REGISTRATION REPORT

Part B
Section 6
Mammalian Toxicology

Detailed summary of the risk assessment

Product code: A23109A
Product name: ORONDIS VIP
Chemical active substances:
Metalaxyl-M, 174.4 g/L
Oxathiapiprolin, 30 g/L

Central Zone
Zonal Rapporteur Member State: Poland

CORE ASSESSMENT
(New authorisation)

Applicant: Syngenta
Submission date: June 2022
MS Finalisation date: March 2023 (initial Core Assessment)
November 2023 (final Core Assessment)

Version history

When	What
June 2022	Applicant submission
March 2023	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.
November 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. Not agreed or not relevant information are ~~struck through~~ and shaded for transparency. Following changes have been added: - Summary of results relating to the toxicity studies for IN-E8S72 were updated (Table 6.4 4) - Updated estimated operator exposure and worker exposure (longer term exposure) to include potential exposure (Tables 6.6-3 and 6.6-5) - Clarifications made to Table 6.6-8 and 6.6-9 to indicate the levels of risk mitigation applied - Updated table 6.3-2, Additional toxicological information relevant for classification/labelling of A23109A - Tables 6.5-1, 6.5-2 and 6.5-3 have been updated to indicate dilution ratio - Updated list of data submitted/referred by the applicant, but already evaluated at the EU peer review - Additional text to clarify skin sensitisation classification added to the conclusion of A 2.7

Table of Contents

Reviewer general comment:

This part of dossier summarizes data related to the toxicological and NDE assessment for the plant protection product ORONDIS VIP (product code A23109A a dispersible concentrate (DC) containing 174.4 g/L metalaxyl-M and 30 g/L oxathiapiprolin) which has been submitted to support registration according art. 33 of 1107/2009 in Poland also for zonal registration and interzonal for which PL was designated zRMS/izRMS. Intended use of PPP is as fungicide on field and protected vegetable crops.

ORONDIS VIP (A23109A) is a new plant protection product, which is intended to be authorized in Member States for the first time. There is no duplication of vertebrate studies and extrapolation to data of similar formulations is not possible. 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. Since to fact that A23109A is a new plant protection product there is no EU derogation allowing for these data points to be addressed by extrapolation from existing data; therefore in order to obtain approval new tests were required and the study reports are provided.
Regarding evaluation of the toxicity potential (product A23109A) submitted by the Applicant based on additivity method it must be noted that provisions of Regulation 1272/2008 indicate that the *in vivo* tests are overriding the estimation of the calculation method (ATEmix) also due to fact that mentioned below *in vitro* tests (e.g. OECD 439, is not suitable for agrochemical), ZRMS PL decided to summarize assessment of toxicological hazards for A23109A considering only available *in vivo* tests.
Discussing *in vitro* skin irritating study (Toth-Gönczöl, 2020, VV-876289) zRMS/izRMS decided not to take it into account due to the following information available in TG OECD 439 rev. 14 June 2021 INITIAL CONSIDERATIONS AND LIMITATIONS Subsection 8: p.2 (..) *data indicates a lack of applicability of the RhE based in vitro skin irritation test for agrochemical formulations* (47). (..)
See also: Kolle S.N, van Ravenzwaay B. and Landsiedel R. (2017). Regulatory accepted but out of domain: In vitro skin irritation tests for agrochemical formulations. Regul. Toxicol. Pharmacol 89, 125-130.

Therefore, considering mentioned above information's ZRMS decided to conclude assessment for the A23109A for all hazard categories taking into account *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 ORONDIS VIP (A23109A).

Notifier submit additional genotoxicity studies to evaluate (for details refer point A 2.11) clastogenic nor aneugenic potential of CGA62826 (A 2.11.1); NOA409045 (A 2.11.2); Metalaxyl-M (A 2.11.3); CGA226048(A 2.11.4). Studies outcome allow to conclude that there was no evidence of clastogenicity or aneugenicity of the tested substance.

6 Mammalian Toxicology (KCP 7)

6.1 Summary

Table 6.1-1: Information on A23109A *

Product name and code	ORONDIS VIP/A23109A
Formulation type	Dispersible concentrate (DC)
Active substance(s) (incl. content)	Metalaxyl-M (174.4 g/L) Oxathiapiprolin (30 g/L)
Function	Fungicide
Product already evaluated as the 'representative formulation'	No

during the approval of the active substance(s)	
Product previously evaluated in another MS according to Uniform Principles	No

* Information on the detailed composition of A23109A 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 A23109A according to Regulation (EC) No 1272/2008

Hazard class(es), categories	Skin sensitisation, Sub-category 1B Eye irritation, Sub-category 2
Hazard pictograms or Code(s) for hazard pictogram(s)	GHS07
Signal word	Warning
Hazard statement(s)[1]	H317 May cause an allergic skin reaction H319 Causes serious eye irritation
Precautionary statement(s)	**Prevention:** P261 Avoid breathing mist or vapours. P280 Wear protective gloves. **Response** P264 Wash thoroughly after handling P305 + P351 + P338 If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P337 + P313 If eye irritation persists: Get medical advice/ attention. P333 + P313 If skin irritation or rash occurs: Get medical advice/ attention. P362 + P364 Take off contaminated clothing and wash it before reuse. P391 Collect spillage. **Disposal:** P501 Dispose of contents/container to an approved waste disposal plant.
Additional labelling phrases	To avoid risks to man and the environment, comply with the instructions for use. [EUH401]

[1]: Differences in hazard statements may arise between the B.6 and the SDS, due to the difference in Regulation (EC) No 1272/2008 and the GHS for classification.

Table 6.1-3: Summary of risk assessment for operators, workers, residents and bystanders for A23109A

	Result	PPE / Risk mitigation measures
Operators	Acceptable	Gloves during mixing and loading, and during application for handheld manual and knapsack spray applications to low crops
Workers	Acceptable	None
Residents	Acceptable	None
Bystanders	Acceptable	None

No unacceptable risk for operators, workers, residents and bystanders was identified when the product is used as intended. Gloves are required by operators during mixing and loading, and during application for handheld manual and knapsack spray applications to low crops. Additionally, due to the inate hazard of the formulation operators are required to wear protective gloves/protective clothing/eye protection/face protection (P280).

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 (d)**	**Remarks: (e.g. safener/synergist (L/ha)) critical gap for operator, worker, resident or bystander exposure based on [Exposure model]**	**Acceptability of exposure assessment**			
			Method / Kind (incl. application technique ***	**Max. number (min. interval between applications) a) per use b) per crop/ season**	**Max. application rate kg as/ha a) Metalaxyl-M b) Oxathiapiprolin**	**Water L/ha min / max**			**Operator**	**Worker**	**Residents**	**Bystander**
PL-1	Baby leaves (BBCH 12-49)	F	Spraying, LCTM & LCHH	a) 2 b) 2	a) 0.0872 b) 0.015	200 - 800	10	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				a

* Use number(s) in accordance with the list of all intended GAPs in Part B, Section 0 should be given in column 1. Use pattern listed for Poland only, as it represents the most critical GAP in CEU. The justification of the critical use selection can be found in section 6.6.1.

** 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 (this includes hand held manual and knapsack application)

a Bystander assessment has not been undertaken as no AAOEL (termed RVAAS [Reference Value Acutely toxic Active Substance] in the EFSA model) has been set for metalaxyl-M or oxathiapiprolin

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

There is no data gap.

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)

	Metalaxyl-M	**Oxathiapiprolin**
Common Name	Metalaxyl-M	Oxathiapiprolin
CAS-No.	70630-17-0	1003318-67-9
Classification and proposed labelling		
With regard to toxicological endpoints (according to the criteria in Reg. 1272/2008, as amended)	**Hazard classes (s), categories**: Acute toxicity (Oral) Category 4 H302 Serious eye damage Category 1 H318 **Code(s) for hazard pictogram(s):** GHS05, GHS07 **Signal word:** Danger **Hazard statement(s)**: H302 Harmful if swallowed	**Hazard classes (s), categories**: n/a **Code(s) for hazard pictogram(s):** n/a **Signal word:** n/a **Hazard statement(s):** n/a **Precautionary statement(s):** n/a

	Metalaxyl-M	Oxathiapiprolin
	H318 Causes serious eye damage **Precautionary statement(s):** **Prevention:** P264 Wash skin thoroughly after handling. P270 Do not eat, drink or smoke when using this product. P280 Wear eye protection/ face protection. **Response:** P301 + P312 + P330 IF SWALLOWED: Call a POISON CENTRE/doctor if you feel unwell. Rinse mouth. P305 + P351 + P338 + P310 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTRE/doctor	
Additional C&L proposal	This substance/mixture contains no components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) at levels of 0.1% or higher.	This substance is not considered to be very persistent, bioaccumulating and toxic (PBT). This substance is not considered to be very persistent and very bioaccumulating (vPvB).
Agreed EU endpoints		
AOEL systemic	0.08 mg/kg bw/d (corrected for 100X UF, no correction for oral absorption)	0.04 mg/kg bw/d (corrected for 100X UF and 30% oral absorption)
Reference	EFSA Journal 2015;13(3):3999	EFSA Journal 2016;14(7):4504
Conditions to take into account/critical areas of concern with regard to toxicology		
According to Report/EFSA Conclusion for active substance	An issue is also listed as a critical area of concern the active substance is not expected to meet the approval criteria provided for in Article 4 of Regulation (EC) No 1107/2009. *"The technical specification is not supported by the toxicological assessment due to one relevant impurity CGA226048 that has been shown to be potentially clastogenic and that was not tested at appropriate levels in the toxicological studies."* An ongoing EU evaluation is currently being finalised by the active substance RMS Belgium under Article 7 (Application to amend the conditions of approval /Submission of documentation 17th July 2019) showing that impurity CGA226048 (2-[(2,6-dimethyl-phenyl)-(2-methoxyacetyl)-amino]-propionic acid 1-methoxycarbonyl-ethyl ester) is non-genotoxic and non-relevant and an updated RAR has been made available in May 2021 for public commenting. Studies demonstrating the lack of clastogenic potential of CGA226048 are submitted here for transparency. Based on the studies' results the maximum limit for CGA226048 of 0.18 g/kg, as currently set in the Metalaxyl-M approval regulation, can be removed as they confirm that the impurity is devoid of genotoxic potential. This area of concern has already been assessed and full	None

	Metalaxyl-M	Oxathiapiprolin
	summaries of these studies are described in detail in Appendix 2. **Reference** KCA 5.4.2, Dunton J, 2015 (VV-411540) KCA 5.4.2, Dunton J, 2017 (VV-468462)	

6.3 Toxicological Evaluation of Plant Protection Product

Acute Toxicity Estimate (ATE) calculations have been conducted and are provided in the Part C document. Syngenta has also conducted acute toxicity studies on the formulation as these studies are required for registration in other countries outside of Europe. Where classification proposals have varied between the ATE calculation approach and the animal data generated it is Syngenta's approach to base the product classification on the animal data, in accordance with CLP guidance.

Reviewer comment: Since that the provisions of Regulation 1272/2008 indicate that the *in vivo* tests are overriding the estimation of the calculation method (ATE, Additivity method) also due to fact that mentioned below *in vitro* tests (e.g. OECD 439 is not suitable for agrochemical, see our detailed comment in the Preface p.5), ZRMS PL decided to summarize assessment of toxicological hazards for A23109A considering available *in vivo* tests.

A summary of the toxicological evaluation for A23109A is given in the following tables. Full summaries of studies on the product that have not been previously considered within an EU peer review process are described in detail in Appendix 2.

Table 6.3-1: Summary of evaluation of the studies on acute toxicity including irritancy and skin sensitisation for A23109A

Type of test, species, model system (Guideline)	Result	ATE & Additivity Calculation Result	Acceptability	Classification [1] (acc. to the criteria in Reg. 1272/2008)	Reference
LD_{50} oral, rat (OECD 425)	> 2000 mg/kg bw	~~>2000 mg/kg Not classified~~	Yes	None	xxxxxxxx, 2021, VV-888460
LD_{50} dermal, rat (OECD 402)	>2000 mg/kg bw	~~>2000 mg/kg Not classified~~	Yes	None	xxxxxxxx, 2020, VV-885797
LC_{50} inhalation, rat (OECD 403)	> 5.46 mg/L air	~~> 5.0 mg/L Not classified~~	Yes	None	xxxxxxxx, 2021, VV-915563
Skin irritation, *in vitro* (OECD 439)	Non-irritant	- ~~n/a~~ Not classified	No	None	xxxxxxxx, 2020, VV-876289
Skin irritation, rabbit (OECD 404)	Non-irritant	~~n/a Not classified~~	Yes	None	xxxxxxxx, 2021, VV-904073
Eye irritation, rabbit (OECD 405)	Moderate irritant Category 2	~~Eye irritant Category 1~~	Yes	H319[1, 2]	xxxxxxxx, 2020, VV-868398
Skin sensitisation, mouse (OECD 429, LLNA)	Sensitising Category 1B	~~n/a Not classified~~	Yes	H317[1]	xxxxxxxx, 2020, VV-885070
Supplementary studies for combinations of plant protection products	No data – not required	No data – not required		-	-

[1] Proposed acute toxicity classifications are based on A23109A study results.

2: Differences in hazard statements may arise between the B.6 and the SDS, due to the difference in Regulation (EC) No 1272/2008 and the GHS for classification.

Table 6.3-2: Additional toxicological information relevant for classification/labelling of A23109A

	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)	Metalaxyl-M (ISO) (>= 10 - <20% (w/w))	Hazard statement(s) Acute tox. 4; H302 Eye Dam. 1; H318	MSDS** Reg. 1272/2008	Hazard statement ~~Skin sens. 1B; H317~~ Not applicable
	Oxathiapiprolin (>= 2.5 - <10% (w/w))	Hazard statement n/a		
Toxicological properties of non-active substance(s) (relevant for classification of product)	None	Hazard statement n/a		Hazard statement Not applicable
Further toxicological information	No data – not required			

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

6.4 Toxicological Evaluation of Groundwater Metabolites

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

6.4.1 Metalaxyl-M metabolites

6.4.1.1 NOA409045

An overview of the results of the accepted toxicological studies for groundwater metabolite NOA409045 (and the R/S racemate CGA62826) is given in the following table. Full summaries of studies on the metabolite that have not previously been considered within an EU peer review process are described in detail in Appendix 2 (A 2.11 Other/Special Studies).

Table 6.4-1: Summary of the results of toxicity studies for NOA409045

Type of test, species (Guideline)	Result	Acceptability	Reference*
Ames test [CGA62826] (OECD 471)	Non-genotoxic	Yes	Deparade, 1997 VV-353872*
Gene mutation test in chinese hamster ovary [CGA62826] (92/69/EEC B.17)	Non-genotoxic	Yes	Ogorek, 1998 VV-312522*
Gene mutation in mammalian Cells [CGA62826] (OECD 476)	Non-genotoxic	Yes	Clay, 2006 VV-337156*
Acute Oral Toxicity [CGA62826] (92/69/EEC B.1)	LD_{50} >2000 mg/kg	Yes	Winkler, 1996 VV-372576*
Acute Dermal Toxicity [CGA62826] (92/69/EEC B.3)	LD_{50} >2000 mg/kg	Yes	Winkler, 1996 VV-353936*
28 Day Oral Gavage [CGA62826] (96/54/EEC B.7)	NOAEL = 1000 mg/kg/day	Yes	Fankhauser, 1997 VV-353945*
In vitro cytogenetic test [NOA409045]	Positive - clastogenic	Yes	Bohnenberger, 2014

Type of test, species (Guideline)	Result	Acceptability	Reference*
(OECD 473)			VV-407547*
In vivo mouse micronucleus assay [CGA62826] (OECD 474)	Negative – non genotoxic	Yes	Dunton, 2014 VV-410510
In vivo mouse micronucleus assay [NOA409045] (OECD 474)	Negative – non genotoxic	Yes	Dunton, 2015 VV-28599

* indicates that a study was reviewed at EU level

6.4.1.2 SYN546520

An overview of the results of the accepted toxicological studies for groundwater metabolite SYN546520 (tested as R/S racemate CGA108906) is given in the following table. No detailed summaries are provided as the studies have already been assessed and accepted at EU level.

Table 6.4-2: Summary of the results of toxicity studies for SYN546520

Type of test, species (Guideline)	Result	Acceptability	Reference*
Ames test (OECD 471)	Non-genotoxic	Yes	Ogorek, 1997 VV-353948*
In vitro cytogenetic test (92/69/EEC B.17)	Non-genotoxic	Yes	Deparade, 1998 VV-308272*
In vitro cytogenetic test (OECD 473)	Non-genotoxic	Yes	Czich, 2001 VV-311721*
Gene mutation in mammalian cells (OECD 476)	Non-genotoxic	Yes	Clay, 2001 VV-311524*
Acute Oral Toxicity (92/69/EEC B.1)	LD_{50} >2000 mg/kg	Yes	Hartmann, 1994 VV-372832*
Acute Dermal Toxicity (92/69/EEC B.3)	LD_{50} >2000 mg/kg	Yes	Winkler, 1996 VV-353993*
28 Day Oral Gavage (96/54/EEC B.7)	NOAEL = 1000 mg/kg/day	Yes	Gerspach, 1997 VV-370343*

* indicates that a study was reviewed at EU level

6.4.1.3 CGA67868

An overview of the results of the accepted toxicological studies for groundwater metabolite CGA67868 (described as CGA92370 in the study reports) is given in the following table. No detailed summaries are provided as the studies have already been assessed and accepted at EU level.

Table 6.4-3: Summary of the results of toxicity studies for CGA67868

Type of test, species (Guideline)	Result	Acceptability	Reference*
Ames test [CGA92370] (440/2008/EC B13.14)	Non-genotoxic	Yes	Sokolowski, 2012 VV-402487*
In vitro cytogenetic test[CGA92370] (440/2008/EC B10 & OECD 473)	Non-genotoxic	Yes	Bohnenberger, 2012 VV-402488*
Gene mutation in mammalian Cells [CGA92370] (OECD 476)	Non-genotoxic	Yes	Wollny, 2012 VV-402489*

* indicates that a study was reviewed at EU level

6.4.2 Oxathiapiprolin metabolites

6.4.2.1 IN-E8S72

An overview of the results of the accepted toxicological studies for groundwater metabolite IN-E8S72 is given in the following table. The metabolite IN-E8S72 is predicted to occur in groundwater at concentrations >0.75 µg/L but <10 µg/L (see Chapter 8.8 of the dRR Part B, Section).

General information on the metabolites are provided in Table 6.4-3. The impact of the relevance assessment on whether a particular GAP use leads to acceptable risk or not is presented in the summary of the cGAP evaluation in Chapter 8.1 of the dRR Part B, Section 8 (Environmental fate and behaviour).

No detailed summaries are provided as the studies have already been assessed and accepted at EU level.

Table 6.4-4: Summary of the results of toxicity studies for IN-E8S72

Type of test, species (Guideline)	Result	Acceptability	Reference*
Ames test (OECD 471)	Non-genotoxic	Yes	DuPont-35559*
Gene mutation test in mammalian cells (OECD 476)	~~Positive~~ Non-genotoxic	Yes	DuPont-3556~~0~~1*
In vitro chromosome aberration (OECD 473)	~~Non-genotoxic~~ Positive	Yes	DuPont-3556~~1~~0*
In vivo micronucleus (OECD 474)	Non-genotoxic	Yes	DuPont-36720*
28-day feeding study in rats (OECD 407)	NOAEL 1157 mg/kg bw/day	Yes	DuPont-35562*

* indicates that a study was reviewed at EU level

The relevance of the groundwater metabolite IN-E8S72 has already been assessed and the assessment agreed at EU level (see Oxathiapiprolin, EFSA Journal 2016;14(7):4504), and the relevance assessment is applicable as well for the GAP and groundwater scenarios considered in this dRR (i.e., the conclusions reached at Step 4 and 5 of the relevance assessment made at the EU-level are valid also with regard to the PEC_{GW} calculated for the GAP and groundwater scenarios considered in this dRR). IN-E8S72 is not considered relevant according to the criteria laid down in the EC guidance document SANCO/221/2000 – rev.10. A summary of the relevance assessment is provided in Chapter 10.5 of dRR Part B, Section 10.

