone (Moltox, Inc., Boone, North Carolina, U.S.A.). Protein content: 34.6 mg/mL and 39.2 mg/mL

Methods

Preliminary Toxicity Assay

The preliminary toxicity test was performed with the test concentrations of 10, 50, 100, 250, 500, 750, 1000, 1500, 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 200 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 ± 2ºC in a humidified atmosphere of 5 ± 2% 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 30, 75, 150, 375, 750, 1000, and 1500 µg/mL for the 4-hour S9-activated test condition,

30, 75, 150, 375, 750, and 1000 µg/mL for the 4-hour non-activated test condition, and 25, 50, 100, 200, 250, 260, 270, 280, 290, 300, 400, and 500 µg/mL for the 24-hour non-activated test condition. The standard incubation conditions were 37 ± 2ºC in a humidified atmosphere of 5 ± 2% 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.

For each test condition, micronuclei evaluation was conducted for at least 3 test substance concentrations, the vehicle control, and a positive control. The maximum concentration evaluated for the 4-hour S9-test conditions was the lowest precipitating concentration, which was also the lowest concentration exhibiting toxicity of 55 ± 5% for the 4-hour non-activated test condition. The maximum concentration evaluated for the 24-hour non-activated test condition was the lowest concentration exhibiting toxicity of 55 ± 5%. It is noted that the highest concentration selected for the 4-hour non activated test condition also exhibited toxicity of 55 ± 5%. 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

Calculations

The CBPI were determined using the following formula:

$$CBPI = \frac{1 \text{ x mono-nucleated cells} + 2 \text{ x bi-nucleated cells} + 3 \text{ x multi-nucleated cells}}{\text{total number of cells scored}}$$

$$\% \text{ cytostasis (cytotoxicity)} = 100 - 100 \{(CBPI_t - 1) / (CBPI_c - 1)\}$$

where:

t = test substance treatment culture

c = vehicle control culture

Acceptance Criteria

An assay was considered acceptable for evaluation of test results only if all of the following criteria were satisfied. The metabolically activated and non-activated assays of the test are independent and, if necessary, were repeated separately.

d. Negative Controls: The frequency of cells with micronuclei was within the 95% control limits of the distribution of the historical negative control data. If the concurrent negative control data fell outside the 95% control limits, they were acceptable as long as these data were not extreme outliers (indicative of experimental or human error).

e. Positive Controls: The frequency of cells with micronuclei was significantly greater ($p \leq 0.05$, Fisher's exact test) than the vehicle control response and induced responses compatible with those generated in the historical control database.

f. Cell Proliferation: The CBPI of the vehicle control at harvest was ≥1.4.

Evaluation Criteria

The following conditions were used as a guide to determine a positive response:

- A statistically significant increase ($p \leq 0.05$, Fisher's exact test) in the frequency of cells with micronuclei was seen in one or more treatment groups relative to the vehicle control response.
- The observed increased frequencies were accompanied by a concentration-related increase when evaluated by the trend test ($p \leq 0.05$, Cochran-Armitage test).
- Any of the results were outside the 95% control limit distribution of the historical negative control data.
- Note: Statistically significant values that did not exceed the historical control range for the negative/vehicle control were judged as not being biologically relevant.

The following condition was used as a guide to determine an equivocal response:

- Results observed in any of the assays resulted in statistically significant elevations in micronuclei at more than one test concentration level without demonstrating a dose-responsive trend.

The test substance was judged negative if the following conditions were met:

- There was no statistically significant increase in the frequency of cells with micronuclei in any treatment group relative to the vehicle control group.
- There was no concentration-related increase when evaluated with an appropriate trend test.
- All results were within the 95% control limit of the distribution of the historical negative control database.

Statistics

Statistical analysis was used as a guide to determine whether or not the test substance induced a positive response. Interpretation of the statistical analysis also relied on additional considerations including the magnitude of the observed test substance response relative to the vehicle control response and the presence of a dose-responsive trend. Statistical analysis consisted of a Fisher's exact test (with Bonferroni-Holm Adjustment) to compare the frequency of micronuclei in the test substance-treated groups with the vehicle control response. A Cochran-Armitage test for dose responsiveness was conducted.

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, cyclophosphamide and vinblastine 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

Test substance precipitation was observed at the beginning of treatment at 250 to 2000 µg/mL in all test conditions. Test substance precipitation was observed at the end of treatment at 1500 and 2000 µg/mL in the 4-hour S9-activated test condition and at 750 to 2000 µg/mL in the 4-hour non-activated and 24-hour non-activated test conditions. There were no observed pH changes at the beginning or end of treatment in any test condition. Cytotoxicity of 55 ± 5%, or greater, based on CBPI was only observed in the 24-hour non-activated test condition at 750 µg/mL. It is noted that indexing was not

conducted for 1000 to 2000 µg/mL in the 24-hout test condition due to toxicity observed at lower concentrations.

Micronucleus Assay

Test substance precipitation was observed at the beginning of treatment in the 4-hour S9-activated test condition at 375 to 1500 µg/mL, in the 4-hour non-activated test condition from dose levels 375 to 1000 µg/mL, and in the 24-hour non-activated test condition at 250 to 750 µg/mL. Test substance precipitation was observed at the end of treatment in the 4-hour S9-activated test condition at 1000 and 1500 µg/mL, in the 4-hour non-activated test condition in dose levels 750 and 1000 µg/mL, and in the 24-hour non-activated test condition at 700 and 750 µg/mL. There were no observed pH changes at the beginning or end of treatment in any test condition. Cytotoxicity of 55 ± 5%, or greater, based on CBPI was observed in the 4-hour S9 activated test condition at 1500 µg/mL and in the 4-hour non-activated test condition at 750 and 1000 µg/mL. Cytotoxicity of greater than 55 ± 5%, based on CBPI was observed in the 24-hour non-activated test condition at 300 and 400 µg/mL.

Due to not obtaining concentrations acceptable for micronucleus evaluation in the 24-hour non-activated test condition, two repeat studies were conducted. In the initial repeat, the concentrations chosen for the 24-hour non-activated test condition were 125, 200, 250, 300, 400, 500, 550, 600, 650, 700, and 750 µg/mL. Test substance precipitation was observed at the beginning of treatment at 300 to 750 µg/mL. Test substance precipitation was observed at the end of treatment at 700 and 750 µg/mL. Cytotoxicity of greater than 55 ± 5%, based on CBPI, was observed in the 24-hour non-activated test condition at 500 and 550 µg/mL. Due to not obtaining concentrations acceptable for micronucleus evaluation, a second repeat was conducted at 25, 50, 100, 200, 250, 260, 270, 280, 290, 300, 400, and 500 µg/mL. Test substance precipitation was observed at the beginning of treatment at 100 to 500 µg/mL. Test substance precipitation was not observed at the end of treatment. Cytotoxicity of 55 ± 5%, or greater, based on CBPI was observed in the 24-hour non-activated test condition at 250 and 260 µg/mL.

Osmolality and pH measurements were taken from two test substance concentrations and the vehicle control media for all three treatment medias (complete RPMI, CytoB Media, and S9 Media). The observed changes in osmolality were ≤ 20% and were, therefore, not considered significant.

In the 4-hour non-activated exposure group, the CBPI for the highest test concentration evaluated microscopically for micronuclei, 750 µg/mL, was 1.40, compared with 1.90 for the vehicle control. This represents a 55.7% decrease in cell survival relative to the vehicle control. The concentrations selected for microscopic analysis of micronuclei were 150, 375, and 750 µg/mL and the highest concentration was selected based on the lowest end-of-treatment precipitating concentration and cytotoxicity of 55 ± 5%. A total of 2000 binucleated cells per concentration, vehicle, and positive control were evaluated. The occurrence of binucleated cells with micronuclei in the test substance-treated groups were not significantly increased above that of the vehicle control in any test condition ($p > 0.05$, Fisher's exact test). No dose responsive trend was observed in any test condition ($p > 0.05$, Cochran-Armitage test).

The occurrence of binucleated cells with micronuclei in the MMC (positive control) treatment group (n=76) was statistically significant in relation to the occurrence in the vehicle control (n=15) ($p \leq 0.05$, Fisher's exact test).

In the S9-activated 4-hour exposure group, the CBPI for the highest test concentration evaluated microscopically for micronuclei, 1000 µg/mL, was 1.51, compared with 1.77 for the vehicle control. This represents a 33.4% decrease in cell survival relative to the vehicle control. The concentrations selected for microscopic analysis of micronuclei were 150, 375, and 1000 µg/mL and were selected based on the lowest end-of-treatment precipitating concentration. A total of 2000 binucleated cells per concentration were evaluated. Due to a discrepancy in the 10 µg/mL CP cultures, the 4-hour S9-activated vehicle control cultures and 10 µg/mL CP cultures were re-dropped, and an additional 1000

cells per slide were analyzed. The analysis for both evaluations were added together. The occurrence of binucleated cells with micronuclei in the test substance-treated groups were not significantly increased above that of the vehicle control in any test condition ($p > 0.05$, Fisher's exact test). No dose responsive trend was observed in any test condition ($p > 0.05$, Cochran-Armitage test).

The occurrence of binucleated cells with micronuclei in the CP (positive control) treatment group (n=70) was statistically significant in relation to the occurrence in the vehicle control (n=21) ($p \leq 0.05$, Fisher's exact test).

In the non-activated 24-hour exposure group, the CBPI, in the second repeat micronucleus assay, for the highest test concentration evaluated microscopically for micronuclei, 250 µg/mL, was 1.28, compared with 1.62 for the vehicle control. This represents a 55.0% decrease in cell survival relative to the vehicle control. The concentrations selected for microscopic analysis of micronuclei were 50, 100, and 250 µg/mL and were selected based on a cytotoxicity of 55 ± 5%. A total of 2000 binucleated cells per concentration, vehicle, and positive control were evaluated. The occurrence of binucleated cells with micronuclei in the test substance-treated groups were not significantly increased above that of the vehicle control in any test condition ($p > 0.05$, Fisher's exact test). No dose responsive trend was observed in any test condition ($p > 0.05$, Cochran-Armitage test).

The occurrence of binucleated cells with micronuclei in the VB (positive control) treatment group (n=51) was statistically significant in relation to the occurrence in the vehicle control (n=17) ($p \leq 0.05$, Fisher's exact test).

The summary results of Cytotoxicity (CBPI relative to the vehicle control) and % of micronucleated binucleated cells was observed as:

Table 1: Results of Micronucleus Test

Treatment Time: 4 hours (-S9)					
	Vehicle	150 µg/mL	375 µg/mL	750 µg/mL	Positive control[c]
Cytotoxicity	NA	10.8	19.4	55.7	53.7
Precipitates	No	No	Yes[a,b]	Yes[a,b]	No
CBPI	1.90	1.80	1.72	1.40	1.42
Total number of binucleated cells scored	2000	2000	2000	2000	2000
Total number of binucleated cells with micronucleus	15	14	9	16	76
% of cells with micronucleus	0.8	0.7	0.5	0.8	3.8[d]
Treatment Time: 4 hours (+S9)					
	Vehicle	150 µg/mL	375 µg/mL	1000 µg/mL	Positive control[c]
Cytotoxicity	NA	7.2	5.3	33.4	42.3
Precipitates	No	No	Yes[a]	Yes[a,b]	No
CBPI	1.77	1.71	1.73	1.51	1.44
Total number of binucleated cells scored	4000	2000	2000	2000	4000
Total number of binucleated cells with micronucleus	21	17	23	16	70
% of cells with micronucleus	0.5	0.9	1.2	0.8	1.8[d]
Treatment Time: 24 hours (-S9) (second repeat assay)					
	Vehicle	50 µg/mL	100 µg/mL	250 µg/mL	Positive control[c]
Cytotoxicity	NA	11.4	22.9	55.0	39.6
Precipitates	No	No	Yes[a]	Yes[a]	No
CBPI	1.62	1.55	1.48	1.28	1.37
Total number of binucleated cells scored	2000	2000	2000	2000	2000
Total number of binucleated cells with micronucleus	17	22	20	13	51
% of cells with micronucleus	0.9	1.1	1.0	0.7	2.6[d]

[a] Precipitation observed at the beginning of treatment
[b] Precipitation observed at the end of treatment
[c] 0.4 µg/mL MMC (4-hour -S9); 10 µg/mL CP (4-hour +S9); 6.25 ng/mL VB (24-hour -S9)
[d] Statistically significant increase ($p \leq 0.05$, Fisher's Test)

Table 2: Historical Control Data[a]

Negative Controls			
	4-Hour Non-Activated Test System	24-Hour Non-Activated Test System	4-Hour Activated Test System[b]
Historical Values	% Micronucleated Bi-Nucleate Cells	% Micronucleated Bi-Nucleate Cells	% Micronucleated Bi-Nucleate Cells
95% Control Limits	0.2-1.2	0.3-0.9	0.1-1.2
Mean	0.6	0.6	0.7
Standard Deviation	0.24	0.18	0.17
Range	0.3-1.1	0.3-0.9	0.4-0.9
Positive Controls[c]			
	% Micronucleated Bi-Nucleate Cells		
	4-Hour Non-Activated Test System 0.4 µg/mL Mitomycin C	24-Hour Non-Activated Test System 12.5 ng/mL Vinblastine	4-Hour Activated Test System[b] 10 µg/mL Cyclophosphamide
Historical Values	% Micronucleated Bi-Nucleate Cells	% Micronucleated Bi-Nucleate Cells	% Micronucleated Bi-Nucleate Cells
95% Control Limits	2.8-5.2	1.4-4.0	1.2-2.4
Mean	3.9	2.6	1.8
Standard Deviation	0.65	0.73	0.031
Range	2.8-4.7	1.4-4.0	1.2-2.3

[a] Data are based on studies reported in 2019. Data include all control vehicles. All values are reported as percent micronucleated bi-nucleate cells.
[b] Metabolic activation systems based on PB/BNF-induced rat liver S9.
[c] Values are based on the highest concentration tested in the assay.

CONCLUSION

All criteria for a valid study were met as described in the protocol. From the results of this study, it is concluded that X12314005 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
X12314005	*In vitro* micronucleus	Human peripheral blood lymphocytes	30, 75, 150*, 375*, 750, 1000*, and 1500 µg/mL (4-hour S9-activation), 30, 75, 150*, 375*, 750*, and 1000 µg/mL (4-hour non-activation), and 25, 50*, 100*, 200, 250*, 260, 270, 280, 290, 300, 400, and 500 µg/mL (24-hour non-activation). *Analyzed for MNT	Negative

A 2.14 Special Studies

No further studies were conducted on GF-3307.