6.5 Dermal Absorption (KCP 7.3)

A summary of the dermal absorption rates for the active substances in A23109A are presented in the following table.

Table 6.5-1: Dermal absorption rates for active substances in A23109A

	Metalaxyl-M		Oxathiapiprolin	
	Value	Reference	Value	Reference
Concentrate (MFX 180 g/L, OXTP 30 g/ L)	5.6%	New study reported in Appendix 2	1.5%	New study reported in Appendix 2
Dilution 1 (MFX 0.09 g/L(1/2000), OXTP 0.015 g/L(1/2000))	22%	New study reported in Appendix 2	9.0%	New study reported in Appendix 2
Dilution 2 (MFX 0.047 g/L, (1/3830), OXTP 0.0079 g/L(1/3797)	20%	New study reported in Appendix 2	12%	New study reported in Appendix 2

6.5.1 Justification for proposed values - Metalaxyl-M

Proposed dermal absorption rates for metalaxyl-M are based on a dermal absorption study conducted with the current product. The study results are summarized in the following table. Full summaries of the study on the dermal absorption of metalaxyl-M/A23109A are described in detail in Appendix 2.

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

Test	Concentrate (180 g/L)	Spray dilution (0.09 g/L, 1/2000))	Spray dilution (0.047 g/L , 1/3830)	Formulation in study	Acceptability of study	Justification provided on representativity of study formulation for current product	Acceptability of justification	Reference*
In vitro (human)	5.6%	22%	20%	A23109A	Yes	Yes (see Appendix A 2.10)	Justification accepted. Endpoint can be used for current product	Blackstock and Morrison, 2021, VV-913056

* indicates that a study was reviewed at EU level

6.5.2 Justification for proposed values – Oxathiapiprolin

Proposed dermal absorption rates for oxathiapiprolin are based on a dermal absorption study conducted with the current product. The study results are summarized in the following table. Full summaries of the study on the dermal absorption of oxathiapiprolin/A23109A are described in detail in Appendix 2.

Table 6.5-3: Summary of the results of submitted dermal absorption studies for Oxathiapiprolin

Test	Concentrate (30 g/L)	Spray dilution (0.015 g/L, 1/2000)	Spray dilution (0.0079 g/L, 1/3797)	Formulation in study	Acceptability of study	Justification provided on representativity of study formulation for current product	Acceptability of justification	Reference*
In vitro (human)	1.5%	9.0%	12%	A23109A	Yes	Yes (see Appendix A 2.10)	Justification accepted. Endpoint can be used for current product	Blackstock and Morrison , 2021, VV-913056

* indicates that a study was reviewed at EU level

6.6 Exposure Assessment of Plant Protection Product (KCP 7.2)

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

Product name and code	A23109A	
Formulation type	Dispersible Concentrate (DC)	
Category	Fungicide	
Active substance(s) (incl. content)	**Metalaxyl-M** 174.4 g/L	**Oxathiapiprolin** 30 g/L
AOEL systemic	0.08 mg/kg bw/d	0.04 mg/kg bw/d
Inhalation absorption	100%	100%
Oral absorption	>80%	30%
Dermal absorption	Concentrate: 5.6% (180 g/L) Dilution (1 in 2000): 22% (0.09 g/L) Dilution (1 in 3830): 20% (0.047 g/L)	Concentrate: 1.5% (30 g/L) Dilution (1 in 2000): 9.0% (0.015 g/L) Dilution (1 in 3797): 12% (0.0079 g/L)

6.6.1 Selection of critical use(s) and justification

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

Justification

For operators, the critical GAP is dependent on the amount of product handled and the application method. A23109A is to be applied to a range of outdoor crops using tractor mounted boom sprayers and hand-held sprayers. A critical GAP has been defined for these application methods based on the highest amount of active substance applied.

For outdoor crops the application rate using tractor mounted boom sprayer and hand-held equipment is 0.0872 kg metalaxyl-M /ha and 0.015 kg oxathiapiprolin/ha.

Worker exposure is defined by the task being undertaken and the amount of active substance that is available to be dislodged. Where multiple applications are made, the minimum application interval should be considered. This represents the worst case because previous applications will have less time to dissipate before worker re-entry occurs. According to the EFSA guidance[1], crops that are similar will have similar work tasks undertaken and therefore will result in similar exposures.

The critical GAP for bystanders and residents depends on the method of application, the amount of active substance applied, the spray volume, the number of applications and the interval between applications.

The methods of application with the greatest potential for spray drift are tractor mounted boom spraying. The application rate for all crops treated with a tractor mounted boom sprayer is 0.0872 kg metalaxyl-M /ha and 0.015 kg oxathiapiprolin/ha.

[1] EFSA (European Food Safety Authority), 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 doi: 10.2903/j.efsa.2014.3874

6.6.2 Operator exposure (KCP 7.2.1)

6.6.2.1 Estimation of operator exposure

Comments of zRMS:	NDE calculations performed by the applicant are acceptable and zRMS agrees to the conclusions. The risk for operators is acceptable under conditions of intended uses and considering below mentioned risk mitigation measures such as Work wear (arms, body and legs covered) during M, L and A in the case of tractor boom spray application; while hand-held application is without risk when Work wear (arms, body and legs covered) + gloves M/L and A while Hand-held spray application is without risk when Work wear (arms, body and legs covered) + gloves M/L and A are using.

A summary of the exposure models used for estimation of operator exposure to the active substances during application of A23109A according to the critical use(s) is presented in Table 6.6-2. The outcome of the estimation is presented in and
Table 6.6-3 (longer term exposure). Detailed calculations are in A 2.11.1.

At this time, no acute AOELs have been set for metalaxyl-M and oxathiapiprolin. Consequently, no acute risk assessment has been provided for these active substances.

Table 6.6-2: Exposure models for intended uses

Critical use	Baby leaves (max. 0.5 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 (longer term exposure)

		Metalaxyl-M		Oxathiapiprolin	
Model data	**Level of PPE**	**Total absorbed dose (mg/kg/day)**	**% of systemic AOEL**	**Total absorbed dose (mg/kg/day)**	**% of systemic AOEL**
Tractor mounted boom spray application outdoors to low crops					
Application rate		2 x 0.0872 kg a.s./ha		2 x 0.015 kg a.s./ha	
Spray application (AOEM; 75th percentile) Body weight: 60 kg	Potential exposure	0.0276	34.45	0.0021	5.34
	Work wear (arms, body and legs covered) M/L and A	0.0170	21.22	0.0013	3.23
Hand-held (manual) spray application outdoors to low crops					
Application rate		2 x 0.0872 kg a.s./ha		2 x 0.015 kg a.s./ha	
Spray application (AOEM; 75th percentile) Body weight: 60 kg	Potential exposure	0.3357	419.57	0.1816	453.93
	Work wear (arms, body and legs covered) + gloves M/L and A	0.0332	41.53	0.0183	45.76
Hand-held (knapsack) spray application outdoors to low crops					
Application rate		2 x 0.0872 kg a.s./ha		2 x 0.015 kg a.s./ha	
Spray application (AOEM; 75th	Potential exposure	0.3420	427.53	0.1843	460.68
	Work wear (arms,	0.0336	42.00	0.0187	46.76

		Metalaxyl-M		Oxathiapiprolin	
Model data	**Level of PPE**	**Total absorbed dose (mg/kg/day)**	**% of systemic AOEL**	**Total absorbed dose (mg/kg/day)**	**% of systemic AOEL**
percentile) Body weight: 60 kg	body and legs covered) + gloves M/L and A				

6.6.2.2 Measurement of operator exposure

Since the operator exposure estimations carried out indicated that the acceptable operator exposure level (AOEL) for metalaxyl-M and oxathiapiprolin will not be exceeded under conditions of intended uses and consideration of the above mentioned personal protective equipment (PPE). The intended use of PPE is to account for the combined exposure (Section 6.6.5). 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)

6.6.3.1 Estimation of worker exposure

Comments of zRMS:	NDE calculations performed by the applicant are acceptable and zRMS agrees to the conclusions. Exposure for workers (entry into a previously treated area or handling a crop according to the critical uses) is acceptable under conditions of intended uses considering below mentioned risk mitigation measures such as Work wear, (arms, body and legs covered) but no PPE is used.

Table 6.6-4 shows the exposure model(s) used for estimation of worker exposure after entry into a previously treated area or handling a crop treated with A23109A according to the critical use(s). Outcome of the estimation is presented in
Table 6.**6-5** (longer term exposure). Detailed calculations are in A 2.11.1.

At this time, no acute AOELs have been set for metalaxyl-M and oxathiapiprolin and as there is no available model within the EFSA re-entry model for assessing acute exposure, an acute risk assessment for re-entry workers has not been performed.

Table 6.6-4: Exposure models for intended uses

Critical use	Baby leaves (max. 0.5 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 6.6-5: Estimated worker exposure (longer term exposure)

		Metalaxyl-M		Oxathiapiprolin	
Model data	Level of PPE	Total absorbed dose (mg/kg bw/day)	% of systemic AOEL	Total absorbed dose (mg/kg bw/day)	% of systemic AOEL
Reaching, picking Outdoor Work rate: 8 hours/day, DT50: 30 days DFR: 3 µg/cm2/kg a.s./ha Interval between treatments: 7 days					
Number of applications and application rate		2 x 0.0872 kg a.s./ha		2 x 0.015 kg a.s./ha	

Body weight: 60 kg	Potential exposure	0.0824	102.96	0.0077	19.32
	Work wear (arms, body and legs covered) and gloves TC: 580 cm^2/person/h	0.0082	10.30	0.0008	1.93

6.6.3.2 Refinement of generic DFR value (KCP 7.2)

Since the worker exposure estimations carried out indicated that the acceptable operator exposure level (AOEL) will not be exceeded under conditions of intended uses and considering above mention PPE, a refinement of the generic DFR value was not necessary and was therefore not performed.

6.6.3.3 Measurement of worker exposure

Since the worker exposure estimations carried out indicated that the acceptable operator exposure level (AOEL) for metalaxyl-M and oxathiapiprolin will not be exceeded 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)

6.6.4.1 Estimation of resident and bystander exposure

Comments of zRMS:	Justification of waiving acute risk assessment discussed by the applicant is reliable thus, zRMS agrees to the conclusions. Risk for bystanders and residents is acceptable under conditions of intended uses.

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.
Table 6.6-6 shows the exposure models used for estimation of resident and bystander exposure to metalaxyl-M and oxathiapiprolin. The outcome of the estimations is presented in
Table 6.6-7 (longer term resident exposure). Detailed calculations are in A 2.11.1. At this time, no acute AOELs have been set for metalaxyl-M and oxathiapiprolin. Consequently, no acute risk assessment has been provided for these active substances.

Table 6.6-6: Exposure models for intended uses

Critical use	Baby leaves (max. 0.5 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 6.6-7: Estimated resident exposure (longer term exposure)

		Metalaxyl-M		**Oxathiapiprolin**	
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: 3 µg/cm²/kg a.s./ha Interval between treatments: 7 days Minimum water volume for application: 200 L/ha					
Number of applications and application rate		2 x 0.0872 kg a.s./ha		2 x 0.015 kg a.s./ha	
Resident child Body weight: 10 kg	Drift (75th perc.)	0.0026	3.23	0.0002	0.61
	Vapour (75th perc.)	0.0011	1.34	0.0011	2.68
	Deposits (75th perc.)	0.0006	0.81	0.0001	0.14
	Re-entry (75th perc.)	0.0060	7.49	0.0006	1.41
	Sum (mean)	0.0077	9.68	0.0017	4.23
Resident adult Body weight: 60 kg	Drift (75th perc.)	0.0006	0.77	0.0001	0.14
	Vapour (75th perc.)	0.0002	0.29	0.0002	0.58
	Deposits (75th perc.)	0.0002	0.30	0.0000	0.06
	Re-entry (75th perc.)	0.0033	4.16	0.0003	0.78
	Sum (mean)	0.0034	4.19	0.0005	1.31

6.6.4.2 Measurement of resident and/or bystander exposure

Since the resident and/or bystander exposure estimations carried out indicated that the acceptable operator exposure level (AOEL) for metalaxyl-M and oxathiapiprolin will not be exceeded under conditions of intended uses and considering above mentioned risk mitigation measures, a study to provide measurements of resident/bystander exposure was not necessary and was therefore not performed.

6.6.5 Combined exposure

6.6.5.1 Exposure assessment of metalaxyl-M and oxathiapiprolin in A23109A

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.

Table 6.6-8: Risk assessment from combined exposure (longer term exposure)

Application scenario	Active ingredient	Estimated exposure / AOEL (HQ)
Operators – tractor mounted boom spray application to low crops; using workwear only	Metalaxyl-M	0.2122
	Oxathiapiprolin	0.0323
	Cumulative risk operators (HI)	**0.2445**
Operators – handheld manual spray application to low crops; using workwear only	Metalaxyl-M	0.5109
	Oxathiapiprolin	0.5380
	Cumulative risk operators (HI)	**1.0489**
Operators – handheld knapsack spray application to low crops; using workwear only	Metalaxyl-M	0.6011
	Oxathiapiprolin	0.6037
	Cumulative risk operators (HI)	**1.2048**
	Metalaxyl-M	0.1030

Application scenario	Active ingredient	Estimated exposure / AOEL (HQ)
Workers – reaching and picking; using workwear and gloves	Oxathiapiprolin	0.0193
	Cumulative risk workers (HI)	**0.1223**
Resident - child Buffer zone: 2-3(m) Drift reduction technology: No DT_{50}: 30 days DFR: 3 µg/cm²/kg a.s./ha Interval between treatments: 7 days Minimum water volume for application: 200 L/ha	Metalaxyl-M	
	Drift	0.0323
	Vapour	0.0134
	Deposits	0.0081
	Re-entry	0.0749
	Sum of all pathways	0.0968
	Oxathiapiprolin	
	Drift	0.0061
	Vapour	0.0268
	Deposits	0.0014
	Re-entry	0.0141
	Sum of all pathways	0.0423
	Cumulative risk resident – child (HI)	
	Drift	0.0384
	Vapour	0.0402
	Deposits	0.0095
	Re-entry	0.0890
	Sum of all pathways	0.1391
Resident – adult Buffer zone: 2-3(m) Drift reduction technology: No DT_{50}: 30 days DFR: 3 µg/cm²/kg a.s./ha Interval between treatments: 7 days Minimum water volume for application: 200 L/ha	Metalaxyl-M	
	Drift	0.0077
	Vapour	0.0029
	Deposits	0.0030
	Re-entry	0.0416
	Sum of all pathways	0.0419
	Oxathiapiprolin	
	Drift	0.0014
	Vapour	0.0058
	Deposits	0.0006
	Re-entry	0.0078
	Sum of all pathways	0.0131
	Cumulative risk resident – adult (HI)	
	Drift	0.0091
	Vapour	0.0087
	Deposits	0.0036
	Re-entry	0.0494
	Sum of all pathways	0.0550

The Hazard Index is not expected to present a risk for workers, residents and bystanders following combined exposure to all active substances in A23109A, since the value is < 1. Thus, no further refinement of the assessment is required.

However, the Hazard Index is > 1 for operators applying ORONDIS VIP manually, for which refinement of the assessment is required. Further refinement in the form using gloves during mixing and loading, as well as application, will lower the Hazard Index < 1. Additionally, since this formulation is a sensitiser the use of gloves is considered an obligatory requirement.

Table 6.6-9: Risk assessment from combined exposure (longer term exposure)

Application scenario	Active ingredient	Estimated exposure / AOEL (HQ)
Operators – handheld manual spray application to low crops; using workwear and gloves (during mixing/loading and application)	Metalaxyl-M	0.4153
	Oxathiapiprolin	0.4576
	Cumulative risk operators (HI)	**0.8729**
Operators – handheld knapsack spray application to low crops; using workwear and gloves (during mixing/loading and application)	Metalaxyl-M	0.4200
	Oxathiapiprolin	0.4676
	Cumulative risk operators (HI)	**0.8876**

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	xxxxxxxx	12/01/2021	Metalaxyl-M / Oxathiapiprolin Metalaxyl-M / Oxathiapiprolin DC (A23109A) - Acute Oral Toxicity Study in Rats (Up and Down Procedure) Report No. 20/131-001P Document No. VV-888460 Test Facility xxxxxxxx GLP Unpublished	Y	SYN
KCP 7.1.2	xxxxxxxx	16/12/2020	Metalaxyl-M/Oxathiapiprolin DC (A23109A) – Acute Dermal Toxicity Study in Rats Report No. 20/131-002P Document No. VV-885797 Test Facility xxxxxxxxx GLP Unpublished	Y	SYN
KCP 7.1.3	xxxxxxxx	16/08/2021	Metalaxyl-M / Oxathiapiprolin DC (A23109A) - Acute Inhalation Toxicity Study (Nose-Only) in Rats Report No. 20/131-004P Document No. VV-915563 Test Facility xxxxxxxxx GLP Unpublished	Y	SYN
KCP 7.1.4	xxxxxxxx	26/05/2021	Metalaxyl-M / Oxathiapiprolin DC (A23109A) - Primary Skin Irritation Study in Rabbits Report No. 20/131-006N Document No. VV-904073 Test xxxxxxxxx GLP Unpublished	Y	SYN
~~KCP 7.1.4~~	~~Toth-Gonczol, K.~~	~~14/10/2020~~	~~Metalaxyl-M / Oxathiapiprolin DC (A23109A) – In Vitro Skin Irritation Test in the EPISKIN™ Model~~ ~~Report No. 20/131-043B~~ ~~Document No. VV-876289~~ ~~Test Facility Charles River Laboratories Hungary, Kft.~~ ~~GLP~~ ~~Unpublished~~	~~Y~~	~~SYN~~

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 7.1.5	xxxxxxxx	23/07/2020	Metalaxyl-M / Oxathiapiprolin DC (A23109A) - Acute Eye Irritation Study in Rabbits Report No. 19/269-005N Document No. VV-868398 Test Facility xxxxxxxxxx GLP Unpublished	Y	SYN
KCP 7.1.6	xxxxxxxx	09/12/2020	Metalaxyl-M / Oxathiapiprolin DC (A23109A) – Skin Sensitisation Local Lymph Node Assay Report No. 2119700 Document No. VV-885070 Test Facility xxxxxxxx GLP Unpublished	Y	SYN
KCP 7.3	Blackstock, C. Morrison, C.	27/07/2021	Metalaxyl-M/Oxathiapiprolin DC (A23109A) - The In Vitro Percutaneous Absorption of Radiolabelled Metalaxyl-M and Radiolabelled Oxathiapiprolin in Concentrate Formulation and Two In-Use Dilutions Through Human Split-Thickness Skin Report No. 787369 Document No. VV-913056 Test Facility Charles River Laboratories Edinburgh, Ltd. GLP Unpublished	N	SYN
Metalaxyl-M Active substance data					
~~KCA1 5.4.2~~	~~Dunton, J.~~	~~08/06/2015~~	~~NOA409045 – Oral (Gavage) Mouse Micronucleus Test~~ ~~Report No. BFI0257~~ ~~Document No. VV-28599 , NOA409045_10012~~ ~~Test Facility Sequani Limited~~ ~~GLP~~ ~~Unpublished~~	~~Y~~	~~SYN~~
~~KCA1 5.4.2~~	~~Dunton, J.~~	~~12/08/2014~~	~~CGA62826 – Oral (Gavage) Mouse Micronucleus Test~~ ~~Report No. BFI0255~~ ~~Document No. VV-410510 , CGA062826_10006~~ ~~Test Facility Sequani Limited~~ ~~GLP~~ ~~Unpublished~~	~~Y~~	~~SYN~~

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
~~KCA1 5.4.2~~	~~Dunton, J.~~	~~27/01/2015~~	~~Metalaxyl-M – Oral (Gavage) Mouse Micronucleus Test~~ ~~Report No. BFI0262~~ ~~Document No. VV-411540 , CGA329351_11683~~ ~~Test Facility Sequani Limited~~ ~~GLP~~ ~~Unpublished~~	~~Y~~	~~SYN~~
~~KCA1 5.4.2~~	~~Dunton, J.~~	~~15/09/2017~~	~~CGA226048 – Oral (Gavage) Mouse Micronucleus Test~~ ~~Report No. BFI0633~~ ~~Document No. VV-468462 , CGA226048_10000~~ ~~Test Facility Sequani Limited~~ ~~GLP~~ ~~Unpublished~~	~~Y~~	~~SYN~~

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
Metalaxyl-M Active substance data					
KCA1 5.4.2	Dunton, J.	12/08/2014	CGA62826 - Oral (Gavage) Mouse Micronucleus Test Report No. BFI0255 Document No. VV-410510 , CGA062826_10006 Test Facility Sequani Limited GLP Unpublished	Y	SYN
KCA1 5.4.2	Dunton, J.	08/06/2015	NOA409045 - Oral (Gavage) Mouse Micronucleus Test Report No. BFI0257 Document No. VV-28599 , NOA409045_10012 Test Facility Sequani Limited GLP Unpublished	Y	SYN

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
Metalaxyl-M Active substance data					
KCA1 5.4.2	Dunton, J.	27/01/2015	Metalaxyl-M - Oral (Gavage) Mouse Micronucleus Test Report No. BFI0262 Document No. VV-411540 , CGA329351_11683 Test Facility Sequani Limited GLP Unpublished	Y	SYN
KCA1 5.4.2	Dunton, J.	15/09/2017	CGA226048 - Oral (Gavage) Mouse Micronucleus Test Report No. BFI0633 Document No. VV-468462 , CGA226048_10000 Test Facility Sequani Limited GLP Unpublished	Y	SYN

List of data submitted by the applicant and not relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 7.1.4	Toth-Gönczöl K.	2020	Metalaxyl-M/Oxathiapiprolin DC (A23109A) − *In Vitro* Skin Irritation Test in the EPISKIN™ Model. 20/131-043B, VV-876289 Charles River Laboratories Hungary Kft. H-8200 Veszprém, Szabadságpuszta, hrsz. 028/1., Hungary GLP Unpublished	N	SYN

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

Acute Toxicity Estimate (ATE) and Additivity calculations have been conducted and are provided in the Part C document. Syngenta has also conducted acute toxicity studies on the formulation as at the time of the initial registration these studies were required for registration in other countries. The data from these studies has been used to populate the safety data sheet, therefore classification proposals may vary between the ATE calculation approach and the safety data sheet. It is Syngenta's approach to base the product classification on the animal data. Summaries of the acute toxicity studies are included in this document.

Comments of zRMS:	*In vivo* studies submitted by the applicant to support registration of the product A23109A has been conducted on the same formulation thus bridging approach is not applicable for this registration process. Due to the fact that zRMS PL accept all *in vivo* studies as background to hazard assessment, that is why mentioned above by the applicant Acute Toxicity Estimate (ATE) and Additivity calculations has not been taken into account in the final conclusions regarding toxicity potential by the zRMS PL.

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. There is no deviation from studies protocol. The OECD 425 procedure implements the 3R rules thus study is in line with the suggestions of point 5 of Regulation 284/2013. Results of the study and conclusions are adequate for risk assessment and classification purpose. Study accepted.

A 2.2.1 Study 1

Reference	KCP 7.1.1
Report	Metalaxyl-M / Oxathiapiprolin DC (A23109A) - Acute Oral Toxicity Study in Rats (Up and Down Procedure). xxxxxxxx, 2021 20/131-001P, VV-888460
Guideline(s)	Yes. Acute Oral Toxicity (rat): OECD Test Guideline 425 (2008): EPA OPPTS 870.1100 (2002)
Deviations	No
GLP	Yes
Acceptability	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test material (Lot/Batch No.)	Metalaxyl-M / Oxathiapiprolin DC (A23109A/ ORONDIS VIP), (JHU003-036-001)
Species	Rat, Crl:WI
No. of animals (group size)	5 rats (female)
Dose(s)	2000 mg/kg bw
Exposure	Once by gavage
Vehicle/Dilution	None

Post exposure observation period	14 days
Remarks	None

Results and discussions

Table A 1: Results of acute oral toxicity study in rats of A23109A/ORONDIS VIP

Dose (mg/kg bw)	Toxicological results *	Duration of signs	Time of death	LD_{50} (mg/kg bw) (14 days)
Female rats				
2000	0/5/5	Day 6	Day 14	> 2000

* Number of animals which died/number of animals with clinical signs/number of animals used

Table A 2: Summary of findings of acute oral toxicity study in rats of A23109A/ORONDIS VIP

Mortality	No mortality occurred.
Clinical signs	Yes. Slight to extreme decreased activity (5/5 animals), hunched back (5/5 animals), ataxia (5/5 animals), piloerection (4/5 animals), recumbency (3/5 animals), clonic convulsion (3/5 animals), decreased respiratory rate (1/5 animal), continuous tremors (1/5 animals) and heightened startle response (1/5 animal) were observed from the day of administration. From Day 6 all the animals were symptom-free.
Body weight	Body weight and body weight gain was considered to be normal.
Macroscopic examination	The necropsies performed at the end of the study revealed no apparent findings.

Conclusion

Under the experimental conditions, the oral LD_{50} of Metalaxyl-M / Oxathiapiprolin DC (A23109A/ORONDIS VIP) is greater than 2000 mg/kg bw in female Wistar rats. Thus, no classification is required according to Regulation (EC) No. 1272/2008.

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. OECD 402 procedure is still valid and acceptable. Noted deviation from the study protocol has no impact on the final outcome of the study. Results of the study and conclusions are adequate for risk assessment and classification purpose. Study accepted.

A 2.3.1 Study 1

Reference	KCP 7.1.2
Report	Metalaxyl-M/Oxathiapiprolin DC (A23109A) - Acute Dermal Toxicity Study in Rats. xxxxxxxx, 2020 20/131-002P, VV-885797
Guideline(s)	Yes. OECD 402 (2017): EPA 870.1200 (1998): EC No. 440/2008 (2008)
Deviations	Due to technical error, temperature value (minimum of 18.4 °C) outside the expected range of 19-25°C was recorded during the study. This deviation has no effect on the outcome of the study.
GLP	Yes
Acceptability	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test material (Lot/Batch No.)	Metalaxyl-M / Oxathiapiprolin DC (A23109A/ ORONDISVIP), (JHU003-036-001)
Species	Rat, Crl:WI
No. of animals (group size)	3 rats/female
Dose(s)	2000 mg/kg bw
Exposure	24 hours (dermal, semi-occlusive)
Vehicle/Dilution	None
Post exposure observation period	14 days
Remarks	None

Results and discussions

Table A 3: Results of acute dermal toxicity study in rats of A23109A/ORONDIS VIP

Dose (mg/kg bw)	Toxicological results *	Duration of signs	Time of death	LD_{50} (mg/kg bw) (14 days)
Female rats				
2000	0/0/3	-	Day 14	>2000

* Number of animals which died/number of animals with clinical signs/number of animals used

Table A 4: Summary of findings of acute dermal toxicity study in rats of A23109A/ORONDIS VIP

Mortality:	No mortality occurred.
Clinical signs:	No clinical signs of toxicity were observed.
Body weight:	Body weight and body weight gain was considered to be normal.
Macroscopic examination:	The necropsies performed at the end of the study revealed no apparent findings.

Conclusion

Under the experimental conditions, the dermal LD_{50} of Metalaxyl-M / Oxathiapiprolin DC (A23109A/ORONDIS VIP) is greater than 2000 mg/kg bw in female Wistar rats. Thus, no classification is required according to Regulation (EC) No. 1272/2008.

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 403 procedure is still valid and acceptable. Results of the study and conclusions are adequate for risk assessment and classification purpose. Study accepted.

A 2.4.1 Study 1

Reference	KCP 7.1.3
Report	Metalaxyl-M / Oxathiapiprolin DC (A23109A) - Acute Inhalation Toxicity Study (Nose-Only) in Rats xxxxxxxx, 2021 20/131-004P, VV-915563
Guideline(s)	Yes. OECD 403 (2009); EPA 870.1300 (1998); EC 440/2008, B.2 (2008)
Deviations	No
GLP	Yes

Acceptability	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test material (Lot/Batch No.)	Metalaxyl-M / Oxathiapiprolin DC (A23109A/ ORONDIS VIP), (JHU003-036-001)
Species	Rat, Crl:WI
No. of animals (group size)	10 rats (5 male & 5 female)
Concentration(s)	5.46 mg/L air
Exposure	4 hours (nose only)
Vehicle/Dilution	None
Post exposure observation period	14 days
Remarks	None

Results and discussions

Table A 5: Concentration(s) and exposure conditions

Group	Maximum achievable mean concentration (mg/L air)	Mean Mass Median Aerodynamic Diameter MMAD * (µm)	Geometric Standard Deviation GSD ** (µm)
0.1 (sighting exposure)	6.14	2.40	1.99
1 (main study)	5.46	2.35	2.03

* MMAD = Mass Median Aerodynamic Diameter
** GSD = Geometric Standard Deviation

Table A 6: Results of acute inhalation toxicity study in rats of A23109A/ ORONDIS VIP

Concentration (mg/L air)	Toxicological results *	Duration of signs	Time of death	LC_{50} (mg/L air) (14 days)
Male rats				
5.46	0/5/5	3	Day 14	> 5.46
Female rats				
5.46	0/5/5	3	Day 14	> 5.46

* Number of animals which died/number of animals with clinical signs/number of animals used

Table A 7: Summary of findings of acute inhalation toxicity study in rats of A23109A/ ORONDIS VIP

Mortality	No mortality occurred in either the sighting or main exposure groups.
Clinical signs	*Group 0.1 (Sighting Exposure – 6.14 mg/L)* In the male animals, decreased activity (1/2) (slight), laboured respiration (2/2) (slight), noisy respiration (2/2) (slight), increased respiratory rate (2/2) (slight), fur staining by the test item (on the head) (2/2) and lack of grooming (1/2) were observed from Day 0 up to Day 1. All male animals were symptom-free from Day 2. In the female animals, decreased activity (2/2) (slight or moderate), ataxia (2/2) (slight), hunched back (2/2), laboured respiration (2/2) (slight), noisy respiration (2/2) (slight), increased respiratory rate (2/2) (slight), fur staining by the test item (on the head) (2/2), red-brown staining (2/2) (on the nose) were observed from Day 0 up to Day 2. All female animals were symptom-free from Day 3. *Group 1 (Main Exposure – 5.46 mg/L)* In the male animals, ataxia (5/5) (slight), increased respiratory rate (5/5) (slight), noisy respiration (2/5) (slight), fur staining by the test item (on the head) (5/5) and lack of grooming (4/5), sneezing (1/5), wet

	fur (5/5) (on whole body) were observed from Day 0 up to Day 2. All male animals were symptom-free from Day 3. In the female animals, ataxia (5/5) (moderate), decreased activity (2/5) (slight), increased respiratory rate (5/5) (slight), noisy respiration (4/5) (slight), fur staining by the test item (on the head) (5/5) and lack of grooming (4/5), wet fur (5/5) (on whole body) were observed from Day 0 up to Day 2. All female animals were symptom-free from Day 3. Wet fur and fur staining (as chromodacryorrhea) in the animals were considered to be related to the restraint and exposure procedures or discomfort of the animals but not to be toxicologically significant.
Body weight	*Group 0.1 (Sighting Exposure – 6.14 mg/L)* There was no test item related effect on body weight or body weight gain in male or female animals. *Group 1 (Main Exposure – 5.46 mg/L)* There was no test item related effect on body weight or body weight gain in male or female animals. However, slight body weight losses were noted between Day 7-14 in two female animals.
Macroscopic examination	*Group 0.1 (Sighting Exposure – 6.14 mg/L)* The two males and two females from the sighting exposure survived the 14-day observation period and necropsy revealed no macroscopic lesions. *Group 1 (Main Exposure – 5.46 mg/L)* All animals (5 males and 5 females) survived the 14-day observation period. Necropsy revealed no macroscopic findings.

Conclusion
Under the experimental conditions, the inhalation 4 hour nose only LC_{50} of Metalaxyl-M / Oxathiapiprolin DC (A23109A/ORONDIS VIP) is greater than 5.46 mg/L air in male and female Wistar rats. Thus, no classification is required according to Regulation (EC) No. 1272/2008.

A 2.5 Skin irritation (KCP 7.1.4)

Comments of zRMS:	As it was mentioned in the preface to this report zRMS PL recognized this *in vitro* study as a not applicable for agrochemicals: TG OECD 439 rev. 14 June 2021 INITIAL CONSIDERATIONS AND LIMITATIONS Subsection 8: p.2 (..) *data indicates a lack of applicability of the RhE based in vitro skin irritation test for agrochemical formulations* (47). (..) See also: Kolle S.N, van Ravenzwaay B. and Landsiedel R. (2017). Regulatory accepted but out of domain: In vitro skin irritation tests for agrochemical for-mulations. Regul. Toxicol. Pharmacol 89, 125-130. Thus for this end point "Skin irritation" Reviewer consider an *in vivo* study A.2.5.2 Met-alaxyl-M/Oxathiapiprolin DC (A23109A) - Primary Skin Irritation Study in Rabbits. xxxxx Z., 2021 20/131-006N, VV-904073 as valid for hazard and risk assessment.

A 2.5.1 Study 1 *(in-vitro)*

Reference	KCP 7.1.4
Report	Metalaxyl-M / Oxathiapiprolin DC (A23109A) − *In Vitro* Skin Irritation Test in the EPISKIN™ Model. Toth-Gönczöl K., 2020 20/131-043B, VV-876289
Guideline(s)	Yes. OECD 439 (2019): EC No 640/2012, B.46 (2012).
Deviations	No
GLP	Yes
Acceptability	No

Materials and methods

For this test to be considered valid the following validity criteria needed to be met:

- The mean OD value of the three negative control tissues was between 0.6 and 1.5, and the standard deviation value (SD) of the % viability values was ≤ 18.The acceptable mean percentage viability range for positive controls was 0-40% and the standard deviation value (SD) of the % viability values was ≤ 18.
- The SD calculated from individual % tissue viability values of the three test item treated replicates was <18.The mean OD value of the blank samples (acidified isopropanol) was <0.1.

Test material (Lot/Batch No.)	Metalaxyl-M / Oxathiapiprolin DC (A23109A/ ORONDIS VIP), (JHU003-036-001)
Test system	EpiSkin™ Small Model
No. of replicate wells	3 units
Test item application	10 µL
Test exposure time	15 minutes at room temperature
Vehicle/dilution	None
Remarks	None

Results and discussions

Table A 8: Optical Density (OD) and the Calculated Relative Viability % of the Samples

Substance		Optical Density (OD)		Viability	%SD
		Measured	Blank corrected	(% RV)	
Negative Control:	1	1.269	1.223	96.0	--
Phosphate buffered saline	2	1.306	1.260	98.9	--
	3	1.385	1.340	105.1	--
	mean	--	**1.275**	**100.0**	**4.7**
Positive Control:	1	0.156	0.110	8.7	--
5% (w/v) SDS solution	2	0.095	0.049	3.9	--
	3	0.266	0.221	17.3	--
	mean	--	**0.127**	**10.0**	**6.8**
Test Item:	1	1.336	1.291	101.3	--
Metalaxyl-M / Oxathiapiprolin DC (A23109A)	2	1.459	1.413	110.9	--
	3	1.459	1.414	110.9	--
	mean	--	**1.373**	**107.7**	**5.6**

Note: Mean blank optical density value was 0.045.
Optical density means the mean value of the triplicate wells for each sample (rounded to three decimal places).

All the parameters met the acceptability criteria, therefore the study was considered to be valid.

Criteria for *in vitro* interpretation	Classification
	UN GHS
Mean tissue viability % is ≤ 50 %	Category 2 or Category 1
Mean tissue viability % is > 50 %	Non-Irritant*

*Note: If there is clear evidence that the test item is not corrosive, then it can be determined as No Category according to the UN GHS. It is plausible that some weaker corrosives could be classified as non-irritant in this *in vitro* assay.

Conclusion

In conclusion, under the conditions of this *in vitro* EpiSkin™ irritation assay conducted on Metalaxyl-M / Oxathiapiprolin DC (A23109A/ORONDIS VIP), the results indicate that the test item is non-irritant to skin.

A 2.5.2 Study 2 *(in-vivo)*

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. Results of the study and conclusions are adequate for risk assessment and classification purpose. Study accepted.

Reference	KCP 7.1.4/01
Report	Metalaxyl-M/Oxathiapiprolin DC (A23109A) - Primary Skin Irritation Study in Rabbits. xxxxxx 2021 20/131-006N, VV-904073
Guideline(s)	Yes. OECD 404 (2015): OPPTS 870.2500 (1998); EC No 440/2008, B.4 (2008)
Deviations	No
GLP	Yes
Acceptability	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test material (Lot/Batch No.)	Metalaxyl-M / Oxathiapiprolin DC (A23109A/ ORONDIS VIP), (JHU003-036-001)
Species	Rabbit, New Zealand White
No. of animals (group size)	3 (male)
Initial test using one animal	Yes
Exposure	0.5 mL (4 hours, semi-occlusive)
Vehicle/Dilution	None
Post exposure observation period	3 days
Remarks	None

Results and discussions

Table A 9: Skin irritation of A23109A/ORONDIS VIP

Animal No.		Scores after treatment *				Mean scores (24-72 h)	Reversible (day)
		1 h	24 h	48 h	72 h		
1843	Erythema Oedema	0 0	0 0	0 0	0 0	0 0	- -
1855	Erythema Oedema	0 0	0 0	0 0	0 0	0 0	- -
1841	Erythema Oedema	0 0	0 0	0 0	0 0	0 0	- -

* scores in the range of 0 to 4

Clinical signs:	No clinical signs of toxicity were observed.
Body weight:	Body weight and body weight gain was considered to be normal.

Conclusion
Under the experimental conditions, Metalaxyl-M / Oxathiapiprolin DC (A23109A/ORONDIS VIP) is not a skin irritant. Thus, no classification is required according to Regulation (EC) No. 1272/2008.

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, the OECD 405 procedure is still valid and acceptable. Results of the study and conclusions are adequate for risk assessment and classification purpose. Study accepted.

A 2.6.1 Study 1 *(in-vivo)*

Reference	KCP 7.1.5
Report	Metalaxyl-M/Oxathiapiprolin DC (A23109A) - Acute Eye Irritation Study in Rabbits. xxxxxxx, 2020 19/269-005N, VV-868398
Guideline(s)	Yes OECD Test Guideline 405 (2017), EPA 870.2400 (1998), EC No 2127/735, B.5 (2017) amending EC No 440/2008
Deviations	No
GLP	Yes
Acceptability	Yes

Materials and methods

Test material (Lot/Batch No.)	Metalaxyl-M / Oxathiapiprolin DC (A23109A/ ORONDIS VIP), (JHU003-036-001)
Species	Rabbit, New Zealand White
No. of animals (group size)	3 (male)
Initial test using one animal	Yes
Exposure	0.1 (conjunctival sac of the left eye)
Irrigation (time point)	Physiological saline solution following fluorescein control: 24 hours after test item application as part of the fluorescein observation process.
Vehicle/Dilution	None
Post exposure observation period	7 days
Remarks	None

Results and discussions

Table A 10: Eye irritation of A23109A/ORONDIS VIP

Animal No.		Scores after treatment *					Mean scores (24-72 h)	Reversible (day)
		1 h	24 h	48 h	72 h	7 d		
2097	Corneal opacity	1	1	1	1	0	1.00	7
	Iritis	0	0	0	0	0	0.00	-
	Redness conjunctivae	2	2	2	2	0	2.00	7
	Chemosis conjunctivae	2	2	2	2	0	2.00	7
2087	Corneal opacity	1	1	1	1	0	1.00	7

	Iritis Redness conjunctivae Chemosis conjunctivae	0 2 2	0 2 2	0 2 2	0 2 2	0 0 0	0.00 2.00 2.00	- 7 7
2090	Corneal opacity Iritis Redness conjunctivae Chemosis conjunctivae	1 0 2 2	1 0 2 2	1 0 1 1	1 0 1 1	0 0 0 0	1.00 0.00 1.33 1.33	7 - 7 7

* scores in the range of 0 to 4 for cornea opacity and chemosis, 0 to 3 for redness of conjunctivae and 0 to 2 for iritis

Clinical signs:	No clinical signs of toxicity were observed.
Body weight:	Body weight and body weight gain was considered to be normal.