Appendix 3 Exposure calculations

A 3.1 Operator exposure calculations (KCP 7.2.1.1)

A 3.1.1 Calculations for fenpicoxamid

Table A 1: Input parameters considered for the estimation of operator exposure: Fenpicoxamid (0.075 kg a.s./ha)

Substance	Fenpicoxamid	Formulation = Soluble concentrates, emulsifiable concentrate, etc.	Application rate-0.075 kg a.s. /ha	Spray dilution = 0.75 g a.s./l	Vapour pressure = low volatile substances having a vapour pressure of
Scenario	Cereals / Outdoor / Downward spraying / Vehicle-mounted			Buffer = 2-3	Number applications = 1, Application interval = 365 days
Percentage Absoprtion	Dermal for product = 0.33	Dermal for in use diluation = 12	Oral = 12	Inhalation = 100	
RVNAS	0.05 mg/kg bw/day		RVAAS	0.2 mg/kg bw/day	
DFR	3 µg a.s./cm2 per kg a.s./ha		DT50	30 days	

Table A 2: Estimation of operator exposure towards active substance (no PPE) according to EFSA guidance (0.075 kg a.s./ha)

Operator Model		Mixing, loading and application AOEM			
Potential exposure	Longer term systemic exposure mg/kg bw/day		0.0031	% of RVNAS	6.27%
	Acute systemic exposure mg/kg bw/day		0.0246	% of RVAAS	12.29%
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		0.0020	% of RVNAS	4.08%
	Acute systemic exposure mg/kg bw/day		0.0156	% of RVAAS	7.81%

Table A 3: Estimation of operator exposure towards active substance (PPE) according to EFSA guidance (0.075 kg a.s./ha)

Operator Model		Mixing, loading and application AOEM			
Potential exposure	Longer term systemic exposure mg/kg bw/day		0.0031	% of RVNAS	6.27%
	Acute systemic exposure mg/kg bw/day		0.0246	% of RVAAS	12.29%
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		0.0004	% of RVNAS	0.73%
	Acute systemic exposure mg/kg bw/day		0.0086	% of RVAAS	4.32%

A 3.1.2 Calculations for prothioconazole

Table A 4: Input parameters considered for the estimation of operator exposure: Prothioconazole (0.15 kg a.s./ha)

Substance	Prothioconazole	Formulation = Soluble concentrates, emulsifiable concentrate, etc.	Application rate-0.15 kg a.s. /ha	Spray dilution = 1.5 g a.s./l	Vapour pressure = low volatile substances having a vapour pressure of
Scenario	Cereals / Outdoor / Downward spraying / Vehicle-mounted			Buffer = 2-3	Number applications = 1, Application interval = 365 days
Percentage Absoprtion	Dermal for product = 25	Dermal for in use diluation = 70	Oral = 100	Inhalation = 100	
RVNAS	0.2 mg/kg bw/day		RVAAS	mg/kg bw/day	
DFR	3 µg a.s./cm2 per kg a.s./ha		DT50	30 days	

Table A 5: Estimation of operator exposure towards active substance (no PPE) according to EFSA guidance (0.15 kg a.s./ha)

Operator Model		Mixing, loading and application AOEM			
Potential exposure	Longer term systemic exposure mg/kg bw/day		0.1791	% of RVNAS	89.54%
	Acute systemic exposure mg/kg bw/day		1.0599	% 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		0.1113	% of RVNAS	55.67%
	Acute systemic exposure mg/kg bw/day		0.4886	% of RVAAS	

Table A 6: Estimation of operator exposure towards active substance (PPE) according to EFSA guidance (0.15 kg a.s./ha)

Operator Model		Mixing, loading and application AOEM			
Potential exposure	Longer term systemic exposure mg/kg bw/day		0.1791	% of RVNAS	89.54%
	Acute systemic exposure mg/kg bw/day		1.0599	% 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		0.0049	% of RVNAS	2.46%
	Acute systemic exposure mg/kg bw/day		0.0710	% of RVAAS	

A 3.1.3 Calculations for PTZ-desthio

Table A 7: Input parameters considered for the estimation of operator exposure: PTZ-desthio (0.136 kg a.s./ha)

Substance	Des thio	Formulation = Soluble concentrates, emulsifiable concentrate, etc.	Application rate-0.136 kg a.s. /ha	Spray dilution = 1.36 g a.s./l	Vapour pressure = low volatile substances having a vapour pressure of
Scenario	Cereals / Outdoor / Downward spraying / Vehicle-mounted			Buffer = 2-3	Number applications = 1, Application interval = 365 days
Percentage Absoprtion	Dermal for product = 25	Dermal for in use diluation = 14	Oral = 100	Inhalation = 100	
RVNAS	0.01 mg/kg bw/day		RVAAS	mg/kg bw/day	
DFR	0.63 µg a.s./cm2 per kg a.s./ha		DT50	30 days	

Table A 8: Estimation of operator exposure towards active substance (no PPE) according to EFSA guidance (0.136 kg a.s./ha)

Operator Model	Mixing, loading and application AOEM			
Potential exposure	Longer term systemic exposure mg/kg bw/day	0.1511	% of RVNAS	1510.64%
	Acute systemic exposure mg/kg bw/day	0.8911	% 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	0.0931	% of RVNAS	931.37%
	Acute systemic exposure mg/kg bw/day	0.3648	% of RVAAS	

Table A 9: Estimation of operator exposure towards active substance (PPE) according to EFSA guidance (0.136 kg a.s./ha)

Operator Model	Mixing, loading and application AOEM			
Potential exposure	Longer term systemic exposure mg/kg bw/day	0.1511	% of RVNAS	1510.64%
	Acute systemic exposure mg/kg bw/day	0.8911	% 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	0.0030	% of RVNAS	30.44%
	Acute systemic exposure mg/kg bw/day	0.0285	% of RVAAS	

A 3.2 Worker exposure calculations (KCP 7.2.3.1)

A 3.2.1 Calculations for fenpicoxamid

Table A 10: Input parameters considered for the estimation of worker exposure: Fenpicoxamid (0.075 kg a.s./ha)

Crop type	Cereals		
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	0.075	kg a.s./ha	*i_AppRate*
Number of applications	1		*i_AppNo*
Interval between multiple applications	365	days	*i_AppInt*
Half-life of active substance	30	days	*d_HalflifeAS*
Multiple application factor	1.0		*d_MAF*
Dermal absorption of the product	0.33%		*i_AbsorpProduct*
Dermal absorption of the in-use dilution	12.00%		*i_AbsorpInuse*
Dislodgeable foliar residue (i_AppRate*i_DFR)	0.225	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*

Table A 11: Estimation of worker exposure towards active substance according to EFSA guidance

	Potential exposure	Work wear - arms, body and legs covered	Working wear and gloves	Comments
Total systemic exposure (mg a.s./day)	0.6750000	0.0756000	no TC available for this assessment	
Total systemic exposure per kg body weight (mg/kg bw/day)	0.0112500	0.0012600		
% of RVNAS	22.50%	2.52%		

A 3.2.2 Calculations for prothioconazole

Table A 12: Input parameters considered for the estimation of worker exposure: Prothioconazole (0.15 kg a.s./ha)

Crop type	Cereals		
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	0.15	kg a.s./ha	*i_AppRate*
Number of applications	1		*i_AppNo*
Interval between multiple applications	365	days	*i_AppInt*
Half-life of active substance	30	days	*d_HalflifeAS*
Multiple application factor	1.0		*d_MAF*
Dermal absorption of the product	25.00%		*i_AbsorpProduct*
Dermal absorption of the in-use dilution	70.00%		*i_AbsorpInuse*
Dislodgeable foliar residue (i_AppRate*i_DFR)	0.45	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*

Table A 13: Estimation of worker exposure towards active substance according to EFSA guidance

	Potential exposure	Work wear - arms, body and legs covered	Working wear and gloves	Comments
Total systemic exposure (mg a.s./day)	7.8750000	0.8820000	no TC available for this assessment	
Total systemic exposure per kg body weight (mg/kg bw/day)	0.1312500	0.0147000		
% of RVNAS	65.63%	7.35%		

A 3.2.3 Calculations for PTZ-desthio

Table A 14: Input parameters considered for the estimation of worker exposure: PTZ-desthio (0.136 kg a.s./ha)

Crop type	Cereals		
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	0.136	kg a.s./ha	*i_AppRate*
Number of applications	1		*i_AppNo*
Interval between multiple applications	365	days	*i_AppInt*
Half-life of active substance	30	days	*d_HalflifeAS*
Multiple application factor	1.0		*d_MAF*
Dermal absorption of the product	25.00%		*i_AbsorpProduct*
Dermal absorption of the in-use dilution	14.00%		*i_AbsorpInuse*
Dislodgeable foliar residue (i_AppRate*i_DFR)	0.08568	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*

For PTZ-desthio two DFR studies have been conducted by BCS (Stuke 2013 and Stuke 2015, appendix 4.2)). Crop and substance specific DFR values (0.63 µg/cm²/kg a.s./ha) have been used for the calculation in the EFSA calculator.

Table A 15 Estimation of worker exposure towards active substance according to EFSA guidance

	Potential exposure	Work wear - arms, body and legs covered	Working wear and gloves	Comments
Total systemic exposure (mg a.s./day)	0.5355000	0.0599760	no TC available for this assessment	
Total systemic exposure per kg body weight (mg/kg bw/day)	0.0089250	0.0009996		
% of RVNAS	89.25%	10.00%		

A 3.3 Resident and bystander exposure calculations (KCP 7.2.2.1

A 3.3.1 Calculations for Fenpicoxamid

Table A 16: Input parameters considered for the estimation of longer term resident exposure (0.075 kg a.s./ha)

Croptype	Cereals		
Application method	Downward spraying		
Application equipment	Vehicle-mounted		*i_AppEquip*
Formulation type	Soluble concentrates, emulsifiable concentrate, etc.		*i_FormVal*
Buffer strip	2-3	m	*i_Buffer*
Application rate of the product	0.075	kg a.s./ha	*i_AppRate*
Concentration of active substance (in-use dilution for liquid applications)	0.75	g a.s./l	*d_ConcAS*
Dermal absorption of product	0.33%		*i_AbsorpProduct*
Dermal absorption of in-use dilution	12.00%		*i_AbsorpInuse*
Oral absorption	12.00%		*i_AbsorpOralInuse*
Dislodgeable foliar residue (i_AppRate*i_DFR)	0.225	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	0.47	ml spray dilution/person	
Resident dermal spray drift exposure 75th percentile - child	0.327	ml spray dilution/person	
Resident inhal. spray drift exposure 75th percentile - adult	0.00010	ml spray dilution/person	
Resident inhal. spray drift exposure 75th percentile - child	0.00022	ml spray dilution/person	
Resident dermal spray drift exposure mean - adult	0.22318	ml spray dilution/person	
Resident dermal spray drift exposure mean - child	0.18	ml spray dilution/person	
Resident inhal. spray drift exposure mean - adult	0.00009	ml spray dilution/person	
Resident inhal. spray drift exposure mean - child	0.00017	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)	5.60%		
Drift percentage on surface (mean)	4.10%		
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) - ad	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*

Table A 17: Estimation of resident exposure towards fenpicoxamid (EFSA Model)

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)	0.0242976	0.0107000	0.0013835	0.0151875	0.0372339
Total systemic exposure per kg body weight (mg/kg bw/day)	0.0024298	0.0010700	0.0001383	0.0015188	0.0037234
% of RVNAS	4.86%	2.14%	0.28%	3.04%	7.45%

1.2 Adult

	Spray drift	Vapour	Surface deposits	Entry into treated crops	All pathways (mean)
Total systemic exposure (mg a.s./day)	0.0347610	0.0138000	0.0036792	0.0506250	0.0733969
Total systemic exposure per kg body weight (mg/kg bw/day)	0.0005794	0.0002300	0.0000613	0.0008438	0.0012233
% of RVNAS	1.16%	0.46%	0.12%	1.69%	2.45%

Table A 18: Input parameters considered for the estimation of acute bystander exposure: Fenpicoxamid (0.075 kg a.s./ha)

Croptype	Cereals		
Application method	Downward spraying		
Application equipment	Vehicle-mounted		*i_AppEquip*
Formulation type	luble concentrates, emulsifiable concentrate, etc.		
Application rate of the product	0.075	kg a.s./ha	*i_AppRate*
Buffer strip	2-3	m	*i_Buffer*
Concentration of active substance (in-use dilution for liquid applications)	0.75	g a.s./l	*d_ConcAS*
Dermal absorption of product	0.33%		*i_AbsorpProduct*
Dermal absorption of in-use dilution	12.00%		*i_AbsorpInuse*
Oral absorption	12.00%		*i_AbsorpOralInuse*
Dislodgeable foliar residue (i_AppRate*i_DFR)	0.225	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*
Bystander dermal spray drift exposure - adult	1.21	ml spray dilution/person	
Bystander dermal spray drift exposure - child	0.74	ml spray dilution/person	
Bystander inhal. spray drift exposure - adult	0.00050	ml spray dilution/person	
Bystander inhal. spray drift exposure - child	0.00112	ml spray dilution/person	
Exposure duration	2	hours	*d_ByExpDur*
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/kg bw/day	*d_BreathRAd*
Breathing rate child (1-3 year old)	1.07	m^3/kg bw/day	*d_BreathRCh*
Drift percentage on surface (90th percentile)	8.50%		
Turf transferable residues percentage	5.00%		*d_Turf*
Transfer coeff. of surface deposits-adult	14500	cm^2/hour	*d_ByTCAd*
Transfer coeff. of surface deposits-child (1-3 year old)	5200	cm^2/hour	*d_ByTCCh*
Saliva extraction percentage	50.00%		*d_SalExt*
Surface area of hands mouthed	20	cm^2	*d_AreaHM*
Frequency of hand to mouth activity	20	events/hour	*d_ByFreqHM*
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 - a	7500	cm^2/h	*d_TcEntryAd*
Transfer coefficient for entry into treated crops - cl	2250	cm^2/h	*d_TcEntryCh*

Table A 19: Estimation of acute bystander exposure towards fenpicoxamid (EFSA Model)

1.1 1-3 year old child

	Spray drift	Vapour	Surface deposits	Entry into treated crops
Total systemic exposure (mg a.s./day)	0.0554520	0.0107000	0.0041693	0.0151875
Total systemic exposure per kg body weight (mg/kg bw/day)	0.0055452	0.0010700	0.0004169	0.0015188
% of RVAAS	2.77%	0.54%	0.21%	0.76%

1.2 Adult

	Spray drift	Vapour	Surface deposits	Entry into treated crops
Total systemic exposure (mg a.s./day)	0.0896730	0.0138000	0.0110925	0.0506250
Total systemic exposure per kg body weight (mg/kg bw/day)	0.0014946	0.0002300	0.0001849	0.0008438
% of RVAAS	0.75%	0.12%	0.09%	0.42%

A 3.3.2 Calculations for Prothioconazole

Table A 20: Input parameters considered for the estimation of longer term resident exposure: Prothioconazole (0.15 kg a.s./ha)

Croptype	Cereals		
Application method	Downward spraying		
Application equipment	Vehicle-mounted		*i_AppEquip*
Formulation type	Soluble concentrates, emulsifiable concentrate, etc.		*i_FormVal*
Buffer strip	2-3	m	*i_Buffer*
Application rate of the product	0.15	kg a.s./ha	*i_AppRate*
Concentration of active substance (in-use dilution for liquid applications)	1.5	g a.s./l	*d_ConcAS*
Dermal absorption of product	25.00%		*i_AbsorpProduct*
Dermal absorption of in-use dilution	70.00%		*i_AbsorpInuse*
Oral absorption	100.00%		*i_AbsorpOralInuse*
Dislodgeable foliar residue (i_AppRate*i_DFR)	0.45	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	0.47	ml spray dilution/person	
Resident dermal spray drift exposure 75th percentile - child	0.327	ml spray dilution/person	
Resident inhal. spray drift exposure 75th percentile - adult	0.00010	ml spray dilution/person	
Resident inhal. spray drift exposure 75th percentile - child	0.00022	ml spray dilution/person	
Resident dermal spray drift exposure mean - adult	0.22318	ml spray dilution/person	
Resident dermal spray drift exposure mean - child	0.18	ml spray dilution/person	
Resident inhal. spray drift exposure mean - adult	0.00009	ml spray dilution/person	
Resident inhal. spray drift exposure mean - child	0.00017	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)	5.60%		
Drift percentage on surface (mean)	4.10%		
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) - ad	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*

Table A 21: Estimation of resident exposure towards prothioconazole (EFSA Model)

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)	0.2818770	0.0107000	0.0165060	0.1771875	0.3192973
Total systemic exposure per kg body weight (mg/kg bw/day)	0.0281877	0.0010700	0.0016506	0.0177188	0.0319297
% of RVNAS	14.09%	0.54%	0.83%	8.86%	15.96%

1.2 Adult					
	Spray drift	Vapour	Surface deposits	Entry into treated crops	All pathways (mean)
Total systemic exposure (mg a.s./day)	0.4048200	0.0138000	0.0429240	0.5906250	0.7084445
Total systemic exposure per kg body weight (mg/kg bw/day)	0.0067470	0.0002300	0.0007154	0.0098438	0.0118074
% of RVNAS	3.37%	0.12%	0.36%	4.92%	5.90%

A 3.3.3 Calculations for PTZ-desthio

Table A 22: Input parameters considered for the estimation of longer term resident exposure: PTZ-desthio (0.136 g/ha) using crop and substance specific DFR values (0.63 µg/cm²/kg a.s./ha)

Croptype	Cereals		
Application method	Downward spraying		
Application equipment	Vehicle-mounted		*i_AppEquip*
Formulation type	Soluble concentrates, emulsifiable concentrate, etc.		*i_FormVal*
Buffer strip	2-3	m	*i_Buffer*
Application rate of the product	0.136	kg a.s./ha	*i_AppRate*
Concentration of active substance (in-use dilution for liquid applications)	1.36	g a.s./l	*d_ConcAS*
Dermal absorption of product	25.00%		*i_AbsorpProduct*
Dermal absorption of in-use dilution	14.00%		*i_AbsorpInuse*
Oral absorption	100.00%		*i_AbsorpOralInuse*
Dislodgeable foliar residue (i_AppRate*i_DFR)	0.08568	µg a.s./cm²	*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/m³	*d_AirCon*
Resident dermal spray drift exposure 75th percentile - adult	0.47	ml spray dilution/person	
Resident dermal spray drift exposure 75th percentile - child	0.327	ml spray dilution/person	
Resident inhal. spray drift exposure 75th percentile - adult	0.00010	ml spray dilution/person	
Resident inhal. spray drift exposure 75th percentile - child	0.00022	ml spray dilution/person	
Resident dermal spray drift exposure mean - adult	0.22318	ml spray dilution/person	
Resident dermal spray drift exposure mean - child	0.18	ml spray dilution/person	
Resident inhal. spray drift exposure mean - adult	0.00009	ml spray dilution/person	
Resident inhal. spray drift exposure mean - child	0.00017	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	m³/day/kg	*d_BreathRAd*
Breathing rate child (1-3 year old)	1.07	m³/day/kg	*d_BreathRCh*
Drift percentage on surface (75th percentile)	5.60%		
Drift percentage on surface (mean)	4.10%		
Turf transferable residues percentage	5.00%		*d_Turf*
Transfer coeff. of surface deposits-adult	7300	cm²/hour	*d_ReTCAd*
Transfer coeff. of surface deposits-child (1-3 year old)	2600	cm²/hour	*d_ReTCCh*
Saliva extraction percentage	50.00%		*d_SalExt*
Surface area of hands mouthed	20	cm²	*d_AreaHM*
Frequency of hand to mouth activity	9.5	events/hour	*d_ReFreqHM*
Ingestion rate for mouthing of grass per day	25	cm²	*d_MouthGrass*
Dislodgeable residues percentage transferability for object to mouth	20.00%		*d_DRP*
Transfer coefficient for entry into treated crops (75th percentile) - ad	7500	cm²/h	*d_TcEntryAd*
Transfer coefficient for entry into treated crops (75th percentile) - chi	2250	cm²/h	*d_TcEntryCh*
Transfer coefficient for entry into treated crops (mean) - adult	5980	cm²/h	*d_TcEntryAd*
Transfer coefficient for entry into treated crops (mean) - child	1794	cm²/h	*d_TcEntryCh*

For PTZ-desthio two DFR studies have been conducted by BCS (Stuke 2013 and Stuke 2015, appendix 4.2)). Crop and substance specific DFR values (0.63 µg/cm²/kg a.s./ha) have been used for the calculation in the EFSA calculator.