Conclusion
Under the experimental conditions, Metalaxyl-M / Oxathiapiprolin DC (A23109A/ORONDIS VIP) is a moderate irritant eye irritant. Thus, a Category 2, H319 (causes serious eye irritation) classification is required according to Regulation (EC) No. 1272/2008.

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.

A 2.7.1 Study 1

Reference	KCP 7.1.6
Report	Metalaxyl-M/Oxathiapiprolin DC (A23109A) – Skin Sensitisation Local Lymph Node Assay xxxxxxx, 2020 2119700, VV-885070
Guideline(s)	Yes. OECD 429 (2010); EC 440/2008 B.42 (2017); EPA 870.2600 (2003)
Deviations	No
GLP	Yes
Acceptability	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test material (Lot/Batch No.)	Metalaxyl-M / Oxathiapiprolin DC (A23109A/ ORONDIS VIP), (JHU003-036-001)
Species	Mouse, CBA/CaOlaHsd
No. of animals (group size)	Pre-test: 4 female mice (2 for each pre-test) Main Study: 16 female mice
Pre-test	Topical application at concentrations of 50 and 100% (Pre-test 1); 25 and 50% (Pre-test 2) in 1% aqueous Pluronic®
Exposure (concentration(s), no. of applications)	Topical induction - concentrations of 10, 25 and 50% in 1% aqueous Pluronic®
Vehicle	1% aqueous Pluronic®

Pretreatment prior to topical application	No
Reliability check	α-hexyl cinnamaldehyde dissolved in acetone/olive oil (4+1 v/v). The periodic positve control experiment was not performed concurrently, but performed 3 months prior to study conduct.
Remarks	None

Results and discussions

Table A 11: Results of skin sensitisation study of A23109A/ORONDIS VIP

Test substance concentration	**Group Calculation**	
	Mean DPM per animal (2 lymph nodes) [a] ±S.D.	**Stimulation Index[1]**
Vehicle Control Group (1% aqueous Pluronic®)	1987.0 ±441.5	1.0
10% metalaxyl-M / oxathiapiprolin DC (A23109A)	1712.8 ±404.9	0.9
25% metalaxyl-M / oxathiapiprolin DC (A23109A)	3511.0 ±547.6	1.8***
50% metalaxyl-M / oxathiapiprolin DC (A23109A)	6932.0 ±256.4	3.5***
Calculated EC3 value for metalaxyl-M / oxathiapiprolin DC: 42.6%		
Historical positive control data (Oct 2015 – Apr 2020)		
α-hexyl cinnamaldehyde (25% in acetone:olive oil [4:1 v/v])	*n* Mean (±SD)[b] Observed range	10 9.5 ±3.5 5.59 – 17.16

a Mean DPM/animal was determined by dividing the sum of the measured values from lymph nodes of all animals within a group by the number of animals in that group (4 animals)
b calculated from S.I values presented in study report (Appendix 2)
1 The stimulation index is derived by dividing the DPM of each experimental group by the DPM of the vehicle control group. A stimulation index of greater than or equal to 3.0 generally indicates a positive response.
*** Statistically significant vs. concurrent vehicle control ($p \leq 0.001$)

Clinical signs:	No clinical signs of toxicity were observed.
Body weight:	Body weight and body weight gain was considered to be normal.

Conclusion

Under the experimental conditions, Metalaxyl-M / Oxathiapiprolin DC (A23109A/ORONDIS VIP) is a moderate skin sensitiser. Thus, a Category 1B, H317 (may cause an allergic skin reaction) classification is required according to Regulation (EC) No. 1272/2008.

For skin sensitising substances, an assessment needs to be made whether the substance has the potential to cause significant sensitisation in humans (Sub-category 1A). If Sub-category 1A can be excluded, it can be presumed that the substance merits Sub-category 1B (moderate skin sensitiser) classification. Based on moderate potency on animals and according to GHS criteria, the product has been classified as Cat. 1B.

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

Not relevant.

A 2.9 Data on co-formulants (KCP 7.4)

A 2.9.1 Material safety data sheet for each co-formulant

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

A 2.9.2 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 2.10 Studies on dermal absorption (KCP 7.3)

A 2.10.1 Study 1 – Metalaxyl-M/oxathiapiprolin in A23109A/ORONDIS VIP

Comparative dermal absorption, in vitro using rat and human skin

Comments of zRMS:	Study is considered to be acceptable and dermal absorption for a.s. ~~azoxystrobin~~ metalaxyl-M/oxathiapiprolin are covered by this study. DA values obtained from the study are reliable and can be used for risk assessment.

Reference	KCP 7.3
Report	Metalaxyl-M/Oxathiapiprolin DC (A23109A) - The In Vitro Percutaneous Absorption of Radiolabelled Metalaxyl-M and Radiolabelled Oxathiapiprolin in Concentrate Formulation and Two In Use Dilutions Through Human Split Thickness Skin. Blackstock, C. and Morrison, C., 2021 787369, VV-913056
Guideline(s)	OECD 428 (2004)
Deviations	None
GLP	Yes
Acceptability	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test material	Name (Batch No.)	[^{14}C]-Metalaxyl-M (ATS-21-3777)
	Test preparation	radioformulation
	Specific activity	102.2 µCi/mg
	Radiochemical purity	99.4%
	Name (Batch No.)	[^{14}C]-Oxathiapiprolin (ZA0-175900-036)
	Test preparation	radioformulation
	Specific activity	48.0 µCi/mg
	Radiochemical purity	98.5%
Product	Name (Lot/Batch No.)	A23109A (JHU003-036-001)
	Alternative Name	Metalaxyl-M/Oxathiapiprolin DC (180/30)

	Concentration a.s.	185 g/L Metalaxyl-M and 30.6 g/L Oxathiapiprolin
Blank product	Name (Lot/Batch No.)	EXF22949A (JHU003 061 002)
		EXF22947A (JHU003 061 001)

Test system		
Diffusion cell	Cell type	Dynamic
	(if dynamic) Flow rate	1.5 mL/h ± 0.15 mL/h with the expection of testing [^{14}C]-Oxathiapiprolin in spray dilution 1 and 2 when the flow rate was lowered to 0.75 mL/h ± 0.15 mL/h
	Exposed skin area	0.64 cm²
	Cover	Not occluded
Membrane	Skin type	Dermatomed
	Skin thickness range	380-410 µm
	Skin donors age	28-59
	Skin donors sex	M + F
	Location	Abdomen
	Source	*Ex vivo*
	Integrity test	Electrical resistance (> 7.7 kΩ)
Receptor	Receptor medium	Phosphate buffered saline containing polyoxyethylene 20 oleyl ether (PEG, ca 6%, w/v), sodium azide (ca 0.01%, w/v), streptomycin (ca 0.1 mg/mL) and penicillin (ca 100 units/mL), pH 7.35-7.49
	Solubility in receptor medium	Yes
Sample Time	Exposure time	6 h
	Observation time	24 h
Sampling	Sample intervals	[^{14}C]-Metalaxyl-M formulation concentrate, spray dilution 1 and spray dilution 2 and [^{14}C]-Oxathiapiprolin formulation concentrate; 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 18, 20, 22 h and 24 h [^{14}C]-Oxathiapiprolin spray dilution 1 and spray dilution 2 : 4, 8, 12, 16, 20 h and 24 h
Washing		6h and 24 h
Final Procedure	Tape stripping	20
	TS1-2 analysed separately	Y
Remarks:		

	[^{14}C]-Metalaxyl-M		
Tested doses	Concentrate	Spray dilution 1	Spray dilution 2
Target concentration [mg/ml]	180	0.09	0.047
Area dose [µg/cm²]	1867	0.938	0.486
Total dose [µg/cell]	10	10	10
Specific activity [kBq/ml]	102.2	102.2	102.2
No. of donors	4	4	4
No of cells used/valid cells*	8/7*	8/8	8/8

* *Cell 5 Excluded due to suspected leakage at the junction between the donor and receptor chamber*

	[^{14}C]- Oxathiapiprolin		
Tested doses	Concentrate	Spray dilution 1	Spray dilution 2
Target concentration [mg/ml]	30	0.015	0.0079
Area dose [µg/cm²]	330.1	0.148	0.0773
Total dose [µg/cell]	10	10	10
Specific activity [kBq/ml]	48.0	48.0	48.0
No. of donors	4	3	3
No of cells used/valid cells*	8/7[1]	8/6[2]	8/6[3]

[1] *Cell 30 Excluded due to suspected leakage at the junction between the donor and receptor chamber.*
[2] *Cells 37 and 38 Excluded due to suspected leakage at the junction between the donor and receptor chamber.*
[3] *Cells 45 and 46 Excluded due to suspected leakage at the junction between the donor and receptor chamber.*

Results and discussions

Table A 12: ***In-vitro*** **dermal penetration of Metalaxyl-M formulated and [^{14}C]-Oxathiapiprolin as A23109A/ORONDIS VIP through human skin - Recovery data (±SD)**

Dose group	[^{14}C]-Metalaxyl-M			[^{14}C]-Oxathiapiprolin		
	FC	SD1	SD2	FC	SD1	SD2
Target concentration [g/L]	180	0.09	0.047	30	0.015	0.0079
Mean actual applied concentration [g/L]	187	0.0938	0.0486	33.0	0.0148	0.00773
	Recovery [%]					
	Mean ± SD					
Number of replicates	7	8	8	7	6	6
Dislodgeable dose	96.03 ± 2.18	81.39 ± 5.86	84.90 ± 5.90	97.61 ± 1.92	97.26 ± 4.00	96.50 ± 6.04
Skin washing after 6 h	95.48	78.11	81.54	96.80	91.24	86.61
Skin washing after 24 h	0.39	3.09	2.92	0.54	5.65	8.74
Donor chamber wash	0.16	0.19	0.44	0.27	0.36	1.14
Dose associated to skin						
Tape strips 1 - 2	0.02	0.07	0.16	0.13	0.48	0.44
Tape strips 3 - 20	0.02	0.20	0.27	0.37	2.40	3.17
Exposed skin	0.47	2.09	1.30	0.29	2.15	2.14
Unexposed skin	0.06	0.33	0.14	0.17	0.39	0.13
Absorbed dose	3.16 ± 2.03	14.85 ± 5.29	13.21 ± 5.81	0.05 ± 0.06	2.39 ± 1.05	2.87 ± 1.80
Receptor fluid	3.05	14.36	12.89	0.01	1.66	2.16
Receptor chamber wash	0.09	0.38	0.26	0.04	0.56	0.60
Receptor Rinse	0.03	0.10	0.06	<0.01	0.16	0.11
Total recovery[1]	99.76± 0.82	98.93 ± 2.50	99.97 ± 1.02	98.63 ± 1.28	105.07 ± 3.33	105.25 ± 3.75
Absorption essentially complete at end of study (>75% absorption within half the study duration) [%Absorption at $t_{0.5}$] [2]	N 63.68	Y 79.76	Y 85.44	N 34.36	N 58.36	N 63.48
If no: Absorption estimates = absorbed dose + exposed skin + tape strips 3-20	3.66 ± 2.15	N/A	N/A	0.71 ± 0.87	6.93 ± 2.11	8.18 ± 3.71
If yes: Absorption estimates = absorbed dose + exposed skin	N/A	16.94 ± 5.85	14.51 ± 6.19	N/A	N/A	N/A
Absorption estimate normalised[3]	N	N	N	N	N	N
k	0.92	0.84	0.84	0.92	1.0	1.0
Mean + k*SD[4]	5.64	21.85	19.71	1.51	9.04	11.89
Absorption estimates used for risk assessment[5]	5.6	22	20	1.5	9.0	12

1 Values may not calculate exactly due to rounding of figures
2 In accordance with the EFSA Guidance on Dermal Absorption (EFSA Journal 2017;15(6):4873) the radioactivity in the second tape-strip pool (3rd to nth tape strip) is considered potentially absorbable if less than 75% of the absorption occurred in the first half of the study (see Table 7.6.2-1) Finally, the skin preparation is also considered potentially absorbable.
3 According to the EFSA Guidance on Dermal Absorption, cells with insufficient recovery (< 95%) can be corrected by normalisation of absorption estimate to 100% recovery; explanation should be included.
4. In accordance with the EFSA Guidance on Dermal Absorption, the standard deviation aligned to the appropriate k factor was added to the mean% dermal penetration.
5 Relevant absorption estimate was rounded to the required number of significant figures.
N/A: not applicable

Remarks
Cells 5, 30, 37, 38, 45 and 46 were rejected due to suspected leakage at the junction between the donor and receptor chamber.

The study was performed in accordance with the protocol and protocol amendment 1 for Charles River Study No. 787369 with the following deviation.

Protocol Section 11.1 stated that split-thickness skin would be prepared by cutting to a depth of 200-400 µm. Skin cut to a depth of 410 µm from one donor was dosed with each of the oxathiapiprolin test preparations. An additional 10 µm thickness is very unlikely to affect the results and any material would still appear in the risk assessment value. The results have been reviewed and this assessment has been confirmed by the data, therefore, there was no impact on this study.

Conclusion/endpoint:
The study demonstrated that the amount of metalaxyl-M absorbed through human split-thickness skin membranes over 24 h (following a 6 h exposure) from the formulation concentrate (180 g/L) and the intended in-use concentrations (0.09 g/L and 0.047 g/L) was 3.16%, 14.85%, and 13.21% of the applied dose, respectively, as measured in the receptor fluid, receptor rinse and receptor chamber wash. The dermal penetration estimates to be used for risk assessment were set at 5.6%, 22% and 20% for the formulation concentrate, spray dilution 1 and spray dilution 2 respectively based on the EFSA Guidance on Dermal Absorption (2017) criteria.

The study demonstrated that the amount of oxathiapiprolin absorbed through human split-thickness skin membranes over 24 h (following a 6 h exposure) from the formulation concentrate (30 g/L) and the intended in-use dilutions (0.015 g/L and 0.0079 g/L) was 0.05%, 2.39%, and 2.87% of the applied dose, respectively, as measured in the receptor fluid, receptor rinse and receptor chamber wash. The dermal penetration estimates to be used for risk assessment were set at 1.5%, 9.0% and 12% for the formulation concentrate, spray dilution 1 and spray dilution 2 respectively based on the EFSA Guidance on Dermal Absorption (2017) criteria.

A 2.11 Other/Special Studies

A 2.11.1 CGA62826: Oral (Gavage) Mouse Micronucleus Test

Comments of zRMS:	In the zRMS opinion it is important to prove if BM has been reached by the tested compound. This issues was discussed during a.s. renewal (DRAR 2013). Since there was agreement among both RMS and co-RMS on the fact that for Metalaxyl-M BM was reached, thus based on genotoxic data it is concluded that Metalaxyl-M is devoid of clastogenic properties *in-vivo*. Considering this zRMS is in the opinion that under the experimental conditions reported the test substance CGA62826 to be non-clastogenic or aneugenic in this bone marrow micronucleus assay. Study is valid and reliable.

Report author	Dunton, J
Report year	2014
Report title	CGA62826 – Oral (Gavage) Mouse Micronucleus Test.
Report No	BFI0255
Guidelines followed in study	OECD 474 (1997). Guideline for the testing of chemicals: Mammalian erythrocyte micronucleus test.
Major deviations from test guideline	None
Guidance in force at time of submission of supplementary dossier	OECD 474 (2016). Guideline for the testing of chemicals: Mammalian erythrocyte micronucleus test.
Differences between old and current guideline	The 2016 version of OECD 474 details 3 acceptable dosing and sampling regimens; the 1997 details 2 acceptable dosing and sampling regimens; OECD 474 2016 specifies clear requirements for demonstration of laboratory proficiency and maintenance of historical control data. Precise acceptance and evaluation criteria are specified in the 2016 version and comparisons to historical control data are required for both control and treated cultures. The OECD 474 2016 guideline specifies 4000 PCE should be scored for micronuclei and a total of 500 erythrocytes assessed for determination of toxicity. In the 1997 version these numbers were 2000 PCE and 200 eythrocytes respectively.
Previous evaluation	No
GLP/Officially recognised testing facilities	Yes

Reference	KCA 5.4.2
Report	CGA62826: Oral (Gavage) Mouse Micronucleus Test Dunton J., 2014 Report No. BFI0255 Syngenta File No. CGA062826_10006
Guideline(s)	OECD 474 (1997): OPPTS 870.5395 (1998): 2000/32/EC 440/2008 B.12 (2008)
Deviations	No
GLP	Yes
Acceptability	Yes

EXECUTIVE SUMMARY

CGA62826 was tested to evaluate its potential to cause damage to chromosomes or cell division apparatus, or to cause cell cycle interference, leading to micronucleus formation in polychromatic erythrocytes in the bone marrow of young adult mice.

In all phases, the dosing of the vehicle and test item was by oral (gavage) administration twice, separated by approximately 24 hours.

In the range-finding phase, a group of 3 male and 3 female mice was given CGA62826 as a suspension in 1.0 % w/v aqueous carboxymethylcellulose with 0.1 % v/v Tween 80, at 2000 mg/kg/day for males and females, which is the regulatory test guideline maximum dose level. 2000 mg/kg/day was well tolerated in

both male and female mice. As no difference in toxicity was observed between the sexes in the range-finder, only males were dosed in the main study.
A proof of exposure phase was conducted to demonstrate that the bone marrow was exposed to the test item. This was demonstrated by analysis of test item in the whole blood of treated animals. Blood samples were obtained via the orbital sinus route from all animals in the range-finding phase at 1 hour and 4 hours post-second dose and at termination. In each case, 0.1 mL samples were taken into tubes containing K_2EDTA. CGA62826 was recovered from mouse blood:water [1:1 (v/v)] using an appropriate analytical procedure, and the processed samples analysed by LC-MS/MS to confirm exposure to the test item. The presence of CGA62826 was confirmed by analysis of the study samples alongside samples of blank matrix and matrix spiked with the test item.
For the main study phase, three groups, each of 6 male mice were dosed with 500, 1000 or 2000 mg/kg/day CGA62826 on two successive days, separated by approximately 24 hours.
A group of 6 male mice (negative control) was dosed with the vehicle alone and a positive control group, also of 6 male mice, was given a single 4 mg/kg intraperitoneal dose of Mitomycin C (MMC).
Animals were humanely killed approximately 24 hours after their second dose. Bone marrow was harvested from each animal and smears prepared. The stained slides were coded, 2000 polychromatic erythrocytes (PCE) per animal were scored for the presence of micronuclei and the group frequencies were statistically analysed.
There were no statistically significant increases in micronucleus frequency in male mice treated at any dose level of CGA62826, compared with the negative control group.
There was no evidence of a statistically significant reduction in the PCE/NCE ratio in male mice treated with CGA62826, indicating a lack of toxicity of CGA62826 to the bone marrow. However, proof of exposure to the bone marrow was demonstrated in the range finding phase.
The animals dosed with MMC, the positive control item, had statistically significant increases in the number of micronucleated cells compared to the concurrent control group, which demonstrated that the test system was capable of detecting a known clastogen and that the scorers were capable of detecting micronuclei. There was no statistically significant decrease in the PCE/NCE ratio in the positive control group, indicating a lack of toxicity to the bone marrow.