Table A 23: Estimation of resident exposure towards PTZ-Desthio (EFSA Model)

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)	0.0513531	0.0107000	0.0060547	0.0120488	0.0530740
Total systemic exposure per kg body weight (mg/kg bw/day)	0.0051353	0.0010700	0.0006055	0.0012049	0.0053074
% of RVNAS	51.35%	10.70%	6.05%	12.05%	53.07%
1.2 Adult					
	Spray drift	Vapour	Surface deposits	Entry into treated crops	All pathways (mean)
Total systemic exposure (mg a.s./day)	0.0735162	0.0138000	0.0138992	0.0401625	0.0909661
Total systemic exposure per kg body weight (mg/kg bw/day)	0.0012253	0.0002300	0.0002317	0.0006694	0.0015161
% of RVNAS	12.25%	2.30%	2.32%	6.69%	15.16%

A 3.4 Combined exposure calculations for fenpicoxamid and prothioconazole

No further calculations are needed. All details are provided in chapter 6.2.3 .

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)

A 4.2 DFR studies (KCA 6.10)

A 4.2.1 DFR studies for prothioconazole-desthio

Following foliar spray treatment the dislodgeable foliar residues of prothioconazole (PTZ) and its main metabolite prothioconazole-desthio (PTZ-desthio) were determined on wheat. Two studies including three supervised residue trials has been conducted: One in Southern Europe (Portugal) and two in Northern Europe (Germany, Northern France). Summaries of both studies and results are presented below.

1) DFR study on wheat, conducted in Germany with an application rate of 200 g PTZ/ha[5]

Reference	KCA 6.10/01
Report	Stuke, S.; 2013; Determination of the dislodgeable foliar residues (DFR) of prothioconazole in/on wheat after spray application of JAU 6476 & KWG 4168 EC 460 in the field in Germany, Bayer Crop Science; Lab Study No. M-455270-01-1; Company Report No. M-455270-01-1; Unpublished
Guideline(s)	US EPA OPPTS 875.2100 Foliar Dislodgeable Residue Dissipation (formerly US EPA Pesticide Assessment Guidelines Subdivision K: Reentry Protection, Series 132-1 (a))
Deviations	not specified
GLP	Yes
Acceptability	Yes
Duplication (if vertebrate study)	N/A

[5] Under evaluation in PTZ renewal process

In the study 12-2901 the magnitude of the dislodgeable foliar residues of PTZ and PTZ-desthio in washings of leaves after two spray applications with PTZ+SPX EC 460 was determined.
The study included one supervised residue trial conducted in Northern Europe (Germany) during the 2012 season.
The actual application data are presented in the following table. These data reflect the intended application scheme, or, if minor deviations occurred, these were within the acceptable range.

Table A-11. Application summary

Trial no. Country	Formulation	Appl. mode	Application						
			No. of appl.	Interval (days)	Growth stage (BBCH code)	Test item rate (L/ha)	Water rate (L/ha)	Active substance	Appl. rate (kg a.s./ha)
12-2901-01 Germany	PTZ+SPX EC 460	SPI	2	14	47 - 61	1.25	150	PTZ	0.2

Appl.: Application
SPI: Spraying

The analyses were conducted according to the following analytical method(s):

Table A-12. Summary of analytical method criteria relevant to this study

Active substance	Analytes	Method number	Limit of quantitation [µg/L]	Limit of quantitation [µg/cm^2]	Sample material	Measurement principle
PTZ	PTZ	01354/M001	5	0.005	leaf washings	HPLC-MS/MS
	PTZ-desthio					

No residues above the LOQ were found in the control samples.

Results for PTZ-desthio were neither corrected for laboratory nor for field spike recoveries.

The levels of residues of PTZ-desthio are summarised in the table A-15 together with the findings from the second field study conducted in France and Portugal.

2) DFR study on wheat, conducted in Northern France and Portugal with an application rate of 188 g PTZ/ha[6]

Reference	KCA 6.10/02
Report	Stuke, S.; 2015; Determination of the dislodgeable foliar residues (DFR) of prothioconazole and BYF 00587 in/on wheat after spraying of Bixafen & Prothioconazole EC 225 in the field in France (North) and Portugal, Bayer Crop Science; Lab Study No. 14-2907; Company Report No. M-507834-01-1; Unpublished
Guideline(s)	US EPA OPPTS 875.2100 Foliar Dislodgeable Residue Dissipation
Deviations	not specified
GLP	Yes
Acceptability	Yes
Duplication (if vertebrate study)	N/A

Material and methods

In the study 14-2907 the magnitude of the dislodgeable foliar residues of PTZ and PTZ-desthio in washings of leaves after two spray applications with BIX+PTZ EC 225 was determined.
The study included two supervised residue trial conducted in Southern Europe (Portugal) and Northern Europe (France) during the 2014 season.
The actual application data are presented in the following table. These data reflect the intended application scheme, or, if minor deviations occurred, these were within the acceptable range.

Table A-13. Application summary

Trial no. Country	Formulation	Appl. mode	Application						
			No. of appl.	Interval (days)	Growth stage (BBCH code)	Test item rate (L/ha)	Water rate (L/ha)	Active substance	Appl. rate (kg a.s./ha)
14-2907-01 France	Bixafen & Prothioconazole EC 225	SPI	1	-	47	1.25	200	PTZ	0.188
			2	14	65				
14-2907-02 Portugal	Bixafen & Prothioconazole EC 225	SPI	1	-	47	1.25	200	PTZ	0.188
			2	14	65				

Appl.: Application
SPI: Spraying

The leaf samples were taken randomised from the three topmost leaf level.
The analyses were conducted according to the following analytical method(s):

Table A-14. Summary of analytical method criteria relevant to this study

Active substance	Analytes	Method number	Limit of quantitation [µg/L]	Limit of quantitation [µg/cm^2]	Sample material	Measurement principle
PTZ	PTZ	01354/M001	10	0.01	leaf washings	HPLC-MS/MS
	PTZ-desthio					

[6] Under evaluation in PTZ renewal process

The average laboratory recoveries for PTZ were within the acceptable range of 92% – 103% with an overall average of 96%.

The overall average field spike recoveries for PTZ as sum of PTZ and PTZ-desthio (expressed as PTZ) in trial 14-2907-02 (Portugal) and in trial 14-2907-01 (France) were 50% and 57%, respectively.

The average laboratory recoveries for PTZ-desthio were within the acceptable range of 86% – 103% with an overall average of 92%.

The overall average field spike recoveries for PTZ-desthio in trial 14-2907-01 (Portugal) and in trial 14-2907-01 (France) were 81% and 83%, respectively.

No residues above the LOQ were found in the control samples.

The levels of residues of PTZ-desthio are summarised in the following Table A-45 together with the findings from the field trial conducted in Germany.

Table A-15.

Results and Discussion

In both studies average field spike recoveries for PTZ were very low and not within an acceptable range. Consequently no residue values for PTZ from both studies were taken into account for the exposure calculations of workers to PRODUCT X and are thus not presented in Table A-45, but can be found in the study reports. Instead, default tier 1 values according to EFSA (3 µg/cm²/kg a.s. applied/ha) are used to assess worker exposure.

The results from both studies for PTZ-desthio are summarised in the following Table A-15.

Table A-16. Residue summary of both DFR studies on wheat.

Trial No. Country	DALT	Residues [µg/cm²] Average values of sub-plots T1, T2 and T3		
		Germany (0.200 kg a.s./ha)	Portugal (0.1875 kg a.s./ha)	France (0.1875 kg a.s./ha)
		PTZ-desthio	PTZ-desthio	PTZ-desthio
12-2901 Germany 14-2907-01 France 14-2907-02 Portugal	-0	< 0.005	< 0.010	< 0.010
	0	0.096	0.090	0.057
	1	0.083	0.012	0.039
	2	0.012 (d3)	< 0.010	0.012
	7	< 0.005	< 0.010	< 0.010
	14/-0	< 0.005	< 0.010 (d13/-1)	< 0.010
	0	***0.116***	0.100	0.064
	1	0.067	0.046	< 0.010
	3	0.008	0.024	< 0.010
	7	< 0.005 (d8)	< 0.010 (d6)	< 0.010
	14	< 0.005	< 0.010 (d15)	< 0.010

DALT = Days after last treatment; a.s. = active substance; "-0": before the last application

After each treatment in all trials dislodgeable foliar residues of PTZ-desthio declined rapidly leading to residue values below the LOQ within 7 days after application.

Conclusion

The maximum measured average residue values in all three trials were found directly after the application in Germany and amounted to ***0.116 µg/cm²*** for PTZ-desthio. However, using a pre-cautionary approach the highest DFR value for prothioconazole-desthio from across both studies of 0.125 µg/cm² will be used to refine the worker exposure assessment. Taking into account an application rate of 0.200 kg PTZ/ha the normalised DFR value leads to **0.63 µg PTZ-desthio/cm²/kg a.s./ha.**

Appendix 5 Previous data presented to zRMS to support GF-3307 classification, but no longer relied upon

~~**Table 0-1: Justified proposals for classification and labelling for GF-3307 according to Regulation (EC) No 1272/2008**~~

~~Hazard class(es), categories~~	~~Skin irritation Cat 2 Eye irritation Cat 1 Chronic aquatic Cat 1~~
~~Hazard pictograms or Code(s) for hazard pictogram(s)~~	~~GHS05, GHS07, GHS09~~
~~Signal word~~	~~Danger~~
~~Hazard statement(s)~~	~~H315 Causes skin irritation H318 Causes serious eye damage H410 Very toxic to aquatic life with long lasting effects~~
~~Precautionary statement(s)~~	~~P261 Avoid breathing mist/vapours/spray P280 Wear protective gloves/clothing/eye/face protection P302/352 IF ON SKIN: Wash with plenty of water P305/351/338 IF IN EYES: Rinse cautiously with water for several minutes P501 Dispose of contents/container in accordance with applicable regulations~~
~~Additional labelling phrases~~	~~To avoid risks to man and the environment, comply with the instructions for use. [EUH401]~~

~~6.7 Toxicological Evaluation of Plant Protection Product~~

~~A summary of the toxicological evaluation for GF-3307 is given in the following two tables. No *in vivo* toxicology studies were conducted using GF-3307. Acute toxicity was evaluated as per the CLP 1272/2008 calculation method as well as the use of *in vitro* studies assessing dermal and ocular irritation as summarised in Table 6.3-1 & 6.3-2. 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.~~

~~A second approach using "read across" from data on a similar formulation has also been included. The formulation GF-3521 has the same coformulants and active ingredient fenpicoxamid, all at levels similar to those found in GF-3307. A major difference between the two formulations is the presence of a second active ingredient proprioconazole in GF-3521 and a second active ingredient prothioconazole in GF-3307. Detailed comparison between GF-3521 and GF-3307 is provided in dRR Part C.~~
~~Read across is also presented from data on GF-3309, a formulation similar to GF-3307. The formulation GF-3309 has the same coformulants and active ingredient fenpicoxamid, all at levels similar to those found in GF-3307. A major difference between the two formulations is the presence of a second active ingredient pyraclostrobin in GF-3309. Detailed comparison between GF-3309 and GF-3307 is provided in dRR Part C.~~

~~Full summaries of studies on the product GF-3307 as well as those conducted on GF-3521 and GF-3309 that have not been previously considered within an EU peer review process are described in detail in Appendix 2. Detailed assessments using the CLP calculation method are described in dRR Part C.~~

Table 6.7-1: Summary of evaluation of the studies on acute toxicity including irritancy and skin sensitisation for GF-3307

Type of test, species, model system (Guideline)	Result	Acceptability	Classification (acc. to the criteria in Reg. 1272/2008)	Reference
LD_{50} oral, rat (CLP Calculation)	7092 mg/kg bw	Yes / No / Supplementary	None	dRR Part C
LD_{50} dermal, rat (CLP Calculation)	12987 mg/kg bw	Yes / No / Supplementary	None	dRR Part C
LC_{50} inhalation, rat (CLP Calculation)	15.74 mg/L (mist) 129.12 mg/L (vapor)	Yes / No / Supplementary	None	dRR Part C
Skin irritation, *in vitro* EpiDerm skin model (OECD 439)	Irritant	Yes / No / Supplementary	H315 (category 2)	R. S. Settivari, and L. K. Sosinski., 2016
Eye irritation, *in vitro* Neutral Red Release Assay	Irritant	Yes / No / Supplementary	H318 (category 1)	R. S. Settivari, and N. R. Visconti, 2016
Skin sensitisation	Non-sensitising	Yes / No / Supplementary	None	dRR Part C
Supplementary studies for combinations of plant protection products	No data – not required	Yes / No / Supplementary		

Table 6.7-2: Additional toxicological information relevant for classification/labelling of GF-3307

	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)	Fenpicoxamid (50 g/L)	None	Fenpicoxamid: EFSA Journal 2018;16(1):5146	Hazard statement(s): Not applicable
Toxicological properties of active substance(s) (relevant for classification of product)	Prothioconazole	None	SANCO/3923 /07 – final 10 December 2007	Hazard statement(s): Not applicable
Toxicological properties of non-active substance(s) (relevant for classification of product)	See part C, point 1.3.2	See part C, point 1.3.2	See part C, point 1.3.2	See part C, point 1.3.2
Further toxicological information	No data – not required			

Table 6.7-3: Summary of evaluation of the studies on acute toxicity for GF-3521

Type of test, species, model system (Guideline)	Result	Acceptability	Classification (acc. to the criteria in Reg. 1272/2008)	Reference
LD_{50} oral, rat	2000>LD_{50}<5000 mg/kg bw	Yes / No / Supplementary	None	Patel, 2017a
LD_{50} dermal, rat	LD_{50} > 5000 mg/kg bw	Yes / No / Supplementary	None	Patel, 2017b
LC_{50} inhalation, rat	LD_{50} > 5.48 mg/L	Yes / No / Supplementary	None	Patel, 2017c

Skin irritation, rabbit (OECD 404)	Mild Irritant	Yes / No / Supplementary	None	Patel, 2017d
Eye irritation (OECD 405)	Irritant	Yes / No / Supplementary	Cat 2	Patel, 2017e
Skin sensitisation (Contains no classified substances)	Sensitising (based on properties of proprioconazole)	Yes / No / Supplementary	Cat 1B	Patel, 2017f
Supplementary studies for combinations of plant protection products	No data – not required			

Table 6.7-3: Summary of evaluation of the studies on acute toxicity for GF-3309

Type of test, species, model system (Guideline)	Result	Acceptability	Classification (acc. to the criteria in Reg. 1272/2008)	Reference
LD_{50} oral, rat (OECD 423)	300 < LD_{50} < 2000 mg/kg bw	Yes / No / Supplementary	Cat 4	Verma, 2018a
LD_{50} dermal, rat (OECD 402)	LD_{50} > 2000 mg/kg bw	Yes / No / Supplementary	None	Verma, 2018b
LC_{50} inhalation, rat (OECD 436)	5 < LD_{50} < 12.5 mg/L	Yes / No / Supplementary	None	Verma, 2018c
Skin irritation, Rabbit (OECD 404)	Not Irritant	Yes / No / Supplementary	None	Verma, 2018d
Eye irritation, Rabbit (OECD 405)	Irritant	Yes / No / Supplementary	Cat 2	Verma, 2018e
Skin sensitisation, Mouse (OECD 429)	Non Sensitising	Yes / No / Supplementary	None	Verma, 2018f
Supplementary studies for combinations of plant protection products	No data – not required			

A comparison of the classification outcomes between the calculation method and those from the *in vivo* studies used for read-across purposes demonstrate a broadly similar output for acute oral, dermal and inhalation endpoints. With regards to classification for skin and eye irritation, the *in vitro* studies give rise to a more conservative classification for GF-3307, in comparison to using read across data. For skin sensitisation, GF-3521 was found to be a skin sensitiser in the LLNA study. The positive result was solely based on the properties of propiconazole within the formulation as no other components within GF-3521 are sensitisers. This is confirmed by the negative result obtained with GF-3309 which is a mixture of fenpicoxamid and pyroclostrobin, both known as non skin sensitizers. Therefore GF-3307 is not proposed to be classified as skin sensitiser. The read across approach is consistent with the calculation method.

Based on both the calculation and read across methods outlined above it can be concluded that GF-3307 would have low acute oral, dermal and inhalation toxicity. It is likely that GF-3307 would be irritating to both the skin and eyes. Therefore proposed classification regarding toxicology is:

- Skin irritation Cat 2 - H315
- Eye irritation Cat 1 - H318

A 5.1 Statement on bridging possibilities

Acute oral, dermal and inhalation toxicity studies along with skin sensitisation were not performed with GF-3307. A toxicity estimate for each of these end-points was calculated using the approach defined in the Regulation EC 1272/2008. However, *in vitro* skin and eye irritation studies were performed and

detailed below.

A second approach using "read across" from data on two similar formulations has also been included. The formulations GF-3521and GF-3309 have the same coformulants and active ingredient fenpicoxamid, all at levels similar to those found in GF-3307. A difference between the formulations is the presence of a second active ingredient propiconazole in GF-3521 and pyraclostrobin for GF-3309. Detailed comparison between GF-3521/GF-3309 and GF-3307 is provided in dRR Part C.
In vivo acute toxicology data on GF-3521 and GF-3309 are presented to support the current application. These studies have been generated to support application of GF-3521 and GF-3309 in another geography where these data are requested to grant approval.

Comments of zRMS:	Comment on statement; acceptable or not

A 5.2 Acute oral toxicity (KCP 7.1.1)

Comments of zRMS:	Comment on study; acceptable or not; deficiencies, corrections, according to recent guidelines or not, used in evaluation or only as additional information

A 5.2.1 Calculation approach (Regulation EC 1272/2008)

An acute oral toxicity study with GF-3307 was not performed. Acute toxicity estimate via the oral route was calculated using the approach defined in the Regulation EC 1272/2008. Based on the acute toxicity of the individual components, the estimated oral LD50 of GF-3307 is 7092 mg/kg bw. Composition and calculation details are provided in dRR Part C.

Conclusion

The oral LD_{50} of GF-3307 is estimated to be 7092 mg/kg bw in rats. Thus, no classification is required according to Regulation (EC) No. 1272/2008.

A 5.2.2 Read across approach using data on GF-3521

Reference	KCP 7.1.1/01
Report	Patel, M. R.; 2017; Acute Oral Toxicity Study of GF-3521 in Rats; Jai Research Foundation, Valvada, Gujarat, India; Lab Study No. 401-1-01-15424; DAS Study No. 161065 ; 11 March 2017; Unpublished
Guideline(s)	Yes: OECD 423 (2001), OPPTS 870.1100 (2002), EC B.1 (2008), JMAFF 2-1-1 (2000)
Deviations	None
GLP	Yes
Acceptability	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

Test Item(s)

Test item (Common name):	GF-3521
Purity:	4.9 wt% XDE-777 AI (50 g/L); 8.0 wt% Propiconazole AI (82 g/L)
Description (physical state):	Amber- brown liquid
Lot/batch no.:	201500340-15-1 (TSN312215)
Compound stability:	Not applicable
Vehicle and/or positive control:	Not applicable

Test System

Species:	Rat (*Rattus norvegicus*)
Strain:	Wistar (RCCHan:WIST)
Age and weight at dosing:	8-10 weeks Weight (g): Minimum 144.6, maximum 169.7
Source:	Animal Breeding Facility, Jai Research Foundation
Housing:	2-3 rats/cage
Feed and water:	Feed: Teklad certified Global High Fibre Rat and Mice Feed manufactured by Envigo, U.S.A. *ad libitum* with the exception of overnight fasting and three hours post-dosing Water: UV-sterilized water filtered through Reverse Osmosis water filtration system *ad libitum*
Environmental conditions:	Temperature: 20 to 23°C Humidity: 57 to 66% relative humidity Air changes: Minimum 15 air changes/hour Photoperiod: 12 h dark/12 h light
Acclimation period:	6 to 10 days

Study Design

In-life dates

Start: 12 November 2016 End: 22 December 2016

Animal assignment and treatment

Animal assignment is shown in Table 1.

Table 1:Animal assignment

Dose (mg/Kg body weight)	Females
5000	3
2000	6

Following an overnight fast, rats were given a single dose of GF-3521 by gavage. The Test Item was a liquid end-use product and was tested undiluted (at a constant concentration) and dose volume was adjusted according to the dose and body weight to permit constant dose administration.

One female rat (set I) was given a single dose of 5000 mg GF-3521/kg body weight. As no mortality was observed, another two rats were given same dose of 5000 mg GF-3521/kg body weight. As two rats were found dead, three female rats (set II) were administered with the lower dose of 2000 mg GF-3521/kg body weight. As no mortality was observed at this dose level, a third set of three female rats (set III) was administered with same dose of 2000 mg GF-3521/kg body weight. Absence of mortality was confirmed at this dose level and, in turn, further testing was not required

Animals were observed daily and weighed weekly for 14 days. Survivors were sacrificed and a necropsy was performed in all animals.

RESULTS AND DISCUSSION

Mortality

Mortality data are presented in the table below:

Table 2:Dose, mortality/animals treated

Dose (mg/Kg body weight)	Mortality – Female Rats (# affected /total)	Time range of deaths (hours or days)
5000	2/3	1 day after dosing
2000	0/6	NA

N/A: not applicable

Two rats were found dead treated with 5000 mg GF-3521/kg body weight following dosing. No mortality was observed in rats treated with 2000 mg GF-3521/kg body weight.

Clinical Observations

The clinical sign of lethargy was observed on day 1 in the rats treated at the dose level of 5000 mg/kg body weight. No signs of toxicity were observed in rats treated at the dose level of 2000 mg/kg body weight.

Body Weight

All surviving rats treated with GF-3521 at the dose level of 5000 and 2000 mg/kg body weight showed no effect on body weight.

Necropsy Observations

External

External examination of found dead and terminally sacrificed animals did not reveal any abnormality.

Internal

Internal examination of found dead animals revealed liver congestion (Animal N° 3) and autolysis (Animal N° 2) whereas terminally sacrificed animals did not reveal any lesion. Lesions observed in the found dead rats could be correlated with the test item used in the study.

CONCLUSION

Two mortalities were observed in the rats treated at the dose level of 5000 mg GF-3521/kg body weight. No mortality was observed in the six rats treated with 2000 mg GF-3521/kg body weight.

The acute oral LD_{50} of GF-3521 in Wistar rats was found between 2000 and 5000 mg/kg body weight.

A 5.2.3 Read across approach using data on GF-3309

Reference	KCP 7.1.1/02
Report	Verma, R.; 2018; Acute Oral Toxicity Study of GF-3309 in Rats; Jai Research Foundation, Valvada, Gujarat, India; Lab Study No. 401-1-01-19441; DAS Study No. 180201; 17 August 2018; Unpublished
Guideline(s)	Yes: OECD 423 (2001), OPPTS 870.1100 (2002), EC B.1 (2008), JMAFF 2-1-1 (2000)
Deviations	None
GLP	Yes
Acceptability	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

Test Item(s)

Test item (Common name):	GF-3309
Purity:	6.2 wt% (63 g/L) Pyraclostrobin; 4.9 wt% (50 g/L) Fenpicoxamid
Description (physical state):	Amber to brown liquid
Lot/batch no.:	ENBK-166226-023-1 (TSN314593)
Vehicle:	Not applicable

Test System

Species:	Rat (*Rattus norvegicus*)
Strain:	Wistar (RCCHan:WIST)
Age and weight at dosing:	8 to 10 weeks Weight (g): Minimum 171.4, Maximum 203.5
Source:	Animal Breeding Facility, Jai Research Foundation
Housing:	1 to 3 rats/cage

Feed and water:	Feed: Teklad certified Global High Fiber Rat/Mice Feed manufactured by Envigo, U.S.A. *ad libitum* with the exception of overnight fasting and three hours post dosing Water: UV sterilized water filtered through reverse osmosis water filtration system *ad libitum*
Environmental conditions:	Temperature: 20 to 23 °C Humidity: 49 to 66% relative humidity Air changes: Minimum 15 air changes/hour Photoperiod: 12 hours dark/12 hours light
Acclimation period:	6 to 13 days

Study Design

In-life dates

Start: 10 April 2018 End: 14 May 2018

Animal assignment and treatment

Animal assignment is shown in Table 1.

Table 1:Animal assignment

Dose (mg/kg body weight)	Females
2000	3
300	6

Following an overnight fast, rats were given a single dose of GF-3309 by gavage. The test item was a liquid end-use product and was tested undiluted (at a constant concentration) and dose volume was adjusted according to the dose and body weight to permit constant dose administration.

Animals were then observed daily and weighed weekly for 14 days. Survivors were sacrificed and a necropsy was performed in all animals.

RESULTS AND DISCUSSION

Mortality

Mortality data are presented in the table below:

Table 2:Dose, mortality/animals treated

Dose (mg/kg body weight)	Mortality - Female Rats (# affected /total)	Time range of deaths (hours or days)
2000	2/3	day 0 to day 2
300	0/6	N/A

N/A: not applicable

Two mortalities were observed in the rats treated with 2000 mg GF-3309/kg body weight while no mortality was observed at 300 mg GF-3309/kg body weight.

Clinical Observations

Clinical sign like lethargy was observed in rats (rat N° 1 and 3) treated at the dose level of 2000 mg/kg body weight while no signs of toxicity were observed in any of the rats treated at the dose level of 300 mg/kg body weight, throughout the 14-day observation period.

Body Weight

Changes in body weight were considered within the expected range for this strain and age of animals and not influenced by the treatment.

Necropsy Observations

External

External examination of terminally sacrificed and found dead animals did not reveal any abnormalities.

Internal

Internal examination of found dead rats (rat N° 1 and 3) revealed liver: reddish discolouration whereas terminally sacrificed rat did not reveal any abnormalities.

CONCLUSION

Two mortalities were observed in the rats treated with 2000 mg GF-3309/kg body weight while no mortality was observed at 300 mg GF-3309/kg body weight. The acute oral LD_{50} of GF-3309 in female Wistar rats was found to be between 300 and 2000 mg/kg body weight. According to the test guideline, cut-off LD_{50} would be 1000 mg/kg body weight.

Test item	Species	Strain	Sex	Route	Method	Result
GF-3309	Rat	Wistar	F	Oral	Gavage (undiluted)	LD_{50} = between 300 and 2000 mg/kg body weight (LD_{50} cut-off value =1000 mg/kg body weight)

GHS classification

Globally Harmonized System of Classification and Labelling of Chemicals (rev. 7, GHS 2017)	Category 4

A 5.3 Acute percutaneous (dermal) toxicity (KCP 7.1.2)

Comments of zRMS:	Comment on study; acceptable or not; deficiencies, corrections, according to recent guidelines or not, used in evaluation or only as additional information

A 5.3.1 Calculation approach (Regulation EC 1272/2008

An acute dermal toxicity study with GF-3307 was not performed. Acute toxicity estimate via the dermal route was calculated using the approach defined in the Regulation EC 1272/2008. Based on the acute toxicity of the individual components, the estimated dermal LD_{50} of GF-3307 is 12897 mg/kg bw. Composition and calculation details are provided in dRR Part C.

Conclusion

The dermal LD_{50} of GF-3307 is estimated to be 12897 mg/kg bw in rats. Thus, no classification is required according to Regulation (EC) No. 1272/2008.

A 5.3.2 Read across approach using data on GF-3521

Reference	KCP 7.1.2/02
Report	Patel, M. R.; 2017; Acute Dermal Toxicity Study of GF-3521 in Rats; Jai Research Foundation, Valvada, Gujarat, India; Lab Study No. 401-1-01-15424; DAS Study No. 161066 ; 16 March 2017; Unpublished
Guideline(s)	Yes: OECD 402 (1987), OPPTS 870.1200 (1998), EC B.3 (2008), JMAFF 2-1-2 (2000)
Deviations	None
GLP	Yes
Acceptability	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

Test Item(s)

Test item (Common name):	GF-3521
Purity:	4.9 wt% XDE-777 AI (50 g/L); 8.0 wt% Propiconazole AI (82 g/L)
Description (physical state):	Amber- brown liquid
Lot/batch no.:	201500340-15-1 (TSN312215)
Compound stability:	Not applicable
Vehicle and/or positive control:	Not applicable

Test System

Species:	Rat (*Rattus norvegicus*)
Strain:	Wistar (RCCHan:WIST)
Age and weight at dosing:	8-11 weeks Weight (g): Male: Minimum 265.0, maximum 294.3; Female: Minimum 221.9, maximum 248.3
Source:	Animal Breeding Facility, Jai Research Foundation
Housing:	2 to 3 rats/cage except on the day of test item application, in which the rats were housed in individual cages following test item application up to patch removal
Feed and water:	Feed: Teklad certified Global High Fibre Rat/Mice Feed manufactured by Envigo, U.S.A. *ad libitum* Water: UV-sterilized water filtered through Reverse Osmosis water filtration system *ad libitum*
Environmental conditions:	Temperature: 20 to 23°C Humidity: 57 to 66% relative humidity Air changes: Minimum 15 air changes/hour Photoperiod: 12 h dark/12 h light
Acclimation period:	6 days

Study Design

In-life dates

Start: 12 November 2016 End: 02 December 2016

Animal assignment and treatment

Animal assignment is shown in Table 1

Table 1: Animal assignment

Dose (mg/Kg body weight)	Males	Females	Combined
5000	5	5	10

A calculated dose volume (1.08 to 1.43 mL) of GF-3521 was applied over the clipped area (approximately 7 × 5 cm area, corresponding to 10% of the body surface) of the rats and observed for a period of 14 days. The test item was held in contact with the skin using porous gauze dressing (not more than 8 ply) and a non-irritating tape (Medi tape 330 hypo-allergenic surgical tape) throughout the 24 h exposure period to prevent any loss of the test item and also to ensure that the rats did not lick or ingest it. At the end of the exposure period (24 hours), the residual test item was removed using cotton soaked in water.

Animals were then observed daily and weighed weekly for 14 days. Survivors were sacrificed and a necropsy was performed in all animals.

RESULTS AND DISCUSSION

Mortality

Mortality data are presented in the table below:

Table 2: Dose, mortality/animals treated

Dose (mg/Kg body weight)	Mortality (# affected/total)			Time range of deaths (hours)	Number with evident toxicity (# affected /total)		
	Male	Female	Combined		Male	Female	Combined
5000	0/5	0/5	0/10	N/A	0/5	0/5	0/10

N/A: not applicable

Clinical Observations

No treatment related clinical signs were observed in any of the rats treated with 5000 mg GF-3521/kg body weight.

Body Weight

Changes in body weight were considered within the expected range for this strain and age of animals and not influenced by the treatment with GF-3521/kg body weight.

Necropsy

External

External examination of terminally sacrificed male and female rats did not reveal any abnormalities of pathological significance

Internal

Visceral examination of male and female rats sacrificed at termination did not reveal any lesions.

In the absence of any pathological lesion in terminally sacrificed animals, it is concluded that the test item did not produce any treatment related effect at the dose level used in the present study.

CONCLUSION

No mortality, adverse clinical observations, effects on body weight, macroscopic external or internal abnormalities at necropsy were observed in any of the animals treated with 5000 mg GF-3521/kg body weight.