In conclusion, it can be stated that there was no evidence of clastogenicity or aneugenicity following oral (gavage) administration of CGA62826 up to the regulatory test guideline maximum dose level of 2000 mg/kg/day in male mice. Therefore, CGA62826 is considered to be non-clastogenic or aneugenic in this bone marrow micronucleus assay.

Materials and methods

Test Material:	CGA62826		
Description:	White powder		
Lot/Batch number:	MLA-342/2 K1, K2		
Purity:	99 % ± 2 % w/w HPLC		
Stability of test compound:	Retest date: 31 March 2018		
Control Materials:			
Negative control (if not vehicle):	N/A	**Final Volume:** N/A	**Route:** N/A
Vehicle:	1.0 % w/v carboxymethylcellulose with 0.1 % v/v Tween 80	**Final Volume:** 10 mL/kg	**Route:** oral
Positive control:	Mitomycin C	**Final Doses:** 4 mg/kg	**Route:** i.p.

Test Animals:

Species	Mouse
Strain	CD-1
Age/weight at dosing	6 - 7 weeks (at start of experiment); mean value 32 g, range 29-36 g
Source	Charles River (UK) Ltd., Margate, Kent, CT9 4LT, England.
Housing	Up to 3/cage
Acclimatisation period	At least 5 days
Diet	Pelleted standard diet, *ad libitum*
Water	Tap water, *ad libitum*
Environmental conditions	Temperature: 19-21 °C Humidity: 46-64 % Photoperiod: 12 hours dark/12 hours light

Test compound administration:

	Dose Levels	**Final Volume**	**Route**
Preliminary:	2000 mg/kg/day (males and females)	10 mL/kg b.w.	oral
Main Study:	500, 1000, 2000 mg/kg/day males only	10 mL/kg b.w.	oral

Study Design and Methods:
Study initiation date: 16 April 2014 (study plan issued).
Experimental start date: 28 April 2014 (start dosing).
Experimental termination date: 11 June 2014 (last day of slide scoring).

Preliminary Toxicity Assay: A maximum tolerated dose (MTD) was determined, based on toxicity observed over a 24 hour observation period following oral (gavage) administration twice, separated by approximately 24 hours.
A proof of exposure phase was conducted to demonstrate that the bone marrow was exposed to the test item. This was demonstrated by analysis of test item in the whole blood of treated animals. Blood samples were obtained via the orbital sinus route from all animals in the range-finding phase at 1 hour and 4 hours post-second dose and at termination of each group. In each case, 0.1 mL samples were taken into tubes containing K_2EDTA. CGA62826 was recovered from mouse blood:water [1:1 (v/v)] using an appropriate analytical procedure and the processed samples analysed by LC-MS/MS to confirm exposure to the test item. The presence of CGA62826 was confirmed by analysis of the study samples alongside samples of blank matrix and matrix spiked with the test item.

Table A 13: Micronucleus Test: Experimental Design

Treatment	Dose level (mg/kg/day) CGA62826	Number of animals
Negative control	0	6
Test substance	500	6
Test substance	1000	6
Test substance	2000	6
Positive control	MMC 4 mg/kg	6

Animals in Groups 1 to 4 were dosed twice, approximately 24 hours apart, with vehicle alone (negative control) or CGA62826. Group 5 animals (positive control) were given a single 4 mg/kg dose of MMC at a dose volume of 5 mL/kg.

Slide Preparation: The range-finder animals were not allowed to recover from the anaesthetic after the terminal blood sampling approximately 24 hours after the second test item and vehicle administration and death was confirmed by cervical dislocation.
The main study animals in Groups 1 to 4 were humanely killed approximately 24 hours after the second test item and vehicle administration. Group 5 animals were humanely killed approximately 24 hours after the single administration of the positive control. The animals were killed by exposure to rising concentrations of carbon dioxide and death was confirmed by cervical dislocation. The femurs from all animals were exposed by dissection of the surrounding muscle and connective tissues and the shank of the bones removed. The bone marrow cells from both femurs of each animal were aspirated into labelled centrifuge tubes using a syringe containing foetal bovine serum. The bone marrow cells were centrifuged, the supernatant withdrawn, and the cells re suspended in a minimal volume of foetal bovine serum. One drop of cell suspension was placed on each of two slides and spread by drawing an edge of a clean glass microscope slide along from the drop to the end of the slide.
All slides were left to air dry and age overnight before fixing for five minutes in methanol. Fixed slides were stained for 20 to 30 minutes in 11.5 % (v/v) Giemsa in Sorensen's buffer pH 6.8, based on the method of Gollapudi and Kamra.

Slide Analysis: A unique, unambiguous code was devised for each animal, including the positive controls. Adhesive labels that covered the animal and group identity were affixed to each slide so that the analyst could see only the study number and the new code. 2000 polychromatic erythrocytes (PCE), including micronucleated PCE (MN-PCE), were counted for each animal. The numbers of normochromatic erythrocytes (NCE) and micronucleated NCE (MN-NCE) were also recorded for the first 1000 cells scored. Only areas of slides of good technical quality and appropriate staining characteristics were scored.

Results and discussions
There was no need to assess toxicity to the bone marrow and bone marrow smears were not analysed in the range-finding phase, as the presence of CGA62826 was confirmed since the study sample chromatograms showed substantial CGA62826 content when compared with those of blank matrix and matrix fortified with CGA62826.

Micronucleus test: There were no adverse clinical observations following administration of CGA62826 to male mice at 500 mg/kg/day (Group 2) or 1000 mg/kg/day (Group 3). Nor were there any adverse clinical observations in Group 1 (negative control) or Group 5 (positive control).
Noisy breathing was observed in one male at 2000 mg/kg/day (Group 4).
There were no statistically significant increases in micronucleus frequency in male mice treated at any dose level of CGA62826, compared with the negative control group.
There was no evidence of a statistically significant reduction in the PCE/NCE ratio in male mice treated with CGA62826, indicating a lack of toxicity of CGA62826 to the bone marrow. However, proof of exposure of the bone marrow was demonstrated in the range finding phase.

The animals dosed with MMC, the positive control item, had statistically significant increases in the number of micronucleated cells compared to the concurrent control group, which demonstrated that the test system was capable of detecting a known clastogen and that the scorers were capable of detecting micronuclei. There was no evidence of a statistically significant decrease in the PCE/NCE ratio in the positive control group, indicating a lack of toxicity to the bone marrow.

Conclusion

There was no evidence of clastogenicity or aneugenicity following oral (gavage) administration of CGA62826 up to the regulatory test guideline maximum dose level of 2000 mg/kg/day in male mice. Therefore, CGA62826 is considered to be non-clastogenic or aneugenic in this bone marrow micronucleus assay.

Preliminary toxicity assay: There were no clinical signs or significant body weight loss observed following administration of CGA62826 at 2000 mg/kg/day.
The regulatory test guideline maximum dose level of 2000 mg/kg/day was tolerated in both male and female mice.

Micronucleus Data: Negative Control vs. Treated Groups – Males

	Negative Control 0 mg/kg/day	**Metalaxyl-M 100 mg/kg/day**	**Metalaxyl-M 200 mg/kg/day**	**Metalaxyl-M 400 mg/kg/day**	**MMC 4 mg/kg**
N	6	6	6	6	6
Mean MN-PCE	1.00	0.67	1.00	0.50	63.67[WW]
SD	0.89	0.52	0.63	0.84	17.13
Mean MN-PCE +SD	1.89	1.18	1.63	1.34	80.80
Mean MN-PCE –SD	0.11	0.15	0.37	-0.34	46.54
Mean MN-PCE ratio	0.63	0.83	0.54	0.69	0.75
SD	0.12	0.16	0.09	0.27	0.36
Mean PCE/NCE +SD	0.76	0.99	0.63	0.96	1.11
Mean PCE/NCE -SD	0.51	0.67	0.45	0.42	0.39

MMC: Mitomycin C
N: number of animals
WW: statistically significant (Wilcoxon's test) $p<0.01$
Note: any discrepancy in this table is due to rounding differences

Mouse Historical Control Data

Males Negative Control							
	N	Mean	SD	Range (mean +/- SD)		Range (min / max)	
PCE	249	2020.8	140.6	1880.2	2161.4	2000	3004
NCE/1000 cells	249	540.5	81.7	458.9	622.2	327	825
MN-PCE	249	1.5	1.5	-0.1	3.0	0	8
MN-NCE	249	0.3	0.6	-0.3	0.9	0	3
PCE/NCE Ratio	249	0.9	0.3	0.6	1.2	0.2	2.1
Males Positive Control							
	N	Mean	SD	Range (mean +/- SD)		Range (min / max)	
PCE	212	2024.5	152.5	1872.1	2177.0	2000	3010
NCE/1000 cells	212	640.6	94.8	545.8	735.4	372	918
MN-PCE	212	110.2	58.6	51.6	168.8	9	354
MN-NCE	212	0.7	0.9	-0.2	1.6	0	6
PCE/NCE Ratio	212	0.6	0.3	0.3	0.9	0.1	1.7

A 2.11.2 NOA409045: Oral (Gavage) Mouse Micronucleus Test

Comments of zRMS:	NOA 409 045 is the R-enantiomere of the previously tested CGA 62 826. It was considered in the DRAR 2013 that a bridging between the racemate and the R-enantiomere was possible. Due to fact that CGA62826 to be non-clastogenic or aneugenic in the bone marrow micronucleus assay it can be concluded that there is no such action for NOA409045. Study (Dunton, J 2015), discussed below supports this observation and allow to conclude that there was no evidence of clastogenicity or aneugenicity following oral (gavage) administration of NOA409045 up to the regulatory test guideline maximum dose level of 2000 mg/kg/day in male mice. Therefore, NOA409045 is considered to be neither clastogenic nor aneugenic in the mouse bone marrow micronucleus assay. Note: The relevance of the groundwater metabolite NOA409045 was already assessed at EU level by the Metalaxyl-M zRMS Belgium (co-RMS Greece) under Article 7 and an updated DRAR has been made available for commenting at MS level in May 2021. The updated DRAR concluded that the groundwater metabolite NOA409045 is considered not relevant according to the criteria laid down in the EC guidance document SANCO/221/2000 –rev.11.

Report author	Dunton, J
Report year	2015
Report title	NOA409045 – Oral (Gavage) Mouse Micronucleus Test.
Report No	BFI0257
Guidelines followed in study	OECD 474 (1997). Guideline for the testing of chemicals: Mammalian erythrocyte micronucleus test.
Major deviations from test guideline	None
Guidance in force at time of submission of supplementary dossier	OECD 474 (2016). Guideline for the testing of chemicals: Mammalian erythrocyte micronucleus test.
Differences between old and current guideline	The 2016 version of OECD 474 details 3 acceptable dosing and sampling regimens; the 1997 details 2 acceptable dosing and sampling regimens; OECD 474 2016 specifies clear requirements for demonstration of laboratory proficiency and maintenance of historical control data. Precise acceptance and evaluation criteria are specified in the 2016 version and comparisons to historical control data are required for both control and treated cultures. The OECD 474 2016 guideline specifies 4000 PCE should be scored for micronuclei and a total of 500 erythrocytes assessed for determination of toxicity. In the 1997 version these numbers were 2000 PCE and 200 eythrocytes respectively.
Previous evaluation	No
GLP/Officially recognised testing facilities	Yes

Reference	KCA 5.4.2
Report	NOA409045: Oral (Gavage) Mouse Micronucleus Test Dunton J., 2015 Report No. BFI0257 Syngenta File No. NOA409045_10012
Guideline(s)	OECD 474 (1997)
Deviations	No
GLP	Yes
Acceptability	Yes

EXECUTIVE SUMMARY

NOA409045 was tested to evaluate its potential to cause damage to chromosomes or cell division apparatus, or to cause cell cycle interference, leading to micronucleus formation in polychromatic erythrocytes in the bone marrow of young adult mice.
In all phases, the dosing of the vehicle and test item was by oral (gavage) administration twice, separated by approximately 24 hours.
In the range-finding phase, groups of three male and/or three female mice were given NOA409045 at 1000 mg/kg/day or 2000 mg/kg/day, in order to confirm the MTD in both male and female mice.
The regulatory test guideline maximum dose level of 2000 mg/kg/day was well tolerated in male mice and the maximum tolerated dose level (MTD) in female mice was 1000 mg/kg/day. As there was no substantial inter-sex differences in toxicity (a difference in MTD of three-fold or greater), the main study was conducted in males only, with the high dose selected as 2000 mg/kg/day, as permitted by the OECD 474 guideline.

A proof of exposure phase was conducted to demonstrate that the bone marrow was exposed to the test item. This was demonstrated by analysis of test item in the whole blood of treated animals. The presence of NOA409045 was confirmed by analysis of the study samples alongside samples of blank matrix and matrix spiked with the test item.
For the main study phase, three groups, each of six male mice were dosed with 500, 1000 or 2000 mg/kg/day NOA409045 on two successive days, separated by approximately 24 hours. A group of six male mice was dosed with the vehicle alone (negative Control) and a positive Control group, also of six male mice, was given a single 4 mg/kg intraperitoneal dose of Mitomycin C (MMC).
Bone marrow was harvested from all surviving animals approximately 24 hours after the final dose administration and smears were prepared. The stained slides prepared for the main study were coded and 2000 polychromatic erythrocytes (PCE) per animal were scored for the presence of micronuclei and the group frequencies were statistically analysed.
There were no statistically significant increases in micronucleus frequency in male mice treated at any dose level of NOA409045, compared with the negative Control group.
There was no evidence of a statistically significant reduction in the PCE/NCE ratio in male mice treated with NOA409045, indicating a lack of toxicity of NOA409045 to the bone marrow. However, exposure of the bone marrow to NOA409045 was demonstrated in the range-finding phase of this study.
The animals dosed with MMC, the positive Control item, had statistically significant increases in the number of micronucleated cells compared to the concurrent Control group, which demonstrated that the test system was capable of detecting a known clastogen and that the scorers were capable of detecting micronuclei. There was no statistically significant decrease in the PCE/NCE ratio in the positive Control group, indicating a lack of toxicity to the bone marrow.

In conclusion, it can be stated that there was no evidence of clastogenicity or aneugenicity following oral (gavage) administration of NOA409045 up to the regulatory test guideline maximum dose level of 2000 mg/kg/day in male mice. Therefore, NOA409045 is considered to be neither clastogenic nor aneugenic in the mouse bone marrow micronucleus assay.

Materials and methods

Test Material: NOA409045
Description: White powder
Lot/Batch number: MES 136/3
Purity: 97 % w/w ± 2 %
Stability of test compound: Retest date : 31 July 2016

Control Materials:

Negative control (if not vehicle) :	N/A	**Final Volume:** N/A	**Route:** N/A
Vehicle:	1.0 % w/v carboxymethyl-cellulose with 0.1 % v/v Tween 80	**Final Volume:** 10 mL/kg	**Route:** oral
Positive control :	mitomycin C	**Final Doses:** 4 mg/kg	**Route:** oral

Test Animals:

Species	Mouse
Strain	CD-1
Age/weight at dosing	6 – 7 weeks (at start of experiment); Main study: range 28-33 g, mean weight 30 g
Source	Charles River (UK) Ltd., Margate, Kent, CT9 4LT, England
Housing	Up to 3/cage
Acclimatisation period	At least 11 days for the range-finding phase and 5 days for the main study
Diet	Pelleted standard diet, *ad libitum*
Water	Tap water, *ad libitum*
Environmental conditions	Temperature: 19-21 °C 45 % to 54 % Photoperiod: 12 hours dark/12 hours light

Test compound administration:

	Dose Levels	**Final Volume**	**Route**
Preliminary:	Range-finding phase: 2000 mg/kg/day (males) 1000, 2000 mg/kg/day (females)	10 mL/kg b.w.	oral
Main Study:	500, 1000, 2000 mg/kg/day males only	10 mL/kg b.w.	oral

Study Design and Methods:
Study initiation date: 03 February 2015 (study plan issued).
Experimental start date: 05 February 2015 (First animal arrival).
Experimental termination date: 27 March 2015 (last day of slide scoring).

Preliminary Toxicity Assay: Dosing was by oral (gavage) administration twice, separated by approximately 24 hours. Groups of three male and/or three female mice were given NOA409045 at 1000 mg/kg/day or 2000 mg/kg/day. The animals were observed periodically for up to 24 hours after the first and second dose. Surviving animals were humanely killed after the terminal proof of exposure bleed.
Since bone marrow is well perfused, exposure of the bone marrow to the test item was indirectly assessed by collection of blood and analysis for NOA409045. Blood samples were obtained via the orbital sinus route from all animals in the range-finding phase at 1 hour and 4 hours after the second dose and at termination of each group. In each case, 0.1 mL samples were taken into tubes containing K_2EDTA and gently flicked to mix the blood and anticoagulant. Immediately following collection of each sample, 0.05 mL of whole blood was accurately measured into a polypropylene tube containing exactly 0.05 mL of deionised water, gently mixed and placed directly onto dry ice and then was stored frozen (≤ -70 °C), prior to analysis. NOA409045 was extracted and the samples were analysed by LC-MS/MS for NOA409045, alongside samples of blank matrix and matrix spiked with the test item.

Table A 14: Micronucleus Test: Experimental Design

Group number	Number of animals	Dose level (mg/kg/day) NOA409045
1	6	Negative Control
2	6	500
3	6	1000
4	6	2000
5	6	Positive Control MMC 4 mg/kg

Animals in Groups 1 to 4 were dosed twice, approximately 24 hours apart, with vehicle alone (negative control) or NOA409045 at a dose volume of 10 mL/kg. Group 5 animals (positive Control) were given a single 4 mg/kg dose of MMC at a dose volume of 5 mL/kg.

Slide Preparation: Surviving range-finder animals were killed after the terminal blood sampling, approximately 24 hours after the second test item administration. The main study animals in Groups 1 to 4 were killed approximately 24 hours after the second test item and vehicle administration. Group 5 animals were killed approximately 24 hours after the single administration of the positive control. Two femurs from each animal were removed. The bone marrow cells from each femur were aspirated into labelled tubes and centrifuged. The supernatant was withdrawn and the cells were re-suspended in a minimal volume of foetal bovine serum. One drop of cell suspension was placed on each of two slides and spread. All slides were left to air dry and age overnight before fixing for five minutes in methanol. Fixed slides were stained for 20 to 30 minutes in 11.5 % (v/v) Giemsa in Sorensen's buffer pH 6.8.

Slide Analysis: A unique, unambiguous code was devised for each animal. Adhesive labels that covered the animal and group identity were affixed to each slide so that the analyst could see only the study number and the new code. 2000 polychromatic erythrocytes (PCE), including micronucleated PCE (MN-PCE), were counted for each animal. The numbers of normochromatic erythrocytes (NCE) and micronucleated NCE (MN-NCE) were also recorded for the first 1000 cells scored. Only areas of slides of good technical quality and appropriate staining characteristics were scored.

Results and discussions

Preliminary toxicity assay: Clinical signs observed in males following administration at 2000 mg/kg/day included decreased activity, partially closed eyes and piloerection. Two males also showed clinical signs which were consistent with aggressive behaviour by a cage mate and included moderate hairloss and scabbing and a wet lesion. At 2000 mg/kg/day in females, signs included decreased activity and closed or partially closed eyes and, in Animal 75, laboured breathing and wet ventral surface were also seen. Animal 75 was killed due to clinical condition one hour after the second dose and was subject to a macroscopic necropsy examination. At necropsy it was found that the stomach and uterus were distended, with gas in the stomach and clear fluid in the uterus. There were no clinical signs observed following administration of NOA409045 at 1000 mg/kg/day.
There was no effect on bodyweight following administration of NOA409045 at either 1000 mg/kg/day or 2000 mg/kg/day.
Based on the results of this phase, it was confirmed that the regulatory test guideline maximum dose level of 2000 mg/kg/day was well tolerated in male mice and the MTD in female mice was considered to be 1000 mg/kg/day. As the difference between the MTD in males and females was less than three-fold, the main study was conducted in male mice only.
Exposure to NOA409045 was confirmed in all range-finder blood samples.