The acute dermal LD_{50} of GF-3521 in Wistar male and female rats was found to be greater than 5000 mg/kg body weight

A 5.3.3 Read across approach using data on GF-3309

Reference	KCP 7.1.2/02
Report	xxxxxxxxxxx Acute Dermal Toxicity Study of GF-3309 in Rats. xxxxxxxxxxx: 180202; August 18, 2018. Unpublished.
Guideline(s)	Yes: OECD 402 (2017)
Deviations	None
GLP	Yes
Acceptability	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

Test Item(s)

Test item (Common name):	GF-3309
Purity:	6.2 wt% (63 g/L) Pyraclostrobin; 4.9 wt% (50 g/L) Fenpicoxamid
Description (physical state):	Amber to brown liquid
Lot/batch no.:	ENBK-166226-023-1 (TSN314593)
Vehicle:	Not applicable

Test System

Species:	Rat (*Rattus norvegicus*)
Strain:	Wistar (RCCHan:WIST)
Age and weight at dosing:	11 to 13 weeks Weight (g): Female: Minimum 245.0, Maximum 261.7
Source:	Animal Breeding Facility, Jai Research Foundation

Housing:	Three rats/cage except from test item application until patch removal, when rats were housed individually.
Feed and water:	Feed: Teklad certified Global High Fiber Rat/Mice Feed manufactured by Envigo, U.S.A. *ad libitum* Water: UV sterilized water filtered through reverse osmosis water filtration system *ad libitum*
Environmental conditions:	Temperature: 20 to 23°C Humidity: 49 to 66% relative humidity Air changes: Minimum 15 air changes/hour Photoperiod: 12 hours dark/12 hours light
Acclimation period:	6 to 13 days

Study Design

In-life dates

Start: 10 April 2018 End: 07 May 2018

Animal assignment and treatment

Animal assignment is shown in Table 1

Table 1:Animal assignment

Dose (mg/kg body weight)	Females
2000	3

Before treatment, the pH of the test item was measured at JRF and found to be 5.32 (1% aqueous solution in distilled water at room temperature), which is considered acceptable for treatment.

A calculated dose volume (0.48 to 0.51 mL) of GF-3309 was applied over the clipped area (approximately 7 × 5 cm area, corresponding to 10% of the body surface) of the rats. The test item was held in contact with the skin using porous gauze dressing (not more than 8 ply) and a non-irritating tape (Medi tape 330 hypo-allergenic surgical tape) throughout the 24-hour exposure period to prevent any loss of the test item and also to ensure that the rats did not lick or ingest it. At the end of the exposure period, the residual test item was removed using cotton soaked in water.

Animals were then observed daily and weighed weekly for 14 days. Survivors were sacrificed and a necropsy was performed in all animals.

RESULTS AND DISCUSSION

Mortality

Mortality data are presented in the table below:

Table 2:Dose, mortality/animals treated

Dose (mg/kg body weight)	Mortality – Female Rats (# affected /total)	Time range of deaths (hours or days)
2000	0/3	N/A

N/A: not applicable

No mortality was observed in rats treated with 2000 mg GF-3309/kg body weight.

Clinical Observations

No treatment related clinical signs were observed in any of the rats treated with 2000 mg GF-3309/kg body weight.

No erythema and oedema were observed at 24, 48 and 72 hours post patch removal in all three rats.

Body Weight

Changes in body weight were considered within the expected range for this strain and age of animals and not influenced by the treatment with 2000 mg GF-3309/kg body weight.

Necropsy

External

External examination of terminally sacrificed female rats did not reveal any abnormalities of pathological significance.

Internal

Visceral examination of female rats sacrificed at termination did not reveal any lesions.

In the absence of any pathological lesion in terminally sacrificed animals, it is concluded that the test item did not produce any treatment related effect at the dose level used in the present study.

CONCLUSION

No mortality, adverse clinical observations, effects on body weight and macroscopic external or internal abnormalities at necropsy were observed in any of the animals treated with 2000 mg GF-3309/kg body weight.

Based on the study results, the acute dermal median lethal dose (LD_{50} value) of GF-3309 in female Wistar rats was found to be greater than 2000 mg/kg body weight.

Test item	Species	Strain	Sex	Route	Method	Result
GF-3309	Rat	Wistar	F	Dermal	Topical (24-hour semi-occlusive exposure)	LD_{50} > 2000 mg/kg body weight

GHS classification

Globally Harmonized System of Classification and Labelling of Chemicals (rev. 7, GHS 2017)	Unclassified

A 5.4 Acute inhalation toxicity (KCP 7.1.3)

Comments of zRMS:	Comment on study: acceptable or not; deficiencies, corrections, according to

	recent guidelines or not, used in evaluation or only as additional information

A 5.4.1 Calculation approach (Regulation EC 1272/2008)

An acute inhalation toxicity study with GF-3307 was not performed. Acute toxicity estimate via the inhalation route was calculated using the approach defined in the Regulation EC 1272/2008. Based on the acute toxicity of the individual components, the estimated inhalation LC_{50} of GF-3307 is 22.52 mg/L for mist and 129.87 mg/L for vapour. Composition and calculation details are provided in dRR Part C

Conclusion

The inhalation LC_{50} of GF-3307 is estimated to be 22.52 (mist) or 129.87 mg/kg bw (vapour) in rats. Thus, no classification is required according to Regulation (EC) No. 1272/2008.

A 5.4.2 Read across approach using data on GF-3521

Reference	KCP 7.1.3/01
Report	xxxxxxxxxxx; Acute Inhalation Toxicity Study of GF-3521 in Rats; xxxxxxxxxxx; 15 March 2017; Un-published
Guideline(s)	Yes: OECD 436 (2009)
Deviations	None
GLP	Yes
Acceptability	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

Test Item(s)

Test item (Common name):	GF-3521
Purity:	4.9 wt% XDE-777 AI (50 g/L); 8.0 wt% Propiconazole AI (82 g/L)
Description (physical state):	Amber brown liquid
Lot/batch no.:	201500340-15-1 (TSN312215)
Compound stability:	Not applicable
Vehicle and/or positive control:	Not applicable

Test System

Species:	Rat (*Rattus norvegicus*)
Strain:	Wistar (RCCHan:WIST)
Age and weight at dosing:	8 to 10 weeks Weight (g): Male: Minimum: 262.1, Maximum: 275.2, Female: Minimum: 202.6, Maximum: 212.3
Source:	Animal Breeding Facility, Jai Research Foundation
Housing:	3 rats/cage
Feed and water:	Feed: Teklad certified Global High Fibre Rat/Mice Feed manufactured by Envigo, U.S.A. *ad libitum*

Water: UV-sterilized water filtered through Reverse Osmosis water filtration system *ad libitum*

Environmental conditions: Temperature: 19 to 23°C
Humidity: 49 to 66% relative humidity
Air changes: Minimum 15 air changes/hour
Photoperiod: 12 h dark/12 h light

Acclimation period: 7 days

Study Design

In-life dates

Start: 14 December 2016 End: 04 January 2017

Animal assignment and treatment

Animal assignment is shown in Table 1.

Table 1: Animal assignment

Dose (mg/L air)	Males	Females	Combined
5.48	3	3	6

Rats were exposed to the test item by nose only exposure for 4 hours.

Animals were observed daily and weighed on test days 1, 3, 7 and 14. Survivors were sacrificed and a necropsy was performed in all animals.

RESULTS AND DISCUSSION

Concentration Details in the Inhalation Chamber

The time-weighted average (TWA) exposure concentration of GF-3521 in the air for rats was 5.48 mg/L. The mass median aerodynamic diameter (MMAD) of GF-3521 aerosols was determined to be 2.94 µm with an average geometric standard deviation (GSD) of 1.61

Mortality

No mortality was observed in rats exposed for 4 hours to an aerosol concentration of 5.48 mg GF-3521/L air (TWA).

Table 2: Dose, mortality/animals treated

Time-Weighted Average (TWA) Concentration (mg/L air)	Mortality (# affected/total)			Time range of deaths (hours)	Number with evident toxicity (# affected/total)		
	Male	Female	Combined		Male	Female	Combined
5.48	0/3	0/3	0/6	NA	0/3	0/3	0/6

N/A: Not applicable

Clinical Observations

No sign of toxicity was observed in any of the rats exposed to aerosol concentration of 5.48 mg GF-3521/L air (TWA).

Body Weight

A slight decrease in body weight was observed following dosing on days 1 and 3 in all animals treated at 5.48 mg/L air. Recovery occurred by day 7.

Necropsy Observations

External

External examination of terminally sacrificed rats did not reveal any abnormality

Internal

Visceral examination of terminally sacrificed rats did not reveal any abnormality.

In the absence of any pathological lesion in terminally sacrificed rats, it is concluded that the test item did not produce any treatment related effect at the dose level used in the present study

CONCLUSION

No mortality was observed in rats following nose only inhalation exposure to aerosol concentration of 5.48 mg GF-3521/L air (TWA).

Under the conditions of this study, the 4 hour acute inhalation (LC_{50}) of GF-3521 in male and female Wistar rats was found to be greater than the time-weighted average (TWA) exposure concentration of 5.48 mg GF-3521/L air.

A 5.4.3 Read across approach using data on GF-3309

Reference	KCP 7.1.4/02
Report	xxxxxxxxxxx; Acute Inhalation Toxicity Study of GF-3309 in Rats; xxxxxxxxxxx 20 August 2018; Unpublished
Guideline(s)	Yes: OECD 436 (2009)
Deviations	None
GLP	Yes
Acceptability	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

Test Item(s)

Test item (Common name):	GF-3309
Purity:	6.2 wt% (63 g/L) Pyraclostrobin; 4.9 wt% (50 g/L) Fenpicoxamid
Description (physical state):	Amber to brown liquid
Lot/batch no.:	ENBK-166226-023-1 (TSN314593)

Test System

Species:	Rat (*Rattus norvegicus*)

Strain:	Wistar (RCCHan:WIST)
Age and weight at dosing:	10 to 11 weeks Weight (g): Male: Minimum 293.8, Maximum 302.1; Female: Minimum 194.1, Maximum 198.7
Source:	Animal Breeding Facility, Jai Research Foundation
Housing:	1-3 rats/cage
Feed and water:	Feed: Teklad certified Global High Fiber Rat/Mice Feed manufactured by Envigo, U.S.A. *ad libitum* Water: UV sterilized water filtered through reverse osmosis water filtration system *ad libitum*
Environmental conditions:	Temperature: 19 to 23°C Humidity: 56 to 66% relative humidity Air changes: Minimum 15 air changes/hour Photoperiod: 12 hours dark/12 hours light
Acclimation period:	7 days

Study Design

In-life dates

Start: 28 April 2018 End: 22 May 2018

Animal assignment and treatment

Animal assignment is shown in Table 1.

Table 1:Animal assignment

Dose (mg/L air)	Males	Females	Combined
5.45	3	3	6

The rats were exposed for 4 h (nose only) followed by a 14 day post-exposure observation period during which animals were observed daily. Body weights were recorded prior to exposure on day 0 and on days 1, 3, 7 and 14 after exposure and at death. Survivors were sacrificed and a necropsy was performed in all animals.

RESULTS AND DISCUSSION

Concentration Details in the Inhalation Chamber

The time-weighted average (TWA) GF-3309 aerosol concentration in the exposure chamber was 5.45 mg/L air. The average mass median aerodynamic diameter (MMAD) of aerosolized GF-3309 was determined to be 3.27 µm with an average geometric standard deviation (GSD) of 1.55.

Mortality

Mortality data are presented in the following table.

Table 2:Dose, mortality/animals treated

Time-Weighted Average (TWA) Concentration (mg/L air)	Mortality (# affected/total)			Time range of deaths (hours)	Number with evident toxicity (# affected/total)		
	Male	Female	Combined		Male	Female	Combined

5.45	2/3	0/3	2/6	N/A	3/3	3/3	6/6

N/A: Not applicable

Two out of six rats (2 males) were found dead after exposure to a time-weighted average concentration of 5.45 mg GF-3309/L air (TWA). One animal was found dead during the exposure time (hour 4); the other at day 2 post-exposure.

Clinical Observations

All animals showed abdominal breathing during and after the 4-hour exposure. The 5 (2 male and 3 female) rats who survived the exposure demonstrated lethargy 2 hours later. The 4 (1 male and 3 female) surviving rats reverted to normal by day 2 post-exposure..

Body Weight

The surviving male rat showed a decrease in body weight on days 1 (~12%) and 3 (~8%) and exceeded initial (day 0) body weight by days 7 and 14. The three female rats showed a decrease in mean body weight on days 1 (~12%) and 3 (~6%) and exceeded their initial (day 0) body weight by days 7 and 14.

Necropsy Observations

External

External examination of found dead and terminally sacrificed rats did not reveal any abnormality of pathological significance.

Internal

Visceral examination of found dead (male) rats revealed lesions such as lungs: reddish discolouration (Animal N° 2 to 3) and liver: reddish discolouration (Animal N° 3) whereas the terminally sacrificed animals did not reveal any lesion.

Lesion observed in the found dead animals could be correlated with the test item used in the present study.

CONCLUSION

Two (males) out of six rats were found dead following nose-only inhalation exposure to an aerosol concentration of 5.45 mg GF-3309/L air (TWA).

The 4-hour acute inhalation median lethal concentration (LC_{50}) of GF-3309 in Wistar rats (male and female combined) was found to be between 5 and 12.5 mg/L air. According to the test guideline, cut-off LC_{50} would be 12.5 mg/L.

Test item	Species	Strain	Sex	Route	Method	Result
GF-3309	Rat	Wistar	M & F	Inhalation	Nose only (4 hour)	LC_{50} = between 5 and 12.5 mg/L air (cut-off value 12.5 mg/L)

GHS classification

Globally Harmonized System of Classification and Labelling of Chemicals (rev. 7, GHS 2017)	Category 5

A 5.5 Skin irritation (KCP 7.1.4)

Comments of zRMS:	Comment on study; acceptable or not; deficiencies, corrections, according to recent guidelines or not, used in evaluation or only as additional information

A 5.5.1 Study 1

Reference	KCP 7.1.4/1
Report	Settivari, R. S., and Sosinski, L. K.; 2016; GF-3307: Evaluation of the Skin Irritation Potential Using the In Vitro EpiDerm Skin Model; Toxicology and Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan, USA; Lab Study No. 140629; DAS Study No. 140101; 26 April 2016; Unpublished
Guideline(s)	Yes: OECD 439
Deviations	None
GLP	No
Acceptability	Yes
Duplication (if vertebrate study)	N/A

Materials and methods

Test Item(s)

Test item (Common name):	GF- 3307
Purity:	4.8 % w/w XDE-777 and 9.4% w/w prothioconazole
Description (physical state):	Information not included in the study report
Lot/batch no.:	F1281-135-1/TSN307579
Vehicle and/or positive control:	Not applicable

Test System

Test cells:	3-D Normal Human Epidermal Keratinocytes (NHEK)
Source:	MatTek Corporation (Ashland, Massachusetts)
Media:	MatTek Corporation
Reagents:	MatTek Corporation

Study Design

Cell culture procedures

The EpiDerm System (EPI-200) consists of normal, human-derived epidermal kerotinocytes which have been cultured to form a multilayered, highly differentiated model of the human epidermis. It consists of organized basal, spinous and granular layers, and a multilayered stratum corneum containing intercellular lamellar lipid layers arranged in patterns analogous to those found *in vivo*. The EpiDerm tissues are cultured on polycarbonate membranes of cell culture inserts (MILLICELLs, 10 mm diameter, 0.6 cm^2 surface) and shipped as kits, containing 24 tissues mounted on agarose.

Preliminary assay

NA

Definitive assays

The test is based on the principle that chemicals with irritant potential can cause cytotoxic response to the *stratum corneum* and the rate of cytotoxicity is proportional to irritation potency. In the assay, the EpiDerm tissue model was incubated with the test chemical for 60 minutes, followed by 42-hour incubation (recovery) under standard cell culture conditions. Following the post-treatment incubation period, cell viability was assessed using the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 – diphenyltetrazolium bromide) assay (Mosmann, 1983). Relative cell viability was calculated for each tissue as % of the mean of the negative control-treated tissues. A test chemical was interpreted as a potential skin irritant or non-irritant (GHS No label), when the cell viability was ≤ or > 50%, respectively (OECD 439, 2013).

Evaluation of Test Results

Data Analysis

Skin irritation potential of the test chemical was determined based on relative cell viability (corrected to negative control values), following exposure and post-exposure incubations. The mean OD_{570} value of the blank wells was calculated. Individual blank corrected OD_{570} values for each test chemical or control tissue were determined by subtracting the mean OD_{570} value of the blank wells from their individual OD_{570} values.
The mean of the corrected OD_{570} values for the negative control was calculated.