Micronucleus test: There were no adverse clinical observations following administration of NOA409045 to male mice, nor were there any adverse clinical observations in Group 1 (negative Control) or Group 5 (positive Control).

There were no statistically significant increases in micronucleus frequency in male mice treated at any dose level of NOA409045, compared with the negative Control group.
There was no evidence of a statistically significant reduction in the PCE/NCE ratio in male mice treated with NOA409045, indicating a lack of toxicity of NOA409045 to the bone marrow. However, exposure of the bone marrow to NOA409045 was demonstrated in the range-finding phase of this study.
The animals dosed with MMC, the positive Control item, had statistically significant increases in the number of micronucleated cells compared to the concurrent Control group, which demonstrated that the test system was capable of detecting a known clastogen and that the scorers were capable of detecting micronuclei. There was no statistically significant decrease in the PCE/NCE ratio in the positive Control group, indicating a lack of toxicity to the bone marrow.

Conclusion

There was no evidence of clastogenicity or aneugenicity following oral (gavage) administration of NOA409045 up to the regulatory test guideline maximum dose level of 2000 mg/kg/day in male mice. Therefore, NOA409045 is considered to be neither clastogenic nor aneugenic in the mouse bone marrow micronucleus assay.

Micronucleus Data: Negative Control vs. Treated Groups – Males

	Negative Control 0 mg/kg/day	**NOA409045 500 mg/kg/day**	**NOA409045 1000 mg/kg/day**	**NOA409045 2000 mg/kg/day**	**MMC 4 mg/kg**
N	6	6	6	6	6
Mean MN-PCE	0.33	0.17	0.33	1.00	42.83[FFF]
SD	0.52	0.41	0.52	0.63	7.22
Mean MN-PCE +SD	0.85	0.57	0.85	1.63	50.06
Mean MN-PCE –SD	-0.18	-0.24	-0.18	0.37	35.61
Mean MN-PCE ratio	0.62	0.63	0.55	0.60	0.48
SD	0.15	0.19	0.15	0.18	0.12
Mean PCE/NCE +SD	0.77	0.82	0.70	0.78	0.60
Mean PCE/NCE -SD	0.48	0.45	0.40	0.42	0.36

MMC: Mitomycin C
N: number of animals
FFF: statistically significant (Fisher Exact test) $p<0.001$
Note: any discrepancy in this table is due to rounding differences

Mouse Historical Control Data

Males Negative Control							
	N	Mean	SD	Range (mean +/- SD)		Range (min / max)	
PCE	297	2017.4	129.0	188.5	2161.4	2000	3004
NCE/1000 cells	297	550.4	83.2	467.2	633.6	327	825
MN-PCE	297	1.4	1.5	-0.1	2.9	0	8
MN-NCE	297	0.3	0.6	-0.3	0.9	0	3
PCE/NCE Ratio	297	0.9	0.3	0.6	1.2	0.2	2.1
Males Positive Control							
	N	Mean	SD	Range (mean +/- SD)		Range (min / max)	
PCE	259	2020.1	138.2	1881.9	2158.3	2000	3010
NCE/1000 cells	259	645.9	93.1	552.7	739.0	372	918
MN-PCE	259	102.3	56.6	45.7	158.9	9	354
MN-NCE	259	0.6	0.9	-0.2	1.5	0	6
PCE/NCE Ratio	259	0.6	0.3	0.3	0.8	0.1	1.7

(Dunton, J. 2015)

Assessment and conclusion by applicant

Assessment:

The study was performed according to the 1997 version of OECD 474 and was compliant with the guideline that was in force at that time. However there are minor deficiencies in the study when it is compared to the current version of OECD 474 (2016). Only 2000 polychromatic erythrocytes (PCE) per dose level and control were analysed for micronuclei and different assay acceptance and evaluation criteria used compared to those recommended by OECD 474 (2016). The historical control data were not described according to the requirements of OECD 474 (2016). Overall, all the differences are considered to have not impacted the integrity or validity of the data generated. The study is scientifically valid.

The test is considered to meet the acceptance criteria as defined by OECD 474 (2016):

- OECD 474 2016 specifies clear requirements for demonstration of laboratory proficiency and maintenance of historical control data. For the current study the performing laboratory has a well-established record in performing the assay.
- HCD should be expressed as 95% (control limit, control interval), previously whole range. In the study report ranges and mean +/- SD are presented. This has no impact on the current study.
- OECD 474 2016 Data acceptance and evaluation criteria are specified and comparisons to historical control data are required for both control and treated cultures. For the current study the negative control response was close to the mean value of the negative control HCD, and the positive control response was similar to the mean positive control HCD response, additionally the positive control response was statistically significant. Hence, the study is fully acceptable.
- The concurrent vehicle control data are acceptable for addition to a historical control database.
- The concurrent positive controls induced a clear increase in micronucleated PCE compared with the concurrent vehicle control.
- OECD 474 2016: Requirement for proof of exposure of target tissue. In the current study bioanalytical data (qualitative determination in blood) are presented. These show the test substance to be systemically bioavailable.
- OECD 474 2016: 4000 PCE should be scored per animal in 5 animals for micronuclei and a 500 erythrocytes per animal assessed for determination of toxicity. In the 1997 version this was 2000 and 200 respectively. The test item was administered up to the MTD above which dose limiting toxicity was observed and systemic exposure was demonstrated by bioanalysis. In the current study 6 animals per treatment group were assessed for micronucleus formation in 2000 PCE per animal, in excess of the 1997 TG requirement. The Positive control gave a clear positive response, hence the sensitivity of the assay is demonstrated. An appropriate number of doses and cells has been analysed. Although <4000 PCE were examined per animal the data are consistently negative at 3 different dose levels. The reduced number of PCE examined per animals is considered to not have affected the sensitivity of the assay, additionally more animals per treatment group were used (six) than specified in the OECD TG (five).
- The criteria for the selection of highest dose are consistent with those described by OECD 474.
- OECD 474 2016: Test for statistical significance should be performed. Statistical analysis of the data was performed.
- OECD 474 2016: Trend test should be performed. A trend test was not performed on the data, however all treated groups had lower mean MN frequencies than the negative control group therefore a trend test would not provide any additional value to data interpretation.
- OECD 474 2016: Definition of "clear negative" and "clear positive" results. In the current study no increases in MN frequency were observed in treated groups, hence the criteria for study interpretation used in the report are satisfactory. Although no trend test was conducted the study may still be considered to be clearly negative.

Conclusion:

The study complies with the data requirements given in Commission Regulation No 283/2013.
The test substance does not induce micronuclei in the bone marrow of orally treated mice.

A 2.11.3 Metalaxyl-M – Oral (Gavage) Mouse Micronucleus Test

Comments of zRMS:	In DRAR 2013 during per review of the active substance metalaxyl-M, genotoxicity potential has been discussed in details. Special focus has been done on micronucleus assay. During experts commenting time (RT 2014) it was agreed that observed outcome of available studies does not trigger a conclusion as 'positive' for *in-vivo* somatic cell clastogenicity. Overall, taking into account all data from the 14 genotoxicity studies, experts considers the substance not genotoxic. *In vivo* Study Dunton, J (2015) Oral (Gavage) Mouse Micronucleus Test supports mentioned above considerations that Metalaxyl-M is devoid of clastogenic properties in-vivo. Noted differences in the TG has been discussed by the Applicant. The study was performed according to the 1997 version of OECD 474 and was compliant with the guideline that was in force at that time. However there are minor deficiencies in the study when it is compared to the current version of OECD 474 (2016). Only 2000 polychromatic erythrocytes (PCE) per dose level and control were analysed for micronuclei and different assay acceptance and evaluation criteria used compared to those recommended by OECD 474 (2016). The historical control data were not described according to the requirements of OECD 474 (2016). zRMS may accept clarification and to consider all the differences as not impacted the integrity or validity of the study.

Report author	Dunton, J
Report year	2015
Report title	Metalaxyl-M – Oral (Gavage) Mouse Micronucleus Test.
Report No	BFI0262
Guidelines followed in study	OECD 474 (1997). Guideline for the testing of chemicals: Mammalian erythrocyte micronucleus test.
Major deviations from test guideline	None
Guidance in force at time of submission of supplementary dossier	OECD 474 (2016). Guideline for the testing of chemicals: Mammalian erythrocyte micronucleus test.
Differences between old and current guideline	The 2016 version of OECD 474 details 3 acceptable dosing and sampling regimens; the 1997 details 2 acceptable dosing and sampling regimens; OECD 474 2016 specifies clear requirements for demonstration of laboratory proficiency and maintenance of historical control data. Precise acceptance and evaluation criteria are specified in the 2016 version and comparisons to historical control data are required for both control and treated cultures. The OECD 474 2016 guideline specifies 4000 PCE should be scored for micronuclei and a total of 500 erythrocytes assessed for determination of toxicity. In the 1997 version these numbers were 2000 PCE and 200 eythrocytes respectively.
Previous evaluation	Yes
GLP/Officially recognised testing facilities	Yes

Reference	KCA 5.4.2
Report	Metalaxyl-M – Oral (Gavage) Mouse Micronucleus Test. Sequani Ltd. Sequani Dunton, J, 2015 Report No.BFI0262 Syngenta File No. CGA329351_11683.
Guideline(s)	OECD 474 (1997): OPPTS 870.5395 (1998): 2000/32/EC 440/2008 B.12 (2008)
Deviations	No
GLP	Yes
Acceptability	Yes

EXECUTIVE SUMMARY

Metalaxyl-M was tested to evaluate its potential to cause damage to chromosomes or cell division apparatus, or to cause cell cycle interference, leading to micronucleus formation in polychromatic erythrocytes in the bone marrow of young adult mice.
In all phases, the dosing of the vehicle and test item was by oral (gavage) administration twice, separated by approximately 24 hours, where appropriate.
In the dose sighting phase, groups of two male mice were given Metalaxyl-M as an emulsion in 0.5 % w/v carboxymethylcellulose with 0.1 % v/v Tween 80 at 300, 500 or 400 mg/kg/day, in order to determine the maximum tolerated dose (MTD).

In the range-finding phase, groups of up to three male and/or three female mice were given Metalaxyl-M at 400 mg/kg/day or 200 mg/kg/day, in order to confirm the MTD in both male and female mice.
The MTD was confirmed as 400 mg/kg/day in male mice and 200 mg/kg/day in female mice. As there was no substantial inter-sex differences in toxicity (a difference in MTD of three-fold or greater), the main study was conducted in males only, with the high dose selected as 400 mg/kg/day.
A proof of exposure phase was conducted to demonstrate that the bone marrow was exposed to the test item. This was demonstrated by analysis of test item in the whole blood of treated animals. The presence of Metalaxyl-M was confirmed by analysis of the study samples alongside samples of blank matrix and matrix spiked with the test item.
For the main study phase, three groups, each of six male mice were dosed with 100, 200 or 400 mg/kg/day Metalaxyl-M on two successive days, separated by approximately 24 hours (Groups 2 to 4). A group of six male mice (negative control - Group 1) was dosed with the vehicle alone and a positive control group (Group 5), also of six male mice, was given a single 4 mg/kg intraperitoneal dose of Mitomycin C (MMC). Animals were humanely killed approximately 24 hours after the first dose (Group 5) or second dose (Groups 1 to 4). Bone marrow was harvested from each animal and smears prepared. The stained slides were coded, 2000 polychromatic erythrocytes (PCE) per animal were scored for the presence of micronuclei and the group frequencies were statistically analysed.
There were no relevant statistically significant increases in micronucleus frequency in male mice treated at any dose level of Metalaxyl-M, compared with the negative control group.
There was no evidence of a statistically significant reduction in the PCE/NCE ratio in male mice treated with Metalaxyl-M, indicating a lack of toxicity of Metalaxyl-M to the bone marrow. However, proof of exposure to the bone marrow was demonstrated in the range-finding phase of the study.
The animals dosed with MMC, the positive control item, had statistically significant increases in the number of micronucleated cells compared to the concurrent control group, which demonstrated that the test system was capable of detecting a known clastogen and that the scorers were capable of detecting micronuclei. There was no statistically significant decrease in the PCE/NCE ratio in the positive control group, indicating a lack of toxicity to the bone marrow.

In conclusion, it can be stated that there was no evidence of clastogenicity or aneugenicity following oral (gavage) administration of Metalaxyl-M up to the MTD of 400 mg/kg/day in male mice. Therefore, Metalaxyl-M is considered to be neither clastogenic nor aneugenic in this bone marrow micronucleus assay.

Materials and methods

Test Material: Metalaxyl-M
Description: Yellowish liquid
Lot/Batch number: SMU4DL761
Purity: 97 %
Stability of test compound: Retest date :31 May 2019

Control Materials:

Negative control (if not vehicle) :	N/A	**Final Volume:** N/A	**Route:** N/A
Vehicle:	0.5 % w/v carboxymethylcellulose with 0.1 % v/v Tween 80	**Final Volume:** 10 mL/kg	**Route:** oral
Positive control :	Mitomycin C	**Final Doses:** 4 mg/kg	**Route:** i.p

Test Animals:

Species	Mouse
Strain	CD-1
Age/weight at dosing	5 – 6 weeks (at start of experiment); Main study: mean weight 31 g, range 26-35 g
Source	Charles River (UK) Ltd., Margate, Kent, CT9 4LT, England
Housing	Up to 3/cage
Acclimatisation period	11 days
Diet	Pelleted standard diet, *ad libitum*
Water	Tap water, *ad libitum*
Environmental conditions	Temperature: 19-21°C Humidity: 46-70% Photoperiod: 12 hours dark/12 hours light

Test compound administration:

	Dose Levels	Final Volume	Route
Dose-Sighting Phase:	300, 500, 400 mg/kg/day (males only)	10 mL/kg b.w.	oral
Range-Finding Phase:	400 mg/kg/day (males and females) 200 mg/kg/day (females only)	10 mL/kg b.w.	oral
Main Study:	100, 200, 400 mg/kg/day males only	10 mL/kg b.w	oral

Study Design and Methods:

Study initiation date: 15 May 2014 (study plan issued).
Experimental start date: 15 May 2014 (first animal arrival).
Experimental termination date: 30 July 2014 (last day of slide scoring).

Preliminary Toxicity Assay: A maximum tolerated dose (MTD) was determined, based on toxicity observed over a 24 hour observation period following oral (gavage) administration twice, separated by approximately 24 hours.

A proof of exposure phase was conducted to demonstrate that the bone marrow was exposed to the test item. This was demonstrated by analysis of test item in the whole blood of treated animals. Blood samples were obtained via the orbital sinus route from all animals in the range-finding phase at 1 hour and 4 hours post-second dose and at termination of each group. In each case, 0.1 mL samples were taken into tubes containing K_2EDTA. Metalaxyl-M was recovered from mouse blood:water [1:1 (v/v)] using an appropriate analytical procedure, and the processed samples analysed by LC-MS/MS to confirm exposure to the compound. The presence of Metalaxyl-M was confirmed by analysis of the study samples alongside samples of blank matrix and matrix spiked with the test item.

Table A 15: Micronucleus Test: Experimental Design

Group number	Number of animals	Dose level (mg/kg/day) Metalaxyl-M
1	6	Negative Control
2	6	100
3	6	200
4	6	400
5	6	Positive Control MMC 4 mg/kg

Animals in Groups 1 to 4 were dosed twice, approximately 24 hours apart, with vehicle alone (negative control) or Metalaxyl-M. Group 5 animals (positive control) were given a single 4 mg/kg dose of MMC at a dose volume of 5 mL/kg.

Slide Preparation: The range-finder animals were not allowed to recover from the anaesthetic after the terminal blood sample approximately 24 hours after the second test item administration and death was confirmed by cervical dislocation. The main study animals in Groups 1 to 4 were humanely killed approximately 24 hours after the second test item and vehicle administration. Group 5 animals were humanely killed approximately 24 hours after the single administration of the positive control. The animals were killed by exposure to rising concentrations of carbon dioxide and death was confirmed by cervical dislocation. The femurs from all animals were exposed by dissection of the surrounding muscle and connective tissues, and the shank of the bones removed. The bone marrow cells from both femurs of each animal were aspirated into labelled centrifuge tubes using a syringe containing foetal bovine serum. The bone marrow cells were centrifuged, the supernatant withdrawn, and the cells re-suspended in a minimal volume of foetal bovine serum. One drop of cell suspension was placed on each of two slides and spread by drawing an edge of a clean glass microscope slide along from the drop to the end of the slide. All slides were left to air dry and age overnight before fixing for 5 minutes in methanol. Fixed slides were stained for 20 to 30 minutes in 11.5 % (v/v) Giemsa in Sorensen's buffer pH 6.8.

Slide Analysis: A unique, unambiguous code was devised for each animal, including the positive controls. Adhesive labels that covered the animal and group identity were affixed to each slide so that the analyst could see only the study number and the new code.
2000 polychromatic erythrocytes (PCE), including micronucleated PCE (MN-PCE), were counted for each animal. The numbers of normochromatic (NCE) and micronucleated NCE (MN-NCE) erythrocytes were also recorded for the first 1000 cells scored. Only areas of slides of good technical quality and appropriate staining characteristics were scored.

Results and discussions
Dose-sighting phase: There were no clinical signs observed following administration of Metalaxyl-M at 300 mg/kg/day. Clinical signs observed following administration at 500 mg/kg/day included decreased activity, slow breathing, piloerection, partially closed eyes, cold to touch, intermittent tremors and prostration. Animals were killed due to clinical condition two hours post first-dose. At 400 mg/kg/day, signs included decreased activity, slow breathing, partially closed eyes and unsteady gait. No significant body weight loss was observed.

Range-finding phase: Clinical signs observed in males following administration at 400 mg/kg/day included decreased activity, unsteady gait, slow breathing, eyes closed or partially closed and intermittent twitching. At 400 mg/kg/day in females, signs included decreased activity, unsteady gait, slow breathing, eyes partially closed, intermittent twitching, prostration and loss of blink and righting reflex. Females were killed due to clinical condition one hour post first-dose. Administration to females at 200 mg/kg/day resulted in decreased activity, unsteady and abnormal gait, eyes partially closed and hunched posture.
Based on the results of this phase, the MTD was considered to be 400 mg/kg/day in males and 200 mg/kg/day in females. As the difference between the MTD in males and females was less than three-fold, the main study was conducted in male mice only.

There was no need to assess toxicity to the bone marrow and bone marrow smears were not analysed in the range-finding phase, as the presence of Metalaxyl-M was confirmed since the study sample chromatograms showed substantial Metalaxyl-M content when compared with those of blank matrix and matrix fortified with Metalaxyl-M.

Micronucleus test: There were no adverse clinical observations following administration of Metalaxyl-M to male mice at 100 mg/kg/day (Group 2). Nor were there any adverse clinical observations in Group 1 (negative control) or Group 5 (positive control). Decreased activity was observed in males following administration at 200 mg/kg/day (Group 3). Clinical signs observed in males following administration at 400 mg/kg/day (Group 4) included decreased activity, unsteady gait, slow breathing, eyes partially closed and intermittent tremors.
There were no statistically significant increases in micronucleus frequency in male mice treated at any dose level of Metalaxyl-M, compared with the negative control group.
There was no evidence of a statistically significant reduction in the PCE/NCE ratio in male mice treated with Metalaxyl-M, indicating a lack of toxicity of Metalaxyl-M to the bone marrow. However, proof of exposure to the bone marrow was demonstrated in the range-finding phase of this study.
The animals dosed with MMC, the positive control item, had statistically significant increases in the number of micronucleated cells compared to the concurrent control group, which demonstrated that the test system was capable of detecting a known clastogen and that the scorers were capable of detecting micronuclei. There was no statistically significant decrease in the PCE/NCE ratio in the positive control group, indicating a lack of toxicity to the bone marrow.

Conclusion
There was no evidence of clastogenicity or aneugenicity following oral (gavage) administration of Metalaxyl-M up to the MTD of 400 mg/kg/day in male mice. Therefore, Metalaxyl-M is considered to be neither clastogenic nor aneugenic in this bone marrow micronucleus assay.

Micronucleus Data: Negative Control vs. Treated Groups – Males

	Negative Control 0 mg/kg/day	**Metalaxyl-M 100 mg/kg/day**	**Metalaxyl-M 200 mg/kg/day**	**Metalaxyl-M 400 mg/kg/day**	**MMC 4 mg/kg**
N	6	6	6	6	6
Mean MN-PCE	1.50	0.83	0.83	1.00	64.50^{WW}
SD	1.05	0.75	0.98	0.89	16.72
Mean MN-PCE +SD	2.55	1.59	1.82	1.89	81.22
Mean MN-PCE –SD	0.45	0.08	-0.15	0.11	47.78
Mean PCE/NCE ratio	0.57	0.63	0.61	0.70	0.41
SD	0.13	0.16	0.09	0.18	0.15
Mean PCE/NCE +SD	0.70	0.79	0.70	0.88	0.57
Mean PCE/NCE -SD	0.44	0.47	0.52	0.52	0.26

MMC: Mitomycin C
N: number of animals
^{WW}: statistically significant (Wilcoxon's test) $p<0.01$
Note: any discrepancy in this table is due to rounding differences

Mouse Historical Control Data

Males Negative Control						
	N	Mean	SD	Range (mean +/- SD)		Range (min / max)	
PCE	249	2020.8	140.6	1880.2	2161.4	2000	3004
NCE/1000 cells	249	540.5	81.7	458.9	622.2	327	825
MN-PCE	249	1.5	1.5	-0.1	3.0	0	8
MN-NCE	249	0.3	0.6	-0.3	0.9	0	3
PCE/NCE Ratio	249	0.9	0.3	0.6	1.2	0.2	2.1
Males Positive Control							

	N	Mean	SD	Range (mean +/- SD)		Range (min / max)	
PCE	212	2024.5	152.5	1872.1	2177.0	2000	3010
NCE/1000 cells	212	640.6	94.8	545.8	735.4	372	918
MN-PCE	212	110.2	58.6	51.6	168.8	9	354
MN-NCE	212	0.7	0.9	-0.2	1.6	0	6
PCE/NCE Ratio	212	0.6	0.3	0.3	0.9	0.1	1.7

Assessment and conclusion by applicant

Assessment:

The study was performed according to the 1997 version of OECD 474 and was compliant with the guideline that was in force at that time. However there are minor deficiencies in the study when it is compared to the current version of OECD 474 (2016). Only 2000 polychromatic erythrocytes (PCE) per dose level and control were analysed for micronuclei and different assay acceptance and evaluation criteria used compared to those recommended by OECD 474 (2016). The historical control data were not described according to the requirements of OECD 474 (2016). Overall, all the differences are considered to have not impacted the integrity or validity of the data generated. The study is scientifically valid.

The test is considered to meet the acceptance criteria as defined by OECD 474 (2016):

- OECD 474 2016 specifies clear requirements for demonstration of laboratory proficiency and maintenance of historical control data. For the current study the performing laboratory has a well-established record in performing the assay.
- HCD should be expressed as 95% (control limit, control interval), previously whole range. In the study report ranges and mean +/- SD are presented. This has no impact on the current study.
- OECD 474 2016 Data acceptance and evaluation criteria are specified and comparisons to historical control data are required for both control and treated cultures. For the current study the negative control response was close to the mean value of the negative control HCD, and the positive control response was similar to the mean positive control HCD response, additionally the positive control response was statistically significant. Hence, the study is fully acceptable.
- The concurrent vehicle control data are acceptable for addition to a historical control database.
- The concurrent positive controls induced a clear increase in micronucleated PCE compared with the concurrent vehicle control.
- OECD 474 2016: Requirement for proof of exposure of target tissue. In the current study bioanalytical data (qualitative determination in blood) are presented. These show the test substance to be systemically bioavailable.
- OECD 474 2016: 4000 PCE should be scored per animal in 5 animals for micronuclei and a 500 erythrocytes per animal assessed for determination of toxicity. In the 1997 version this was 2000 and 200 respectively. The test item was administered up to the MTD above which dose limiting toxicity was observed and systemic exposure was demonstrated by bioanalysis. In the current study 6 animals per treatment group were assessed for micronucleus formation in 2000 PCE per animal, in excess of the 1997 TG requirement. The Positive control gave a clear positive response, hence the sensitivity of the assay is demonstrated. An appropriate number of doses and cells has been analysed. Although <4000 PCE were examined per animal the data are consistently negative at 3 different dose levels. The reduced number of PCE examined per animals is considered to not have affected the sensitivity of the assay, additionally more animals per treatment group were used (six) than specified in the OECD TG (five).
- The criteria for the selection of highest dose are consistent with those described by OECD 474.
- OECD 474 2016: Test for statistical significance should be performed. Statistical analysis of the data was performed.
- OECD 474 2016: Trend test should be performed. A trend test was not performed on the data, however all treated groups had lower mean MN frequencies than the negative control group therefore a trend test would not provide any additional value to data interpretation.

- OECD 474 2016: Definition of "clear negative" and "clear positive" results. In the current study no increases in MN frequency were observed in treated groups, hence the criteria for study interpretation used in the report are satisfactory. Although no trend test was conducted the study may still be considered to be clearly negative.

Conclusion

The study complies with the data requirements given in Commission Regulation No 283/2013.
The test substance does not induce micronuclei in the bone marrow of orally treated mice.

(Dunton, J. 2015)

A 2.11.4 CGA226048 - Oral (Gavage) Mouse Micronucleus Test

Comments of zRMS:	In the Study Dunton (2017) genotoxic potential of CGA226048 has been evaluated. There is no deviation regarding requirements for this type of studies. Under experimental conditions CGA226048 do not cause damage to chromosomes or cell division apparatus, or to cause cell cycle interference, leading to micronucleus (MN) formation in developing reticulocytes (RET) in the bone marrow of young adult mice. CGA226048 is considered to be neither clastogenic nor aneugenic. Study is reliable.

Report author	Janice Dunton
Report year	2017
Report title	CGA226048 - Oral (Gavage) Mouse Micronucleus Test
Report No	BFI0633
Guidelines followed in study	OECD 474 (2016). Guideline for the testing of chemicals: Mammalian erythrocyte micronucleus test.
Major deviations from test guideline	None
Guidance in force at time of submission of supplementary dossier	OECD 474 (2016). Guideline for the testing of chemicals: Mammalian erythrocyte micronucleus test.
Differences between old and current guideline	None
Previous evaluation	Yes
GLP/Officially recognised testing facilities	Yes

Reference	KCA 5.4.2
Report	CGA226048 - Oral (Gavage) Mouse Micronucleus Test. Sequani Ltd. Sequani Dunton, J. (2017) Report No. BFI0633, Syngenta File No. CGA226048_10000.
Guideline(s)	OECD 474 (2016): OPPTS 870.5395 (1998): 2000/32/EC 440/2008 B.12 (2008)
Deviations	No
GLP	Yes
Acceptability	Yes

EXECUTIVE SUMMARY

CGA226048 was tested to evaluate its potential to cause damage to chromosomes or cell division apparatus, or to cause cell cycle interference, leading to micronucleus (MN) formation in developing reticulocytes (RET) in the bone marrow of young adult mice.

In all phases, the dosing of the vehicle and test item was by oral (gavage) administration twice, approximately 24 hours apart.

In the range-finding phase, a group of 3 male and 3 female mice were given CGA226048 as a suspension in the vehicle, 0.5% hydroxypropylmethylcellulose (4000 cps) (HPMC) at 2000 mg/kg/day in order to determine the maximum tolerated dose (MTD) in both male and female mice. The MTD was confirmed as exceeding the guideline regulatory maximum dose level of 2000 mg/kg/day in male and female mice. As there was no inter-sex difference in toxicity, the main study was conducted in males only, with the high dose selected as 2000 mg/kg/day.

Proof of exposure was conducted as part of the range-finding phase to demonstrate that the bone marrow was exposed to the test item, via LC-MS/MS analysis of CGA226048 in the whole blood and plasma from animals taken at 15 minutes, 1, 4 and 24 hours after the second dose. The presence of CGA226048 was confirmed by analysis of the study samples using a validated method.

For the main study phase, 4 groups, each of 6 male mice were dosed with vehicle alone (negative Control) or 500, 1000 or 2000 mg/kg/day CGA226048 on 2 successive days, approximately 24 hours apart. A positive Control group, also of 6 male mice, was given a single 1 mg/kg intraperitoneal injection of Mitomycin C (MMC).
Blood samples were taken from all main study animals approximately 48 hours after the final dose administration. A minimum of 4000 and a maximum of approximately 20000 reticulocytes were scored for the presence of micronuclei for each animal and the frequency of micronucleated reticulocytes (MN-RET) was statistically analysed.
There were no statistically significant increases in MN-RET frequency in male mice given any dose level of CGA226048, compared with the negative Control group.
There were no relevant reductions in the percentage of reticulocytes (% RET) in mice given CGA226048 and, since proof of exposure to the blood and, hence, bone marrow was demonstrated in the range finding phase of the study, this indicated a lack of toxicity of CGA226048 to the bone marrow.
The animals dosed with MMC, the positive Control item, had statistically significant increases in the number of MN-RET compared with the concurrent Control group which demonstrated that the test system was capable of detecting a known clastogen. There was a statistically significant decrease in the % RET in the positive Control group, indicating toxicity to the bone marrow. Animal 29 showed no increase in the number of MN-RET detected and no decrease in the % RET, indicating that there was no apparent effect of the positive Control. It was considered that this animal had been dosed incorrectly and the data from this animal were not included in the statistical analysis.

In conclusion, it can be stated that there was no evidence of clastogenicity or aneugenicity following oral (gavage) administration of CGA226048 up to 2000 mg/kg/day in male mice. CGA226048 is considered to be neither clastogenic nor aneugenic in the mouse micronucleus test.

Materials and methods

Test Material: CGA226048
Description: White to off-white crystalline powder
Lot/Batch number: BPS 659/2
Active Ingredient Content (CGA226048) 99.0 % (± 2 %) (w/w)
Stability of test compound: Retest date: 30 September 2018

Control Materials:

Negative control (if not vehicle) :	N/A	**Final Volume:** N/A	**Route:** N/A
Vehicle:	0.5 % hydroxypropylmethyl-cellulose (4000 cps)	**Final Volume:** 10 mL/kg	**Route:** oral
Positive control :	Mitomycin C	**Final Doses:** 1 mg/kg	**Route:** i.p.

Test Animals:

Species	Mice
Strain	Crl:CD-1
Age/weight at dosing	6 – 7 weeks (at start of experiment); Main study: range 29 g to 37 g mean weight 34 g
Source	Charles River (UK) Ltd., Margate, Kent, CT9 4LT, England
Housing	3/cage
Acclimatisation period	11 days
Diet	Pelleted standard diet, *ad libitum*
Water	Tap water, *ad libitum*
Environmental conditions	Temperature: 19-21 °C Humidity: 48 % to 55 % Photoperiod: 12 hours dark/12 hours light

Test compound administration:

	Dose Levels	Final Volume	Route
Preliminary:	Range-finding phase: 2000 mg/kg/day (males and females)	10 mL/kg b.w.	oral
Main Study:	500, 1000, 2000 mg/kg/day males only	10 mL/kg b.w.	oral

Study Design and Methods:

Study initiation date: 20 March 2017 (study plan issued).
Experimental start date: 30 March 2017 (first animal arrival).
Experimental termination date: 12 July 2017 (last day of analysis).

Preliminary Toxicity Assay: Dosing was by oral (gavage) administration twice, separated by approximately 24 hours. Animals were observed periodically for up to 48 hours after the second dose.
Since bone marrow is well perfused, exposure of the bone marrow to the test item was assessed indirectly by collection of blood and plasma and analysis for CGA226048. Blood samples were obtained via the lateral tail vein from all animals in the range-finding phase at 15 minutes, 1, 4 and 24 hours after the second dose. At each collection, 100 µL samples were taken into tubes containing K_2EDTA anticoagulant and gently flicked to mix. Immediately following collection of each sample, 25 µL of whole blood was accurately measured into a polypropylene tube containing exactly 75 µL of acidified acetonitrile (1 % v/v formic acid in acetonitrile) [(1:3 (v/v)], vortexed and placed directly onto dry ice. Residual blood was placed on a roller to mix and then held in ice until centrifuged (3000 g, 5 minutes, at approximately 4 °C). 25 µL of the resultant plasma was aliquoted into tubes containing exactly 75 µL of acidified acetonitrile within 30 minutes of sampling. All samples were stored frozen (≤ -70 °C), before analysis. Concentrations of CGA226048 were determined using a validated bioanalytical method.

Table A 16 Micronucleus Test: Experimental Design

Group number	Number of animals	Dose level (mg/kg/day) CGA226048
1	6	Negative Control

2	6	500
3	6	1000
4	6	2000
5	6	Positive Control MMC 1 mg/kg

Animals in Groups 1 to 4 were dosed twice, approximately 24 hours apart, with vehicle alone (negative Control) or CGA226048 at a dose volume of 10 mL/kg. Group 5 animals (positive Control) were given a single 1 mg/kg dose of MMC at a dose volume of 5 mL/kg.
Animals were observed periodically for 48 hours after the last dose.

Slide Preparation: Range-finder animals were killed after the terminal blood sampling, approximately 48 hours after the second administration of the test item. The bone marrow cells from the femurs were aspirated into an individually labelled centrifuge tube containing foetal bovine serum and centrifuged. The supernatant was withdrawn and the cells were re-suspended in a minimal volume of foetal bovine serum. One drop of cell suspension was placed on each of two slides and spread. All slides were left to air dry and age overnight before fixing for five minutes in methanol. Fixed slides were stained for 20 to 30 minutes in 11.5 % (v/v) Giemsa in Sorensen's buffer pH 6.8.

Processing of blood samples for micronucleus evaluation: The main study animals in Groups 1 to 4 were killed approximately 48 hours after the second test item or vehicle administration. Group 5 animals were killed approximately 48 hours after the single administration of the positive Control. A terminal blood sample was taken for micronucleus scoring into tubes containing K_2EDTA anticoagulant and the animals were then killed by a Schedule 1 method. Blood samples were diluted in anticoagulant/diluent, supplied by Litron Laboratories, prior to fixation. Blood samples were then fixed in two separate methanol aliquots and stored at ≤ -70 °C for at least 3 days. One set of samples was then washed out of fixative and analysed. The remaining set of samples was transferred to long term storage solution for continued storage at ≤ -70 °C.

Scoring of micronuclei: All samples from the main study, along with quality control samples, were analysed by the same assay programme on a FACSVerse flow cytometer. A minimum of 4000 and a maximum of approximately 20000 RET were scored for the presence of MN for each animal.

Results and discussions
Preliminary toxicity assay: There were no adverse clinical observations and no effects on body weight following administration of CGA226048 at 2000 mg/kg/day.
Based on the results of this phase, the MTD was considered to exceed the guideline regulatory maximum dose level of 2000 mg/kg/day in males and females. As there was no difference in toxicity between males and females, the main study was conducted in male mice only.
Exposure to CGA226048 was confirmed by the presence of CGA226048 in range-finder blood and plasma samples taken 15 minutes, 1 and 4 hours after the second dose. Bone marrow smears were not analysed in the range-finding phase since the presence of CGA226048 was confirmed in the blood and plasma samples.

Micronucleus test: There were no adverse clinical observations following administration of CGA226048 to male mice at any dose level. Nor were there any adverse clinical observations in Group 1 (negative Control) or Group 5 (positive Control).
There were no statistically significant increases in MN-RET frequency in male mice given any dose level of CGA226048, compared with the negative Control group.
There was no evidence of a statistically significant reduction in the % RET in male mice given CGA226048, indicating a lack of toxicity of CGA226048 to the bone marrow. However, proof of exposure to the test item had been confirmed in blood and plasma samples taken in the range finder.
The animals dosed with MMC, the positive Control item, had statistically significant increases in the number of micronucleated cells compared with the concurrent Control group, which demonstrated that the test system was capable of detecting a known clastogen. There was a statistically significant decrease in the % RET in the positive Control group, indicating toxicity to the bone marrow.

Conclusion

There was no evidence of clastogenicity or aneugenicity following oral (gavage) administration of CGA226048 up to 2000 mg/kg/day in male mice. CGA226048 is considered to be neither clastogenic nor aneugenic in the mouse micronucleus test.

Micronucleus Data: Negative Control vs. Treated Groups

	Negative Control 0 mg/kg/day	**CGA226048 500 mg/kg/day**	**CGA226048 1000 mg/kg/day**	**CGA226048 2000 mg/kg/day**	**MMC 1 mg/kg**
N	6	6	6	6	5
Mean RET	19740.33	20230.17	20081.33	20356.67	20252.80
Mean MN-RET	45.50	48.83	44.50	44.50	415.40
Mean MN-RET frequency	0.23	0.24	0.22	0.22	2.01[WW]
Mean MN-RET frequency SD	0.06	0.02	0.05	0.07	0.79
Mean MN-RET frequency -SD	0.17	0.22	0.17	0.15	1.22
Mean MN-RET frequency +SD	0.29	0.26	0.27	0.29	2.80
Mean NCE	981208.17	1043746.67	853176.50	869982.33	5246793.60
Mean % RET	2.04	2.01	2.53	2.42	0.52[WW]
Mean % RET SD	0.37	0.51	0.79	0.55	0.36
Mean % RET -SD	1.67	1.50	1.74	1.87	0.16
Mean % RET +SD	2.41	2.52	3.32	2.97	0.88

MMC: mitomycin C
N: number of animals
WW: statistically significant (Wilcoxon's test) $p<0.01$
Note: any discrepancy in this table is due to rounding differences

Summary of Mouse Negative and Positive Control Data 2015

Males Negative Control							
	N	Mean	SD	95 % Control limit (mean +/- 2SD)		Range (min / max)	
MN-RET Frequency (MN-RET/RET)	45	0.20	0.05	0.09	0.30	0.13	0.33
% RET	45	1.80	0.57	0.65	2.95	1.16	3.32
Males Positive Control[1]							
	N	Mean	SD	95 % Control limit (mean +/- 2SD)		Range (min / max)	
MN-RET Frequency (MN-RET/RET)	30	2.65	0.77	1.10	4.19	1.06	4.24
% RET	30	0.68	1.20	-1.72	3.08	0.09	5.06

Note: any discrepancy in this table is due to rounding differences
Data was generated from individual animals
1: positive Control used was MMC 1 mg/kg administered by intraperitoneal injection
Whilst every effort has been made to ensure the accuracy of these data, they have not been audited by the QA unit.

Assessment and conclusion by applicant

Assessment:
The study was performed according to the 1997 version of OECD 474 and was compliant with the guideline that was in force at that time. However there are minor deficiencies in the study when it is compared to the current version of OECD 474 (2016). Only 2000 polychromatic erythrocytes (PCE) per dose level and control were analysed for micronuclei and different assay acceptance and evaluation criteria used compared to those recommended by OECD 474 (2016). The historical control data were not described according to the requirements of OECD 474 (2016). Overall, all the differences are considered to have not impacted the integrity or validity of the data generated. The study is scientifically valid.
The test is considered to meet the acceptance criteria as defined by OECD 474 (2016):

- OECD 474 2016 specifies clear requirements for demonstration of laboratory proficiency and maintenance of historical control data. For the current study the performing laboratory has a well-established record in performing the assay.
- HCD should be expressed as 95% (control limit, control interval), previously whole range. In the study report ranges and mean +/- SD are presented. This has no impact on the current study.
- OECD 474 2016 Data acceptance and evaluation criteria are specified and comparisons to historical control data are required for both control and treated cultures. For the current study the negative control response was close to the mean value of the negative control HCD, and the positive control response was similar to the mean positive control HCD response, additionally the positive control response was statistically significant. Hence, the study is fully acceptable.
- The concurrent vehicle control data are acceptable for addition to a historical control database.
- The concurrent positive controls induced a clear increase in micronucleated PCE compared with the concurrent vehicle control.
- OECD 474 2016: Requirement for proof of exposure of target tissue. In the current study bioanalytical data (qualitative determination in blood) are presented. These show the test substance to be systemically bioavailable.
- OECD 474 2016: 4000 PCE should be scored per animal in 5 animals for micronuclei and a 500 erythrocytes per animal assessed for determination of toxicity. In the 1997 version this was 2000 and 200 respectively. The test item was administered up to the MTD above which dose limiting toxicity was observed and systemic exposure was demonstrated by bioanalysis. In the current study 6 animals per treatment group were assessed for micronucleus formation in 2000 PCE per animal, in excess of the 1997 TG requirement. The Positive control gave a clear positive response, hence the sensitivity of the assay is demonstrated. An appropriate number of doses and cells has been analysed. Although <4000 PCE were examined per animal the data are consistently negative at 3 different dose levels. The reduced number of PCE examined per animals is considered to not have

affected the sensitivity of the assay, additionally more animals per treatment group were used (six) than specified in the OECD TG (five).