Corrected Individual Tissue OD_{570} = individual tissue OD_{570} – mean blank OD_{570}

For each individual tissue, % viability relative to negative control was calculated by taking the ratio of Corrected Individual OD570 of Test Chemical (or Control) and Corrected mean OD570 of Negative Control. The individual relative viabilities were tabulated for each tissue and the mean and standard deviations for viability values were calculated for the test chemical and control.

Acceptability criteria

The results for negative and positive controls met assay acceptance criteria, suggesting appropriate conduct of the study.
The corrected mean OD_{570} value of the negative control tissues (exposed for 60 minutes) was 2.707 (i.e. ≥ 1.00; criteria set by the tissue manufacturer).
The relative mean viability of positive control (5% SDS) was 5.5% (i.e. < 20% compared to negative control).

RESULTS AND DISCUSSION

Preliminary Assay

Not applicable

Definitive Assays

The mean relative tissue viability for EpiDerm tissues treated with GF-3307 and positive control (5% SDS) were 10.4% and 5.5%, respectively. According to the EpiDerm skin irritancy prediction model, a test substance is considered irritant to skin if the mean tissue viability is ≤ 50%. Therefore, based on the present EpiDerm study results, GF-3307 was interpreted to be an irritant to skin.

Table 3:Epiderm – results

Test article		1 Hr Treatment plus 42 Hr Recovery			Mean Viability %	Classification prediction
		Replicate 1	Replicate 2	Replicate 3		
Test material	GF-3307	9.2	9.0	12.9	10.4	Irritant
Negative control	DPBS	107.8	97.0	95.2	100.0	Non-irritant
Positive control	5% SDS	5.6	5.4	5.6	5.5	Irritant

Conclusion

According to the EpiDerm skin irritancy prediction model, a test substance is considered irritant to skin if the mean tissue viability is ≤ 50%. Therefore, based on the present EpiDerm study results, GF-3307 was interpreted to be an irritant (GHS Cat 1 or 2) to skin.

Test item	Species	Strain	Sex	Route	Method	Result
GF-3307	Human	Not applicable	Not applicable	Dermal	Epiderm - Topical	Mean relative tissue viability: 10.4 %

A 5.5.2 Study 2 Read across approach using data on GF-3521

Reference	KCP 7.1.4/2
Report	xxxxxxxxxxx Dermal Irritation Study of GF-3521 in Rabbits; xxxxxxxxxxx; DAS Study No. 161062 ; 15 March 2017; Unpublished
Guideline(s)	Yes: OECD 404 (2015), OPPTS 870.2500 (1998), EC B.4 (2008), JMAFF 2-1-4 (2000)
Deviations	None
GLP	Yes
Acceptability	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

Test Item(s)

Test item (Common name): GF-3521
Purity: 4.9 wt% XDE-777 AI (50 g/L); 8.0 wt% Propiconazole AI (82 g/L)
Description (physical state): Amber- brown liquid

Lot/batch no.:	201500340-15-1 (TSN312215)
Compound stability:	Not applicable
Vehicle and/or positive control:	Not applicable

Test System

Species:	Rabbit (*Oryctolagus cuniculus*)
Strain:	New Zealand White
Age and weight at dosing:	11 to 12 weeks old Weight (kg): Minimum 1.914, maximum 1.949
Source:	Animal breeding Facility, Jai Research Foundation
Housing:	Individually
Feed and water:	Feed: Teklad certified Global High Fibre Rabbit Feed manufactured by Envigo, U.S.A. *ad libitum* Water: UV sterilized water filtered through Reverse Osmosis water filtration system *ad libitum*
Environmental conditions:	Temperature: 19 to 22°C Humidity: 64 to 65% relative humidity Air changes: Minimum 15 air changes/hour Photoperiod: 12 h dark/12 h light
Acclimation period:	6 to 8 days

Study Design

In-life dates

Start: 22 November 2016 End: 07 December 2016

Animal assignment and treatment

The pH of GF-3521 was found to be 4.67 (1% aqueous solution in distilled water at room temperature), which is considered acceptable for treatment. .

A total of 3 rabbits were assigned to treatment. A sequential testing strategy was adopted. Initially one rabbit was tested. Immediately after administration of the test item, assessments of any initial local pain reactions were made. As severe effects were not observed in the first treated rabbit, two additional rabbits were subsequently treated in an identical manner.

A volume of 0.5 mL GF-3521 was applied evenly to one of the clipped sites of each rabbit and on the other clipped site of each rabbit remained untreated. The latter served as the control site. The treated and the control sites were covered with gauze patches of approximately 6 cm^2 (gauze rolled) which were not more than 8-ply and were secured at the margins by non-irritating tape (Medi tape 330 hypo-allergenic surgical tape) to ensure that the rabbits did not ingest the test item. At the end of the 4 hour exposure period (day 0), the residual test item was removed with cotton soaked in distilled water.

Irritation was scored by the method of Draize (as described in OECD Test Guideline no. 404) at 1, 24, 48, 72 hours and on day 7 post patch removal. General health conditions and body weights were monitored.

RESULTS AND DISCUSSION

Dermal Irritation

At 1 h post patch removal, the treated skin site revealed very slight erythema (barely perceptible) (score of 1) and very slight oedema (barely perceptible) (score of 1) in all rabbits.

At 24 h, 48 h and 72 h post patch removal, the treated skin site revealed well-defined erythema (score of 2) and very slight oedema (barely perceptible) (score of 1) in all three rabbits.

On day 7 post patch removal, treated skin site of all the three rabbits recovered completely and appeared normal. .

Individual animal irritation scores are presented in Table 1.

Table 4: Doses, scoring/animals treated

Rabbit no.	Site of treatment	Site of control	Observations after patch removal											
			Erythema						Oedema					
			Hours				Days		Hours				Days	
			1	24	48	72	7	14	1	24	48	72	7	14
1	Left	Right	1	2	2	2	0	N/A	1	1	1	1	0	N/A
2	Right	Left	1	2	2	2	0	N/A	1	1	1	1	0	N/A
3	Right	Left	1	2	2	2	0	N/A	1	1	1	1	0	N/A

Key: N/A: not applicable/available

Erythema
0: No erythema
1: Very slight erythema (barely perceptible)
2: Well-defined erythema
3: Moderate to severe erythema
4: Severe erythema (beef redness) to eschar formation preventing grading of erythema
Maximum possible: 4

Oedema
0: No oedema
1: Very slight oedema (barely perceptible)
2: Slight oedema (edges of area well defined by raising)
3: Moderate oedema (raised approximately 1 mm)
4: Severe oedema (raised more than 1 mm and extending beyond area of exposure)
Maximum possible: 4

Systemic toxicity

No signs of toxicity were recorded and all animals gained body weight throughout the study.

CONCLUSION

The mean dermal irritation scores at 24, 48 and 72 h post patch removal, for the 3 rabbits respectively, were: 2.00, 2.00, 2.00 for erythema; and 1.00, 1.00, 1.00 for oedema.

Recovery was completed in all rabbits by day 7 post patch removal.

A 5.5.3 Study 3 Read across approach using data on GF-3309

Reference	KCP 7.1.4/03
Report	xxxxxxxxxxx Acute Dermal Irritation Study of GF-3309 in Rabbits; xxxxxxxxxxx 3; 18 August 2018; Unpublished
Guideline(s)	Yes: OECD 404 (2015), OPPTS 870.2500 (1998), EC B.4 (2008), JMAFF 2-1-4 (2000)
Deviations	None
GLP	Yes
Acceptability	Yes
Duplication	No

(if vertebrate study)

MATERIALS AND METHODS

Test Item(s)

Test item (Common name):	GF-3309
Purity:	6.2 wt% (63 g/L) Pyraclostrobin; 4.9 wt% (50 g/L) Fenpicoxamid
Description (physical state):	Amber to brown liquid
Lot/batch no.:	ENBK-166226-023-1 (TSN314593)
Compound stability:	Not applicable
Vehicle and/or positive control:	Not applicable

Test System

Species:	Rabbit (*Oryctolagus cuniculus*)
Strain:	New Zealand White
Age and weight at dosing:	3.5 to 4.5 months Weight (kg): Minimum 1.871, maximum 2.245
Source:	Sainath Agencies, Hyderabad, India
Housing:	Individually
Feed and water:	Feed: Teklad certified Global High Fibre Rabbit Feed manufactured by Envigo, U.S.A. *ad libitum* Water: UV sterilized water filtered through Reverse Osmosis water filtration system *ad libitum*
Environmental conditions:	Temperature: 19 to 22 °C Humidity: 64 to 65% relative humidity Air changes: Minimum 15 air changes/hour Photoperiod: 12 h dark/12 h light
Acclimation period:	6 to 8 days

Study Design

In-life dates

Start: 11 April 2018 End: 22 April 2018

Animal assignment and treatment

Before treatment, the pH of the test item was measured at JRF and found to be 5.32 (1% solution of test item at room temperature), which is considered acceptable for treatment.

A total of 3 rabbits (3 males) were assigned to treatment. A sequential testing strategy was adopted. Initially one rabbit was tested. Immediately after administration of the test item, assessments of any initial local pain reactions were made. As severe effects were not observed in the first treated rabbit, two additional rabbits were subsequently treated in an identical manner.

A volume of 0.5 mL of GF-3309 (undiluted) was applied evenly to one of the clipped sites of each rabbit and the other clipped site of each rabbit remained untreated. The latter served as the control site. The treated and the control sites were covered with gauze patches of approximately 6 cm^2 (gauze rolled) which were not more than 8-ply and were secured at the margins by non-irritating tape (Medi tape 330 hypo-allergenic surgical tape) to ensure that the rabbits did not ingest the test item. At the end of the 4 hour exposure period (day 0), the residual test item was removed with cotton soaked in distilled water.

Irritation was scored by the method of Draize (as described in OECD Test Guideline no. 404) at 1, 24, 48, 72 hours post patch removal. General health conditions and body weights were monitored.

RESULTS AND DISCUSSION

Dermal Irritation

At 1 hour post patch removal, the treated skin site revealed very slight erythema (score of 1) in all the three rabbits.

At 24 hours post patch removal, the treated skin site of all the three rabbits recovered completely and appeared normal until the end of the 72 hours observation period.

The control skin sites of all rabbits were normal with no erythema and no oedema observed throughout the experimental period.

Individual animal irritation scores are presented in Table 1.

Table 5:Doses, scoring/animals treated

Rabbit no.	Site of treatment	Site of control	Observations after patch removal											
			Erythema						Oedema					
			Hours				Days		Hours				Days	
			1	24	48	72	7	14	1	24	48	72	7	14
1	Right	Left	1	0	0	0	N/A	N/A	0	0	0	0	N/A	N/A
2	Right	Left	1	0	0	0	N/A	N/A	0	0	0	0	N/A	N/A
3	Right	Left	1	0	0	0	N/A	N/A	0	0	0	0	N/A	N/A

Key: N/A: not applicable/available

Erythema
0: No erythema
1: Very slight erythema (barely perceptible)
2: Well-defined erythema
3: Moderate to severe erythema
4: Severe erythema (beef redness) to eschar formation preventing grading of erythema
Maximum possible: 4

Oedema
0: No oedema
1: Very slight oedema (barely perceptible)
2: Slight oedema (edges of area well-defined by raising)
3: Moderate oedema (raised approximately 1 mm)
4: Severe oedema (raised more than 1 mm and extending beyond area of exposure)
Maximum possible: 4

Systemic toxicity

No signs of toxicity were recorded and all animals gained body weight throughout the study.

CONCLUSION

In conclusion, based on these study results, GF-3309 caused a minimal dermal reaction in all the three animals, fully reversible by 24 hours post patch removal. No systemic effects were observed.

The individual animal average dermal irritation scores observed at 24, 48 and 72 hours post GF-3309 application were, for each rabbit respectively: 0.00, 0.00, 0.00 for erythema; 0.00, 0.00, 0.00 for oedema.

Test item	Species	Strain	Sex	Route	Method	Result
GF-3309	Rabbit	NZW	M	Dermal	Topical (4 hour, semi-occlusive)	Mean Erythema Scores: 0.00, 0.00, 0.00. Mean Oedema Scores: 0.00, 0.00, 0.00. Recovery completed by 24 hours.

GHS classification

Globally Harmonized System of Classification and Labelling of Chemicals (rev. 7, GHS 2017)	Unclassified

A 5.6 Eye irritation (KCP 7.1.5)

Comments of zRMS:	Comment on study: acceptable or not; deficiencies, corrections, according to recent guidelines or not, used in evaluation or only as additional information

A 5.6.1 Study 1

Reference	KCP 7.1.5/1
Report	Settivari, R. S., Sosinski, L. K.; 2016; Evaluation of the Eye Irritation Potential of GF-3307 Using the *In Vitro* Neutral Red Release Assay; Toxicology and Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan, USA; Lab Study No. 140630; DAS Study No. 140098; 12 May 2016; Unpublished
Guideline(s)	No
Deviations	None
GLP	No
Acceptability	Yes
Duplication (if vertebrate study)	N/A

Materials and methods

Test Item(s)

Test item (Common name):	GF-3307
Purity:	4.8 % w/w XDE-777 and 9.4% w/w prothioconazole
Description (physical state):	Information not included in the study report
Lot/batch no.:	F1281-135-1/TSN307579
Vehicle and/or positive control:	Dulbecco's Phosphate-Buffered Saline (containing Mg and Ca: DPBS)

Test System

Test cells:	Human Keratinocytes (HaCaT cells)
Source:	Givaudan Schweiz AG (Dubendorf, Switzerland)

Media:	Gibco
Reagents:	Sigma-Aldrich (St. Louis, MO)

Study Design

The NRR assay is a screening assay that identifies chemicals that cause immediate cytotoxic damage to cell membranes and thus may be eye irritants (Reader et al., 1989; Balls et al., 1991; Clothier, 1992). In the assay, the cells are incubated with a water soluble weak cationic dye, 3-amino-7-dimethylamino-2-methylphenazine hydrochloride (neutral red; NR), which preferentially accumulates in the lysosomes. Chemicals that cause direct damage to cell membranes lead to cytotoxicity and leakage of the intracellular contents including the NR dye. The amount of dye released from the cells will indicate the degree of membrane damage that has been caused and thus provide a measure of toxicity, i.e., eye irritation potential.

The NRR assay was conducted using the human keratinocyte cell line in a 96-well format, with triplicate wells of each test material concentration for each assay. A total of two independent assay replicates were conducted on separate days. In the assay, the HaCaT cells were preloaded with the NR dye, followed by exposure to the test material or controls for 60 ± 10 seconds. Following treatment, cells were washed up to three times to remove NR dye released from dead or damaged cells. The remaining live cells were then lysed to release dye stored in their lysosomes, as a measure of undamaged cells. The released dye was quantified by measuring optical density at 540 nm. The concentration that resulted in release of 50% (NRR50) of preloaded neutral red compared to controls was calculated for each test material.

GF-3307 was tested at eight concentrations, 0.5, 2.5, 5.0, 7.5, 10, 25, 50, and 100% (5, 25, 50, 75, 100, 250, 500 and 1000 mg/mL). XDE-777 was soluble in PBS only up to 40% and formed a non-pipetable mix above this concentration. Therefore XDE-777 was tested at the following eight concentrations: 0.5, 2.5, 5.0, 7.5, 10, 25, 30, 40% (5, 25, 50, 75, 100, 250, 300, 400 mg/mL, respectively). The test material concentration that resulted in release of 50% of preloaded NR dye (NRR50) compared to negative controls was determined. GF-3307 and XDE-777 were categorized into different ocular irritation categories following a modified ECVAM-classification scheme. Per the original, ECVAM-classification scheme, chemicals are categorized into severe category when the NRR50 is less than 250 mg/mL; moderate irritants when the NRR50 is between 250 and 600 mg/mL and mild irritants/non-irritants when NRR 50 is above 600 mg/mL. Although this classification approach served well for identifying severe irritants, it provides higher number of false positives. Therefore, the ECVAM-suggested NRR classification scheme was modified following in-house testing of more than 60 agrochemical formulations (data not shown).

According to the modified ECVAM-classification approach, test chemicals with NRR50 ≤ 5% (or 50 mg/mL) were considered severe eye irritants (GHS Cat 1); > 5 ≤ 25 % (or > 50 ≤ 250 mg/mL) as irritant (GHS Cat 2) and > 25% (or >250 mg/mL) as non-irritant category (GHS Cat NC).

Results and discussions

Preliminary Assay

Not applicable

Definitive Assays

Results for the positive control and the test materials were evaluated relative to the criteria specified in the protocol. In the two independent assay replicates, the positive control compound, SDS, was positive, and the negative control, PBS, was negative, thereby demonstrating appropriate assay conduct. The NRR results for XDE-777 suggested that the test material interfered with the readings (due to precipitation) at concentrations above 5% (50 mg/mL). Therefore, the NRR50 for XDE-777 was identified to be >50 mg/mL, and the test material was categorized as a non-severe irritant (irritant/non irritant) in the present study. These results, corroborate the *in vivo* findings to a certain degree, however, the interference of test material with readings prevented a firm conclusion on correlation between the NRR and existing *in vivo* data for XDE-777. The NRR assay demonstrated sharp decrease in NRR values for GF-3307 at the lowest concentration tested and a further dose-related decrease in NRR values were noted, with a NRR50 value of 2.6 mg/mL (derived by extrapolation of dose response data). Based on the present NRR results, GF-3307 was identified to possess severe eye irritation potential.

Table 6:NRR50 results

Test Material	NRR50	Ocular Irritancy Classification
GF-3307	2.6 mg/mL	Possess severe irritation potential to eye
XDE-777	>50 mg/mL	Non-severe irritant (Moderate/Mild irritant or Non-irritant)

Conclusion

The NRR assay identified XDE-777 as a non-severe irritant and GF-3307 to possess severe irritation potential to eye.

A 5.6.2 Study 2 Read across approach using data on GF-3521

Reference	KCP 7.1.5/2
Report	xxxxxxxxxxx; Acute Eye Irritation Study of GF-3521 in Rabbits; Jai Research Foundation, Valvada, xxxxxxxxxxx
Guideline(s)	Yes: OECD 405 (2012), OPPTS 870.2400 (1998), EC B.5 (2008), JMAFF 2-1-5 (2000)
Deviations	None
GLP	Yes
Acceptability	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

Test Item(s)

Test item (Common name):	GF-3521
Purity:	4.9 wt% XDE-777 AI (50 g/L); 8.0 wt% Propiconazole AI (82 g/L)
Description (physical state):	Amber-brown liquid
Lot/batch no.:	201500340-15-1 (TSN312215)
Compound stability:	Not applicable
Vehicle and/or positive control:	Not applicable

Test System

Species:	Rabbit (*Oryctolagus cuniculus*)
Strain:	New Zealand White
Age and weight at dosing:	14 to 17 weeks Weight (kg): Minimum 2.320, maximum 2.390
Source:	Animal Breeding Facility, Jai Research Foundation
Housing:	Individually
Feed and water:	Feed: Teklad certified Global High Fiber Rabbit Feed manufactured by Envigo, U.S.A. *ad libitum* Water: UV sterilized water filtered through Kent Reverse Osmosis water filtration system *ad libitum*
Environmental conditions:	Temperature: 19 to 22°C Humidity: 64 to 65% relative humidity Air changes: Minimum 15 air changes/hour Photoperiod: 12 h dark/12 h light
Acclimation period:	7 to 9 days

Study Design

In-life dates

Start: 22 November 2016 End: 08 December 2016

Animal assignment and treatment

The pH of GF-3521 was found to be 4.67 (1% aqueous solution in distilled water at room temperature), which is considered acceptable for treatment.

A total of 3 rabbits (3 males) were assigned to treatment. A sequential testing strategy was adopted. Initially one rabbit was tested. Immediately after administration of the test item, assessments of any initial local pain reactions were made. As severe effects were not observed in the first treated rabbit, two additional rabbits were subsequently treated in an identical manner.

On day 0, approximately 60 minutes prior to GF-3521 application, buprenorphine 0.01 mg/kg body weight was administered by subcutaneous injection (SC). Approximately 5 minutes prior to GF-3521 application, one to two drops of 0.5% proparacaine hydrochloride was applied to each eye.

A volume of 0.1 mL of GF-3521 (undiluted) was instilled in the conjunctival sac after gently pulling the lower lid away from the eyeball. Then the lids were gently held together for about one second in order to prevent loss of the test item. The contralateral (untreated) eye served as the control. In all animals, both the eyes were gently washed with 0.9% normal saline to remove residual test item at 24 h post application.

After 8 to 8.5 h of application, buprenorphine 0.01 mg/kg body weight SC and meloxicam 0.5 mg/kg body weight SC were administered to provide a continued therapeutic level of systemic analgesia. Initial 8-hour post GF-3521 application, buprenorphine 0.01 mg/kg body weight SC was administered every 12 (± 30 minutes) hours, in conjunction with meloxicam 0.5 mg/kg body weight SC every 24 (± 30 minutes) hours, until the ocular lesions resolved.

Irritation was scored by the method of Draize (as described in OECD Test Guideline no. 405) at 1, 24, 48, 72 hours and day 7. Fluorescein staining was used to assess the corneal epithelium damage at 24, 48, 72 h and on day 7 post GF-3521 application in all animals. General health conditions and body weights were monitored.

RESULTS AND DISCUSSION

Eye Irritation

At 1 h post GF-3521 application, the treated eye of all the rabbits revealed conjunctival redness (score of 1) and conjunctival chemosis (score of 1).

At 24 h post GF-3521 application, the treated eye revealed corneal opacity (score of 1) in rabbits 2 and 3; and conjunctival redness (score of 2) in all the rabbits.

At 48 and 72 h post GF-3521 application, the treated eye of all the rabbits revealed corneal opacity (score of 1), conjunctival redness (score of 2); and conjunctival chemosis (score of 1).

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Examination with fluorescein dye and cobalt blue filter post GF-3521 application revealed corneal epithelium damage (10 to 40% of surface involvement) at 24, 48 and 72 h in all three rabbits.

Individual animal irritation scores are presented in Table 1.

Table 7: Grades for ocular lesions (eye treated with the test item)

Rabbit no.	1							2							3						
Site of application	Right							Right							Right						
Reaction post application	Hour				Day			Hour				Day			Hour				Day		
	1	24	48	72	7	14	21	1	24	48	72	7	14	21	1	24	48	72	7	14	21
Conjuctivae (redness)	1	2	2	2	0	N/A	N/A	1	2	2	2	0	N/A	N/A	1	2	2	2	0	N/A	N/A
Conjuctivae (chemosis)	1	1	1	1	0	N/A	N/A	1	1	1	1	0	N/A	N/A	1	1	1	1	0	N/A	N/A
Cornea (degree of opacity)	0	0	1	1	0	N/A	N/A	0	1	1	1	0	N/A	N/A	0	1	1	1	0	N/A	N/A
Iris inflammation	0	0	0	0	0	N/A	N/A	0	0	0	0	0	N/A	N/A	0	0	0	0	0	N/A	N/A

Key: N/A: not applicable/available

Conjunctivae - Redness (refers to palpebral and bulbar conjunctivae; excluding cornea and iris)
0: Normal
1: Some blood vessels hyperaemic (injected)
2: Diffuse, crimson colour; individual vessels not easily discernible
3: Diffuse beefy red
Maximum possible: 3

Chemosis - Swelling (refers to lids and/or nictating membranes)
0: Normal
1: Some swelling above normal
2: Obvious swelling, with partial eversion of lids
3: Swelling, with lids about half closed
4: Swelling, with lids more than half closed
Maximum possible: 4

Opacity: degree of density
0: No ulceration or opacity
1: Scattered or diffuse areas of opacity (other than slight dulling of normal lustre); details of iris clearly visible
2: Easily discernible translucent area; details of iris slightly obscured
3: Nacrous area; no details of iris visible; size of pupil barely discernible
4: Opaque cornea; iris not discernible through the opacity
Maximum possible: 4

Iris
0: Normal
1: Markedly deepened rugae, congestion, swelling, moderate circumcorneal hyperaemia; or injection; iris reactive to light (a sluggish reaction is considered to be an effect
2: Hemorrhage, gross destruction, or no reaction to light
Maximum possible: 2

Systemic toxicity

No signs of toxicity were recorded and all animals gained body weight throughout the study.

CONCLUSION

The three individual animal average eye irritation scores (mean of scores observed at 24, 48 and 72 h post GF-3521 application) were: 2.00, 2.00, 2.00 for conjunctival redness; 1.00, 1.00, 1.00 for conjunctival chemosis; 0.67, 1.00, 1.00 for corneal opacity; and 0.00, 0.00, 0.00 for iris inflammation.

Recovery was completed in all animals by day 7.

A 5.6.3 Study 3 Read across approach using data on GF-3309

Reference	KCP 7.1.5/03
Report	xxxxxxxxxxx; Acute Eye Irritation Study of GF-3309 in Rabbits; xxxxxxxxxxx No. 407-1-01-19445; DAS Study No. 180204; 18 August 2018; Unpublished
Guideline(s)	Yes: OECD 405 (2012), OPPTS 870.2400 (1998), EC B.5 (2008), JMAFF 2-1-5 (2000)
Deviations	None
GLP	Yes
Acceptability	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

Test Item(s)

Test item (Common name):	GF-3309
Purity:	6.2 wt% (63 g/L) Pyraclostrobin; 4.9 wt% (50 g/L) Fenpicoxamid
Description (physical state):	Amber to brown liquid
Lot/batch no.:	ENBK-166226-023-1 (TSN314593)
Vehicle:	Not applicable

Test System

Species:	Rabbit (*Oryctolagus cuniculus*)
Strain:	New Zealand White (NZW)
Age and weight at dosing:	3.5 to 4.5 months Weight (kg): Minimum 2.060, Maximum 2.171
Source:	Sainath Agencies, Hyderabad, India
Housing:	Individually
Feed and water:	Feed: Teklad certified Global High Fiber Rabbit Feed manufactured by Envigo, U.S.A. *ad libitum* Water: UV sterilized water filtered through reverse osmosis water filtration system *ad libitum*
Environmental conditions:	Temperature: 19 to 22 °C Humidity: 64 to 65% relative humidity

	Air changes: Minimum 15 air changes/hour Photoperiod: 12 hours dark/12 hours light
Acclimation period:	7 to 9 days

Study Design

In-life dates

Start:	11 April 2018	End:	04 May 2018

Animal assignment and treatment

The pH of GF-3309 was found to be 5.32 (1% aqueous solution in distilled water at room temperature), which is considered acceptable for treatment.

A total of 3 rabbits (3 females) were assigned to treatment. A sequential testing strategy was adopted. Initially one rabbit was tested. Immediately after administration of the test item, assessments of any initial local pain reactions were made. As severe effects were not observed in the first treated rabbit, two additional rabbits were subsequently treated in an identical manner.

On day 0, approximately 60 minutes prior to the test item instillation, buprenorphine 0.01 mg/kg body weight was administered by subcutaneous injection (SC). Approximately 5 minutes prior to the test item instillation, one or two drops of 0.5% proparacaine hydrochloride was applied to each eye.

A volume of 0.1 mL of GF-3309 was instilled in the conjunctival sac after gently pulling the lower lid away from the eyeball. Then the lids were gently held together for about one second in order to prevent loss of the test item. The contralateral (untreated) eye served as the control. In all animals, both the eyes were gently washed with 0.9% normal saline at 24 hours post instillation.

Approximately 8 hours (± 30 minutes) post instillation, buprenorphine 0.01 mg/kg body weight (SC) and meloxicam 0.5 mg/kg body weight were administered both subcutaneously to provide a continued therapeutic level of systemic analgesia.

Subsequently, buprenorphine 0.01 mg/kg body weight was administered subcutaneously every 12 hours (± 30 minutes), in conjunction with meloxicam 0.5 mg/kg body weight every 24 hours (± 30 minutes), until the ocular lesions resolved.

Irritation was scored by the method of Draize (as described in OECD Test Guideline 405) at 1, 24, 48 and 72 hours and up to 14 days after GF-3309 instillation. Fluorescein staining was used to assess the corneal epithelium damage at 24, 48 and 72 hours and on days 7 and 14 after the test item instillation in all animals. General health conditions and body weights were monitored.

RESULTS AND DISCUSSION

Eye Irritation

At 1 hour post GF-3309 instillation, the treated eye of all the rabbits revealed conjunctival redness [some blood vessels definitely hyperaemic (injected); score of 1] and conjunctival chemosis [some swelling above normal (includes nictitating membranes); score of 1].

At 24, 48 and 72 h post GF-3309 application, the treated eye of all the rabbits revealed conjunctival redness [diffuse, crimson colour, individual vessels not easily discernible; score of 2], conjunctival chemosis [obvious swelling with partial eversion of lids; score of 2] and discharge [any amount different from normal (does not include small amounts observed in inner canthus of normal animals); score of 1].

On day 7 post GF-3309 application, the treated eye of all the rabbits revealed conjunctival redness [diffuse, crimson colour, individual vessels not easily discernible; score of 2], conjunctival chemosis [some swelling above normal (includes nictitating membranes); score of 1 in rabbit N° 1 to obvious swelling with partial eversion of lids; score of 2 in rabbit N° 2 and 3] and discharge [any amount different from normal (does not include small amounts observed in inner canthus of normal animals); score of 1].

On day 14 post GF-3309 instillation the treated eye of all rabbits appeared normal.

No corneal opacity and iritis reactions were observed in any of the rabbits throughout the experimental period.

Examination with fluorescein dye and cobalt blue filter was carried out post GF-3309 application in all rabbits. Rabbit N° 1 revealed 40%, 40%, 35%, 30% and 0%; rabbit N° 2 revealed 40%, 30%, 30%, 25% and 0%; rabbit N° 3 revealed 45%, 35%, 30%, 20% and 0%, corneal epithelium damage at 24, 48 and 72 h and on days 7 and 14, respectively.

Individual animal irritation scores are presented in Table 1.

Table 8:Grades for ocular lesions (eye treated with the test item)

Rabbit no.	1							2							3						
Site of application	Right							Right							Right						
Reaction post application	Hours				Days			Hours				Days			Hours				Days		
	1	24	48	72	7	14	21	1	24	48	72	7	14	21	1	24	48	72	7	14	21
Conjunctivae (redness)	1	2	2	2	2	0	N/A	1	2	2	2	2	0	N/A	1	2	2	2	2	0	N/A
Conjunctivae (chemosis)	1	2	2	2	1	0	N/A	1	2	2	2	2	0	N/A	1	2	2	2	2	0	N/A
Cornea (degree of opacity)	0	0	0	0	0	0	N/A	0	0	0	0	0	0	N/A	0	0	0	0	0	0	N/A
Iris inflammation	0	0	0	0	0	0	N/A	0	0	0	0	0	0	N/A	0	0	0	0	0	0	N/A

Key: N/A: Not applicable

Conjunctivae - Redness (refers to palpebral and bulbar conjunctivae; excluding cornea and iris)
0: Normal
1: Some blood vessels hyperaemic (injected)
2: Diffuse, crimson colour; individual vessels not easily discernible
3: Diffuse beefy red
Maximum possible: 3

Chemosis - Swelling (refers to lids and/or nictating membranes)
0: Normal
1: Some swelling above normal
2: Obvious swelling, with partial eversion of lids
3: Swelling, with lids about half closed
4: Swelling, with lids more than half closed
Maximum possible: 4

Opacity: degree of density
0: No ulceration or opacity
1: Scattered or diffuse areas of opacity (other than slight dulling of normal lustre); details of iris clearly visible
2: Easily discernible translucent area; details of iris slightly obscured
3: Nacrous area; no details of iris visible; size of pupil barely discernible
4: Opaque cornea; iris not discernible through the opacity
Maximum possible: 4

Iris
0: Normal
1: Markedly deepened rugae, congestion, swelling, moderate circumcorneal hyperaemia; or injection; iris reactive to light (a sluggish reaction is considered to be an effect
2: Hemorrhage, gross destruction, or no reaction to light
Maximum possible: 2

Systemic toxicity

No signs of toxicity were recorded and all animals gained body weight throughout the study.

CONCLUSION

GF-3309 caused conjunctival redness (scores of 1 and 2) and conjunctival chemosis (scores of 1 and 2) at 1, 24, 48 and 72 hours and on day 7 post instillation, in all rabbits, which resolved by day 14.

Examination with fluorescein dye and cobalt blue filter performed post application revealed corneal epithelium damage (20 to 45% of surface involvement) at 24, 48 and 72 h and on day 7 in all the three rabbits which resolved by day 14.

The three individual average eye irritation scores (mean of scores observed at 24, 48 and 72 hours post GF-3309 application) were, for each rabbit respectively: 0.00, 0.00, 0.00 for corneal opacity, 0.00, 0.00, 0.00 for iris inflammation, 2.00, 2.00, 2.00 for conjunctival redness, 2.00, 2.00, 2.00 for conjunctival chemosis.