- The criteria for the selection of highest dose are consistent with those described by OECD 474.
- OECD 474 2016: Test for statistical significance should be performed. Statistical analysis of the data was performed.
- OECD 474 2016: Trend test should be performed. A trend test was not performed on the data, however all treated groups had lower mean MN frequencies than the negative control group therefore a trend test would not provide any additional value to data interpretation.
- OECD 474 2016: Definition of "clear negative" and "clear positive" results. In the current study no increases in MN frequency were observed in treated groups, hence the criteria for study interpretation used in the report are satisfactory. Although no trend test was conducted the study may still be considered to be clearly negative.

Conclusion

The study complies with the data requirements given in Commission Regulation No 283/2013.
The test substance does not induce micronuclei in the bone marrow of orally treated mice.

Appendix 3 Exposure calculations

A 3.1 Operator exposure calculations (KCP 7.2.1.1)

A 3.1.1 Calculations for metalaxyl-M

Table A 17: Input parameters considered for the estimation of operator exposure for application to low crops - tractor mounted

Substance	Metalaxyl-M	Formulation = Soluble concentrate, emulsifiable concentrate, etc.	Application rate-0.0872 kg a.s. /ha	Spray dilution = 0.436 g a.s./l	Vapour pressure = low volatile substances having a vapour pressure of <5*10-3Pa
Scenario	Leaf vegetables and fresh herbs / Outdoor / Downward spraying / Vehicle-mounted			Buffer = 2-3	Number applications = 2, Application interval = 7 days
Percentage Absoprtion	Dermal abs. for product = 5.6	Dermal abs. for in use dilution = 22	Oral = 100	Inhalation = 100	
RVNAS	0.08 mg/kg bw/day		RVAAS	Not applicable	
DFR	3 µg a.s./cm^2/kg a.s./ha		DT_{50}	30 days	

Table A 18: Estimation of longer-term operator exposure towards metalaxyl-M according to EFSA guidance

Operator Model	Mixing, loading and application AOEM				
Potential exposure	Longer term systemic exposure (mg/kg bw/day)		0.0276	% of RVNAS	34.45%
	Acute systemic exposure (mg/kg bw/day)		0.1889	% 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)		0.0170	% of RVNAS	21.22%
	Acute systemic exposure (mg/kg bw/day)		0.0796	% of RVAAS	

Table A 19: Input parameters considered for the estimation of operator exposure for application to low crops – manual hand held

Substance	Metalaxyl-M	Formulation = Soluble concentrate, emulsifiable concentrate, etc.	Application rate-0.0872 kg a.s. /ha	Spray dilution = 0.436 g a.s./l	Vapour pressure = low volatile substances having a vapour pressure of <5*10-3Pa
Scenario	Leaf vegetables and fresh herbs / Outdoor / Downward spraying / Manual-Hand held			Buffer = 2-3	Number applications = 2, Application interval = 7 days

Percentage Absoprtion	Dermal abs. for product = 5.6 Dermal abs. for in use dilution = 22	Oral = 100	Inhalation = 100
RVNAS	0.08 mg/kg bw/day	RVAAS	Not applicable
DFR	3 µg a.s./cm^2/kg a.s./ha	DT_{50}	30 days

Table A 20: Estimation of longer-term operator exposure towards metalaxyl-M according to EFSA guidance without PPE

Operator Model	Mixing, loading and application AOEM			
Potential exposure	Longer term systemic exposure (mg/kg bw/day)	0.3357	% of RVNAS	419.57%
	Acute systemic exposure (mg/kg bw/day)	0.5759	% 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)	0.0409	% of RVNAS	51.09%
	Acute systemic exposure (mg/kg bw/day)	0.2538	% of RVAAS	Not applicable

Table A 21: Estimation of longer-term operator exposure towards metalaxyl-M according to EFSA guidance with PPE

Operator Model	Mixing, loading and application AOEM			
Potential exposure	Longer term systemic exposure (mg/kg bw/day)	0.3357	% of RVNAS	419.57%
	Acute systemic exposure (mg/kg bw/day)	0.5759	% 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)	0.0332	% of RVNAS	41.53%
	Acute systemic exposure (mg/kg bw/day)	0.2311	% of RVAAS	Not applicable

Table A 22: Input parameters considered for the estimation of operator exposure for application to low crops – knapsack

Substance	Metalaxyl-M	Formulation = Soluble Concentrate, emulsifiable concentrate, etc.	Application rate-0.0872 kg a.s. /ha	Spray dilution = 0.436 g a.s./l	Vapour pressure = low volatile substances having a vapour pressure of <5*10-3Pa
Scenario	Leaf vegetables and fresh herbs / Outdoor / Downward spraying / Manual-Knapsack			Buffer = 2-3	Number applications = 2, Application interval = 7 days
Percentage Absoprtion	Dermal abs. for product = 5.6	Dermal abs. for in use dilution = 22	Oral = 100	Inhalation = 100	
RVNAS	0.08 mg/kg bw/day		RVAAS	Not applicable	
DFR	3 µg a.s./cm^2/kg a.s./ha		DT_{50}	30 days	

Table A 23: Estimation of longer-term operator exposure towards metalaxyl-M according to EFSA guidance without PPE

Operator Model	Mixing, loading and application AOEM			
Potential exposure	Longer term systemic exposure (mg/kg bw/day)	0.3420	% of RVNAS	427.53%
	Acute systemic exposure (mg/kg bw/day)	0.5454	% 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)	0.0481	% of RVNAS	60.11%
	Acute systemic exposure (mg/kg bw/day)	0.2702	% of RVAAS	Not applicable

Table A 24: Estimation of longer-term operator exposure towards metalaxyl-M according to EFSA guidance with PPE

Operator Model	Mixing, loading and application AOEM			
Potential exposure	Longer term systemic exposure (mg/kg bw/day)	0.3420	% of RVNAS	427.53%
	Acute systemic exposure (mg/kg bw/day)	0.5454	% 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)	0.0336	% of RVNAS	42.00%
	Acute systemic exposure (mg/kg bw/day)	0.2312	% of RVAAS	Not applicable

A 3.1.2 Calculations for oxathiapiprolin

Table A 25: Input parameters considered for the estimation of operator exposure for application to low crops - tractor mounted

Substance	Oxathiapiprolin	Formulation = Soluble Concentrate, emulsifiable concentrate, etc.	Application rate-0.015 kg a.s. /ha	Spray dilution = 0.075 g a.s./l	Vapour pressure = low volatile substances having a vapour pressure of <5*10-3Pa
Scenario	Leaf vegetables and fresh herbs / Outdoor / Downward spraying / Vehicle-mounted			Buffer = 2-3	Number applications = 2, Application interval = 7 days
Percentage Absoprtion	Dermal abs. for product = 1.5	Dermal abs. for in use dilution = 12	Oral = 30	Inhalation = 100	
RVNAS	0.04 mg/kg bw/day		RVAAS	Not applicable	
DFR	3 µg a.s./cm^2/kg a.s./ha		DT_{50}	30 days	

Table A 26: Estimation of longer-term operator exposure towards oxathiapiprolin according to EFSA guidance

Operator Model	Mixing, loading and application AOEM			
Potential exposure	Longer term systemic exposure (mg/kg bw/day)	0.0021	% of RVNAS	5.34%
	Acute systemic exposure (mg/kg bw/day)	0.0251	% 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)	0.0013	% of RVNAS	3.23%
	Acute systemic exposure (mg/kg bw/day)	0.0079	% of RVAAS	Not applicable

Table A 27: Input parameters considered for the estimation of operator exposure for application to low crops – manual hand held

Substance	Oxathiapiprolin	Formulation = Soluble Concentrate, emulsifiable concentrate, etc.	Application rate-0.015 kg a.s. /ha	Spray dilution = 0.075 g a.s./l	Vapour pressure = low volatile substances having a vapour pressure of <5*10-3Pa
Scenario	Leaf vegetables and fresh herbs / Outdoor / Downward spraying / Manual-Hand held			Buffer = 2-3	Number applications = 2, Application interval = 7 days
Percentage Absoprtion	Dermal abs. for product = 1.5	Dermal abs. for in use dilution = 12	Oral = 30	Inhalation = 100	
RVNAS	0.04 mg/kg bw/day		RVAAS	Not applicable	
DFR	3 µg a.s./cm^2/kg a.s./ha		DT_{50}	30 days	

Table A 28: Estimation of longer-term operator exposure towards oxathiapiprolin according to EFSA guidance without PPE

Operator Model	Mixing, loading and application AOEM			
Potential exposure	Longer term systemic exposure (mg/kg bw/day)	0.1816	% of RVNAS	453.93%
	Acute systemic exposure (mg/kg bw/day)	0.2919	% 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)	0.0215	% of RVNAS	53.80%
	Acute systemic exposure (mg/kg bw/day)	0.1352	% of RVAAS	Not applicable

Table A 29: Estimation of longer-term operator exposure towards oxathiapiprolin according to EFSA guidance with PPE

Operator Model	Mixing, loading and application AOEM				
Potential exposure	Longer term systemic exposure (mg/kg bw/day)		0.1816	% of RVNAS	453.93%
	Acute systemic exposure (mg/kg bw/day)		0.2919	% 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)		0.0183	% of RVNAS	45.76%
	Acute systemic exposure (mg/kg bw/day)		0.1264	% of RVAAS	Not applicable

Table A 30: Input parameters considered for the estimation of operator exposure for application to low crops – knapsack

Substance	Oxathiapiprolin	Formulation = Soluble Concentrate, emulsifiable concentrate, etc.	Application rate-0.015 kg a.s. /ha	Spray dilution = 0.075 g a.s./l	Vapour pressure = low volatile substances having a vapour pressure of <5*10-3Pa
Scenario	Leaf vegetables and fresh herbs / Outdoor / Downward spraying / Manual-Knapsack			Buffer = 2-3	Number applications = 2, Application interval = 7 days
Percentage Absoprtion	Dermal abs. for product = 1.5	Dermal abs. for in use dilution = 12	Oral = 30	Inhalation = 100	
RVNAS	0.04 mg/kg bw/day		RVAAS	Not applicable	
DFR	3 µg a.s./cm^2/kg a.s./ha		DT_{50}	30 days	

Table A 31: Estimation of longer-term operator exposure towards oxathiapiprolin according to EFSA guidance without PPE

Operator Model	Mixing, loading and application AOEM			
Potential exposure	Longer term systemic exposure (mg/kg bw/day)	0.1843	% of RVNAS	460.68%
	Acute systemic exposure (mg/kg bw/day)	0.2905	% 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)	0.0241	% of RVNAS	60.37%
	Acute systemic exposure (mg/kg bw/day)	0.1411	% of RVAAS	Not applicable

Table A 32: Estimation of longer-term operator exposure towards oxathiapiprolin according to EFSA guidance with PPE

Operator Model	Mixing, loading and application AOEM			
Potential exposure	Longer term systemic exposure (mg/kg bw/day)	0.1843	% of RVNAS	460.68%
	Acute systemic exposure (mg/kg bw/day)	0.2905	% 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)	0.0187	% of RVNAS	46.76%
	Acute systemic exposure (mg/kg bw/day)	0.1264	% of RVAAS	Not applicable

A 3.2 Worker exposure calculations (KCP 7.2.3.1)

A 3.2.1 Calculations for metalaxyl-M

Table A 33: Input parameters considered for the estimation of worker exposure towards metalaxyl-M according to EFSA guidance – baby leaves

- Crop type	Leaf vegetables and fresh herbs		
- Indoor or outdoor	Outdoor		
- Application method	Downward spraying		
- Application equipment	Vehicle-mounted		
- Worker's task	Reaching, picking		
- Main body parts in contact with foliage	Hand and body		
- Application rate of active substance	0.0872	kg a.s./ha	*i_AppRate*
- Number of applications	2		*i_AppNo*
- Interval between multiple applications	7	days	*i_AppInt*
- Half-life of active substance	30	days	*d_HalflifeAS*
- Multiple application factor	1.9		*d_MAF*
- Dermal absorption of the product	5.60%		*i_AbsorpProduct*
- Dermal absorption of the in-use dilution	22.00%		*i_AbsorpInuse*
- Dislodgeable foliar residue (i_AppRate*i_DFR)	0.2616	µg a.s./cm^2	*d_DFR*
- Working hours	8	hr	*d_WorkHr*
- Dermal transfer coefficient - Total potential exposure	5800	cm^2/hr	*d_DermTcUCV*
- Dermal transfer coefficient - arms, body and legs covered	2500	cm^2/hr	*d_DermTcCV1*
- Dermal transfer coefficient - hands, arms, body and legs covered	580	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*

1. Total

	Potential exposure	Work wear - arms, body and legs covered	Working wear and gloves
Total systemic exposure (mg a.s./day)	4.9420453	2.1301919	0.4942045
Total systemic exposure per kg body weight (mg/kg bw/day)	0.0823674	0.0355032	0.0082367
% of RVNAS	103%	44%	10.30%

A 3.2.2 Calculations for oxathiapiprolin

Table A 34: Input parameters considered for the estimation of worker exposure towards oxathiapiprolin according to EFSA guidance – baby leaves

- Crop type	Leaf vegetables and fresh herbs		
- Indoor or outdoor	Outdoor		
- Application method	Downward spraying		
- Application equipment	Vehicle-mounted		
- Worker's task	Reaching, picking		
- Main body parts in contact with foliage	Hand and body		
- Application rate of active substance	0.015	kg a.s./ha	*i_AppRate*
- Number of applications	2		*i_AppNo*
- Interval between multiple applications	7	days	*i_AppInt*
- Half-life of active substance	30	days	*d_HalflifeAS*
- Multiple application factor	1.9		*d_MAF*
- Dermal absorption of the product	1.50%		*i_AbsorpProduct*
- Dermal absorption of the in-use dilution	12.00%		*i_AbsorpInuse*
- Dislodgeable foliar residue (i_AppRate*i_DFR)	0.045	µg a.s./cm^2	*d_DFR*
- Working hours	8	hr	*d_WorkHr*
- Dermal transfer coefficient - Total potential exposure	5800	cm^2/hr	*d_DermTcUCV*
- Dermal transfer coefficient - arms, body and legs covered	2500	cm^2/hr	*d_DermTcCV1*
- Dermal transfer coefficient - hands, arms, body and legs covered	580	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*

1. Total

	Potential exposure	Work wear - arms, body and legs covered	Working wear and gloves
Total systemic exposure (mg a.s./day)	0.4637032	0.1998721	0.0463703
Total systemic exposure per kg body weight (mg/kg bw/day)	0.0077284	0.0033312	0.0007728
% of RVNAS	19%	8%	1.93%

A 3.3 Resident and bystander exposure calculations (KCP 7.2.2.1)

A 3.3.1 Calculations for metalaxyl-M

Table A 35: Input parameters considered for the estimation of longer term resident exposure – baby leaves

Substance	Metalaxyl-M	Formulation = Soluble Concentrate, emulsifiable concentrate, etc.	Application rate-0.0872 kg a.s. /ha	Spray dilution = 0.436 g a.s./l	Vapour pressure = low volatile substances having a vapour pressure of <5*10-3Pa
Scenario	Leaf vegetables and fresh herbs / Outdoor / Downward spraying / Vehicle-mounted			Buffer = 2-3	Number applications = 2, Application interval = 7 days
Percentage Absoprtion	Dermal abs. for product = 5.6	Dermal abs. for in use dilution = 22	Oral = 100	Inhalation = 100	
RVNAS	0.08 mg/kg bw/day		RVAAS	Not applicable	
DFR	3 µg a.s./cm^2/kg a.s./ha		DT_{50}	30 days	

Table A 36: Estimation of longer term resident exposure towards metalaxyl-M according to EFSA guidance

Resident - child	Spray drift (75th percentile) (mg/kg bw/day)	0.0026	% of RVNAS	3.23%
	Vapour (75th percentile) (mg/kg bw/day)	0.0011	% of RVNAS	1.34%
	Surface deposits (75th percentile) (mg/kg bw/day)	0.0006	% of RVNAS	0.81%
	Entry into treated crops (75th percentile) (mg/kg bw/day)	0.0060	% of RVNAS	7.49%
	All pathways (mean) mg/kg bw/day	0.0077	% of RVNAS	9.68%
Resident - adult	Spray drift (75th percentile) (mg/kg bw/day)	0.0006	% of RVNAS	0.77%
	Vapour (75th percentile) (mg/kg bw/day)	0.0002	% of RVNAS	0.29%
	Surface deposits (75th percentile) (mg/kg bw/day)	0.0002	% of RVNAS	0.30%
	Entry into treated crops (75th percentile) (mg/kg bw/day)	0.0033	% of RVNAS	4.16%
	All pathways (mean) mg/kg bw/day	0.0034	% of RVNAS	4.19%

A 3.3.2 Calculations for oxathiapiprolin

Table A 37: Input parameters considered for the estimation of longer term resident exposure – baby leaves

Substance	Oxathiapiprolin	Formulation = Soluble Concentrate, emulsifiable concentrate, etc.	Application rate-0.015 kg a.s. /ha	Spray dilution = 0.075 g a.s./l	Vapour pressure = low volatile substances having a vapour pressure of <5*10-3Pa
Scenario	Leaf vegetables and fresh herbs / Outdoor / Downward spraying / Vehicle-mounted			Buffer = 2-3	Number applications = 2, Application interval = 7 days
Percentage Absoprtion	Dermal abs. for product = 1.5	Dermal abs. for in use dilution = 12	Oral = 30	Inhalation = 100	
RVNAS	0.04 mg/kg bw/day		RVAAS	Not applicable	
DFR	3 µg a.s./cm^2/kg a.s./ha		DT_{50}	30 days	

Table A 38: Estimation of longer term resident exposure towards oxathiapiprolin according to EFSA guidance

Resident - child	Spray drift (75th percentile) (mg/kg bw/day)	0.0002	% of RVNAS	0.61%
	Vapour (75th percentile) (mg/kg bw/day)	0.0011	% of RVNAS	2.68%
	Surface deposits (75th percentile) (mg/kg bw/day)	0.0001	% of RVNAS	0.14%
	Entry into treated crops (75th percentile) (mg/kg bw/day)	0.0006	% of RVNAS	1.41%
	All pathways (mean) mg/kg bw/day	0.0017	% of RVNAS	4.23%
Resident - adult	Spray drift (75th percentile) (mg/kg bw/day)	0.0001	% of RVNAS	0.14%
	Vapour (75th percentile) (mg/kg bw/day)	0.0002	% of RVNAS	0.58%
	Surface deposits (75th percentile) (mg/kg bw/day)	0.0000	% of RVNAS	0.06%
	Entry into treated crops (75th percentile) (mg/kg bw/day)	0.0003	% of RVNAS	0.78%
	All pathways (mean) mg/kg bw/day	0.0005	% of RVNAS	1.31%

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)

Not applicable.