Test item	Species	Strain	Sex	Route	Method	Result
GF-3309	Rabbit	NZW	F	Eye	Instillation – washing at 24 h post instillation	Mean Redness Scores: 2.00, 2.00, 2.00 Mean Chemosis Scores: 2.00, 2.00, 2.00 Mean Corneal Scores: 0.00, 0.00, 0.00 Mean Iris Scores: 0.00, 0.00, 0.00 Recovery completed by 14 days

GHS classification

Globally Harmonized System of Classification and Labelling of Chemicals (rev. 7, GHS 2017)	Category 2/2A

A 5.7 Skin sensitisation (KCP 7.1.6)

Comments of zRMS:	Comment on study; acceptable or not; deficiencies, corrections, according to recent guidelines or not, used in evaluation or only as additional information

A 5.7.1 Calculation approach (Regulation EC 1272/2008)

A skin sensitisation study with GF-3307 was not performed. Skin sensitisation potential of GF-3307 was estimated using the approach defined in the Regulation EC 1272/2008. As none of the components in GF-3307 are classified for skin sensitisation, estimation of skin sensitisation potential of GF-3307 is not applicable. Composition and calculation details are provided in dRR Part C.

Conclusion

Estimation of the skin sensitisation potential of GF-3307 is not applicable as none of the components in GF-3307 are classified for skin sensitisation. Thus, no classification is required according to Regulation (EC) No. 1272/2008.

A 5.7.2 Read across approach using data from GF-3521

Reference	KCP 7.1.6/01
Report	Patel, M. R.; 2017; Skin Sensitisation Study of GF-3521 by Local Lymph Node Assay in Mice; Jai Research Foundation, Valvada, Guja-rat, India; Lab

	Study No. 401-1-01-15424; DAS Study No. 161064 ; 15 March 2017; Unpublished
Guideline(s)	Yes: OECD 429 (2010), OPPTS 870.2600 (2003), EC B.42 (2008)
Deviations	None
GLP	Yes
Acceptability	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

Test Item(s)

Test item (Common name):	GF-3521
Purity:	4.9 wt% XDE-777 AI (50 g/L); 8.0 wt% Propiconazole AI (82 g/L)
Description (physical state):	Amber–brown liquid
Lot/batch no.:	201500340-15-1 (TSN312215)
Compound stability:	Not applicable
Vehicle and/or positive control:	Vehicle: 1% Pluronic®L-92 Surfactant; Positive control: HCA (α-hexylcinnamaldehyde) 25% (v/v) in 1% Pluronic® L-92.

Test System

Species:	Mouse *(Mus musculus)*
Strain:	CBA/J
Age and weight at dosing:	9 to 10 weeks Weight (g): Minimum 18.7, maximum 26.1
Source:	Animal Breeding Facility, Jai Research Foundation
Housing:	Animals were group-housed during acclimatisation. On the days of test item application (days 0, 1 and 2), the animals were housed in individual cages. From day 3 the animals were group-housed 5 mice/cage. On day 5 post administration of the radiolabelled material, the animals were transferred to the metabolic cages.
Feed and water:	Feed: Teklad certified Global High Fibre Rat/Mice Feed manufactured by Envigo, U.S.A. *ad libitum* Water: UV sterilized water filtered through Kent Reverse Osmosis water filtration system *ad libitum*
Environmental conditions:	Temperature: 20 to 23°C Humidity: 57 to 66% relative humidity Air changes: Minimum 15 air changes/hour Photoperiod: 12 h dark/12 h light
Acclimation period:	7 days

Study Design

In-life dates

Start: 30 November 2016 End: 21 December 2016

Preliminary test and dose selection

In a preliminary test, 4 groups of mice comprising 2 females per group were treated with GF-3521, applied at 5%, 25%, 50% and 100% (v/v) in 1% solution of Pluronic® L-92 for three consecutive days (days 0, 1 and 2). Individual clinical observations (including systemic clinical signs and scoring of irritation) were recorded daily during the experiment. Ear thickness was measured on days 0, 2 and 5. Body weight was recorded on days 0 and 5.

In the preliminary assay, no erythema was observed at the site of application at the dose concentrations of 5%, 25% and 50% (v/v) GF-3521 in 1% solution of Pluronic® L-92 while very slight erythema was observed at 100% GF-3521 in 1% solution of Pluronic® L-92. Ear thickness increase was below 25% on days 2 and 5 at the dose concentration of 5%, 25% and 50% (v/v) GF-3521 in 1% solution of Pluronic® L-92 while ear thickness increase of more than 25% was observed at 100% (undiluted) on day 5. Therefore, dose concentrations of 5%, 25% and 50% (v/v) GF-3521 in 1% solution of Pluronic® L-92 were evaluated in the main study of LLNA.

Animal assignment and treatment

In the main assay, 3 groups of female mice comprising 5 females per group were treated topically for three consecutive days (days 0, 1 and 2) on the dorsal surface of both ears (25 µL/ear) with GF-3521 at concentrations of 5%, 25% and 50% (v/v) in 1% solution of Pluronic® L-92. Female mice from the vehicle control and positive control groups were maintained in similar conditions with treatment of 1% solution of Pluronic® L-92 and 25% (v/v) of HCA in 1% solution of Pluronic® L-92, respectively.

Individual clinical observations (including systemic clinical signs and scoring of irritation) were recorded daily during the experiment. Body weight was recorded on days 0 and 5. On day 5 of treatment, all mice from each group were injected intravenously (tail vein) 250 µL of sterile phosphate buffered saline (PBS) containing approximately (20±1) µCi of tritiated methyl thymidine. On day 5, five hours (5 h) post injection of ^{3}H-methyl thymidine, the animals were euthanized and the draining auricular (local) lymph node from both ears of each animal was excised and collected into PBS. Single cell suspensions of lymph node cells from individual animals were prepared. The uptake of ^{3}H-methyl thymidine into the auricular (local) lymph nodes draining the site of chemical application was measured to assess the lymph node proliferative response.

Statistics

All the parameters characterised by continuous data such as body weight and radioactive disintegrations per minute (DPM) were subjected to Bartlett's test to meet the homogeneity of variance before conducting Analysis of Variance (ANOVA). To compare vehicle and positive control data, Student's t-test was performed to calculate significance.

RESULTS AND DISCUSSION

Clinical Observations and Irritation

No clinical signs were observed in any of the mice from the control, positive control and groups treated at 5%, 25% and 50% (v/v) GF-3521 in 1% solution of Pluronic® L-92.

No erythema was observed at the site of application of control group and at the dose levels of 5%, 25% and 50% (v/v) GF-3521 in 1% solution of Pluronic® L-92. Very slight erythema was

observed in the group treated with 25% (v/v) HCA in 1% solution of Pluronic® L-92 (during days 1 to 4) in all mice (5/5 mouse)).

Body Weight

The mean body weight of positive control as well as GF-3521 treated mice was comparable to that of the control group.

Group Mean DPM

Proliferative responses in the draining lymph nodes were monitored by measuring the incorporation of ^{3}H-methyl thymidine. These analyses revealed group mean DPM mouse values of 841.60, 1281.20, 3796.20, 5660.60 and 9652.80 for the vehicle control (1% L-92), 5%, 25% and 50% (v/v) GF-3521 in 1% solution of Pluronic® L-92 and positive control (25% v/v HCA in 1% solution of Pluronic® L-92), respectively.

A statistically significant increase in DPM was observed at 25% and 50% (v/v) GF-3521 in 1% solution of Pluronic® L-92 and 25% (v/v) HCA in 1% solution of Pluronic® L-92 when compared to control group values.

Stimulation Index (SI Value) and EC_3 Value

Stimulation Index (SI) values calculated for groups treated with GF-3521 were found to be 1.52, 4.51 and 6.73 at the dose concentrations of 5%, 25% and 50% (v/v) in 1% solution of Pluronic® L-92, respectively and 11.47 for 25% (v/v) HCA in 1% solution of Pluronic® L-92 positive control group.

Individual and group mean values are reported in Table 1.

Table 9: Dose concentration, group mean DPM value and Stimulation Index

Test Material/ Dose concentration	Animal #	Individual Animal DPM	Group Mean +/- SE (DPM)	Stimulation Index (SI)
Vehicle (1% Pluronic® L-92)	1	1336	841.60 ± 422.02	(1)
	2	1133		
	3	246		
	4	818		
	5	675		
GF-3521 5% v/v in vehicle	6	1319	1281.20 ± 391.31	1.52
	7	1258		
	8	1147		
	9	800		
	10	1882		
GF-3521 25% v/v in vehicle	11	4393	3796.20 ± 999.48††	4.51
	12	4833		
	13	2850		
	14	2606		
	15	4299		
GF-3521 50% v/v in vehicle	16	4750	5660.60 ± 1716.00††	6.73
	17	5935		
	18	8548		
	19	4496		
	20	4574		
HCA (Positive control) 25% v/v in 1% Pluronic® L-92	21	11546	9652.80 ± 2793.28††	11.47
	22	7708		
	23	10402		
	24	5910		
	25	12698		

††= Significantly higher than control ($p \leq 0.01$)

CONCLUSION

The SI obtained for GF-3521 at 25% and 50% (v/v) in 1% solution of Pluronic® L-92 concentration showed a greater than threefold increase over the control value with an EC_3 value found to be 14.90%. Therefore, GF-3521 is a dermal sensitiser.

A 5.7.3 Read across approach using data from GF-3309

Reference	KCP 7.1.6/02
Report	xxxxxxxxxxx; Skin Sensitisation Study of GF-3309 by Local Lymph Node Assay in Mice; Jai Research Foundation, xxxxxxxxxxx; 18 August 2018; Unpublished
Guideline(s)	Yes: OECD 429 (2010), OPPTS 870.2600 (2003), EC B.42 (2008)
Deviations	None
GLP	Yes

Acceptability	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

Test Item(s)

Test item (Common name):	GF-3309
Purity:	6.2 wt% (63 g/L) Pyraclostrobin; 4.9 wt% (50 g/L) Fenpicoxamid
Description (physical state):	Amber to brown liquid
Lot/batch no.:	ENBK-166226-023-1 (TSN314593)

Vehicle/Control Item(s)

Vehicle/Negative control:	1% Pluronic® L92
Positive control:	α-hexylcinnamaldehyde, 25% v/v in 1% Pluronic® L92

Test System

Species:	Mouse *(Mus musculus)*
Strain:	CBA/J
Age and weight at dosing:	10 to 12 weeks Weight (g): Minimum 19.3, Maximum 23.4
Source:	Animal Breeding Facility, Jai Research Foundation
Housing:	Group-housed during acclimatisation; individually caged on the days of test item application (days 0, 1 and 2); 5 mice/cage from day 3; 5 mice/cage in metabolic cages from day 5 (post injection of radiolabelled material)
Feed and water:	Feed: Teklad certified Global High Fiber Rat/Mice Feed manufactured by Envigo, U.S.A. *ad libitum* Water: UV sterilized water filtered through reverse osmosis water filtration system *ad libitum*
Environmental conditions:	Temperature: 20 to 23°C Humidity: 57 to 66% relative humidity Air changes: Minimum 15 air changes/hour Photoperiod: 12 hours dark/12 hours light
Acclimation period:	7 days

Study Design

In-life dates

Start:	10 April 2018	End:	23 May 2018

Formulation procedure

Procedure:	The Test Item and the Positive Control Item were freshly dissolved/suspended in the vehicle. An adjustment was not made for the purity of the Test or Positive Control Item.

Stability in the vehicle:	Unknown
Formulation analysis:	Concentration/homogeneity check not performed
Concentrations used:	see description below

Preliminary test and dose selection

In a preliminary test, 4 groups of female mice comprising 2 females per group were treated topically for three consecutive days (days 0, 1 and 2) on the dorsal surface of both ears (25 µL/ear) with GF-3309 at concentrations of 10%, 25%, 50% (v/v) in 1% Pluronic® L92 and 100% GF-3309 (undiluted).

Individual clinical observations (including systemic clinical signs and scoring of irritation) were recorded daily during the experiment. Ear thickness was measured on days 0, 2 and 5. Body weight was recorded on days 0 and 5.

In the preliminary assay, an increase of >25% in ear thickness was observed at 25%, 50% (v/v) in 1% Pluronic® L92 and 100% GF-3309 (undiluted) while an increase of <25% in ear thickness was observed at 10% (v/v) in 1% Pluronic® L92. Erythema was observed at 25% and 50% (v/v) in 1% Pluronic® L92 and 100% GF-3309 (undiluted). Therefore, dose concentrations of 2.5%, 5.0% and 10% (v/v) in 1% Pluronic® L92 were evaluated in the main study of LLNA.

Animal assignment and treatment

In the main assay, 3 groups of female mice comprising 5 females per group were treated topically for three consecutive days (days 0, 1 and 2) on the dorsal surface of both ears (25 µL/ear) with GF-3309 at concentrations of 2.5%, 5.0% and 10% (v/v) in 1% Pluronic® L92. Female mice from the vehicle control and positive control groups were maintained in similar conditions with treatment of 1% Pluronic® L92 and 25% (v/v) of HCA in 1% Pluronic® L92, respectively.

Individual clinical observations (including systemic clinical signs and scoring of irritation) were recorded daily during the experiment. Body weight was recorded on days 0 and 5. On day 5 of treatment, all mice from each group were injected intravenously (tail vein) with 250 µL of sterile phosphate buffered saline (PBS) containing approximately (20±1) µCi of tritiated methyl thymidine (^{3}H-TdR). On day 5, 5 hours post injection of ^{3}H-TdR, the animals were euthanized and the draining auricular (local) lymph node from both ears of each animal was excised and collected into PBS. Single cell suspensions of lymph node cells from individual animals were prepared. The uptake of ^{3}H-TdR into the auricular (local) lymph nodes draining the site of chemical application was measured to assess the lymph node proliferative response.

RESULTS AND DISCUSSION

Clinical Observations and Irritation

No signs of toxicity were observed in any of the mice from all groups, including controls.

No erythema was observed at the site of application at the dose levels of 2.5%, 5.0% and 10% (v/v) in 1% Pluronic® L92. In all mice treated with 25% (v/v) HCA, a local reaction consisting of erythema (score of 1) was observed from days 1 to 5.

Body Weight

No effect on body weight was observed in mice treated with GF-3309, positive control and vehicle control.

Group Mean DPM

Proliferative responses in the draining lymph nodes were monitored by measuring the incorporation of ^{3}H-methyl thymidine. These analyses revealed group mean DPM mouse values of 1637.60, 1608.80, 2508.40, 4342.80 and 9063.00 for the vehicle control (1% L92), 2.5%, 5.0% and 10.0% (v/v) in 1% Pluronic® L92 treated groups, and positive control (25% v/v HCA), respectively.

Stimulation Index (SI Value) and EC_3 Value

Stimulation Index (SI) values calculated for groups treated with GF-3309 were found to be 0.98, 1.53 and 2.65 at the dose concentrations of 2.5%, 5.0% and 10.0% (v/v) in 1% Pluronic® L92, respectively, and 5.53 for 25% (v/v) HCA positive control group.

The SI obtained for GF-3309 showed a less than threefold increase over the control value at all the tested concentrations. Therefore, EC_3 value cannot be calculated.

Individual and group mean values are reported in Table 1.

Table 10: Dose concentration, group mean DPM value and Stimulation Index

Test Material/ Dose concentration	Animal #	Individual Animal DPM	Group Mean +/- SE (DPM)	Stimulation Index (SI)*
Vehicle (1% Pluronic® L92)	1	1375	1637.60 ± 476.95	(1)
	2	2166		
	3	2028		
	4	1621		
	5	998		
2.5% (v/v) in 1% Pluronic® L92	6	967	1608.80 ± 564.87	0.98
	7	1863		
	8	2262		
	9	1065		
	10	1887		
5.0% (v/v) in 1% Pluronic® L92	11	793	2508.40 ± 1068.67	1.53
	12	3032		
	13	3666		
	14	2452		
	15	2599		
10.0% (v/v) in 1% Pluronic® L92	16	4911	4342.80 ± 627.75	2.65
	17	4647		
	18	4727		
	19	3380		
	20	4049		
HCA (Positive control) 25% (v/v) in 1% Pluronic® L92	21	5721	9063.00 ± 3545.45	5.53
	22	5885		
	23	8270		
	24	13626		
	25	11813		

CONCLUSION

The SI obtained for GF-3309 at all tested concentrations showed a less than threefold increase over the control value. Therefore, GF-3309 did not demonstrate dermal sensitisation potential in the local lymph node assay.

Test item	Species	Strain	Sex	Route	Method	Result
GF-3309	Mouse	CBA/J	F	Dermal	Topical – Local lymph node assay	Dermal non-sensitiser SI = 0.98, 1.53 and 2.65 at 2.5%, 5.0% and 10% (v/v), respectively.

GHS classification

Globally Harmonized System of Classification and Labelling of Chemicals (rev. 7, GHS 2017)	Unclassified