DRAFT REGISTRATION REPORT

Part B

Section 6

Mammalian Toxicology

Detailed summary of the risk assessment

Product code: A9873C

Product name: Wakil XL

Chemical active substances:

Cymoxanil, 100 g/kg

Fludioxonil, 50 g/kg

Metalaxyl-M, 169.6 g/kg

~~United Kingdom~~

Great Britain (GB)

NATIONAL ASSESSMENT

~~(Renewal of authorisation)~~

Submitted to support Article 7 amendment of approval of Metalaxyl-M in GB

Applicant: Syngenta

Submission date: 21/10/2021

Finalisation date: 31/01/2024

Version history

When	What
October 2021	Applicant submission to support amendment of approval under Article 7 of retained Regulation (EC) No 1107/2009
December 2023	HSE (GB) assessment added in green boxes

This is an application from Syngenta for the renewal of WAKIL XL (A9873C) under Article 43 of Regulation (EC) No. 1107/2009 following the renewal of EU approval of the active substance metalaxyl-M.

No equivalence assessment is required.

This application follows the data requirements for the active substance laid down in Regulation (EU) No. 544/2011 and the data requirements for the plant protection product laid down in Regulation (EU) No. 545/2011, also called 'old' data requirements. Metalaxyl-M is an 'AIR-2' substance which approval has been renewed in accordance with Regulation (EU) No 1141/2010, therefore Regulations (EU) No 283/2013 and (EU) No 284/2013 are not applicable to the renewal of authorizations for metalaxyl-M-containing plant protection products (derogation by Commission Regulation (EU) No 2015/1475; further details in the guidance document SANTE/11509/2013 rev. 5.2).

Following the renewal of EU approval of the active substance metalaxyl-M, the submission for the product renewal of WAKIL XL (A9873C) was made by 01 September 2020, in accordance with Article 43 of Regulation (EC) No 1107/2009.

All data relied on are provided with this application. The reference lists at Appendix 1 of dRR Part B Sections 1-10 define the data owner and data access. Data protection is a national concern and is addressed in Part A, Appendix 4.

The guidance on Renewal of Authorization according to Art 43 (SANCO/2010/13170 rev 14) requests that within the dRR 'changes to the risk assessment are highlighted'. This is the first submission of WAKIL XL (A9873C) in the dRR format of April 2015, consequently all of the summary text is previously unreviewed and should be considered as 'changed'. To facilitate the review, Syngenta has highlighted the summaries of reports not previously reviewed by the zRMS in yellow.

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	**HSE Chemicals Regulation Division (CRD), UK**
Reviewer's comments	The applicant, Syngenta Crop Protection AG, submitted this application to amend the conditions of approval of metalaxyl-M in accordance to Article 7 of Regulation 1107/2009 in Great Britain (GB). On the 5 May 2020 the Commission Implementing Regulation (EU) 2020/617 renewing the approval of the active substance metalaxyl-M, and restricting the use of seed treated with a plant protection product containing it to be sown only in greenhouses, was published[1]. The renewal of metalaxyl-M applies since 1 June 2020. Since this was before

[1] Commission Implementing Regulation (EU) 2020/617 of 5 May 2020 renewing the approval of the active substance metalaxyl-M, and restricting the use of seeds treated with plant protection products containing it, in accord-

	UK withdrawal from the EU, the Commission Implementing Regulation for the renewal of metalaxyl-M applies direct in GB. Two representative formulations were considered in the renewal of approval for metalaxyl-M, 'Apron XL' (A9642C) and 'Ridomil Gold Mz'/68 WG Fubol Gold' (A9651D). For this Article 7 amendment application in GB, two different formulations have been considered. The formulation 'Vibrance SB' (A20607B) containing 14.4 g/L metalaxyl-M, 22.5 g/L fludioxonil and 15.0 g/L sedaxane to support the field seed treatment use on sugar and fodder beet, and the formulation 'Wakil XL' (A9873C) containing 169.6 g/Kg metalaxyl-M, 100 g/Kg cymoxanil and 50 g/Kg fludioxonil) to support the field seed treatment use on peas (vining) are the basis of this Article 7 application for metalaxyl-M to GB. The applicant has re-submitted the draft registration reports prepared for the product renewals of 'Vibrance SB' and 'Wakil XL' under Article 43 of Regulation No 1107/2009 following the renewal of approval of the active substance metalaxyl-M. The information and data submitted within these draft registration reports have been considered previously by HSE for the applications for authorisation of a new product under Article 33 of Regulation No 1107/2009. Where relevant, re-evaluation of data or information has not occurred where studies have been performed in accordance with the current requirements and the results have been deemed acceptable. This draft registration report has been provided by the applicant, where required, comments have been inserted in green boxes by HSE or the text amended by the HSE in green (applicant's text has been struck through in green where necessary). HSE notes that the product authorisations for 'Vibrance SB' and 'Wakil XL' were withdrawn in GB by the applicant. This was based on the approval restriction provided for in Commission Implementing Regulation (EU) 2020/617 that only the treatment of seeds intended to be sown in greenhouses may be authorised. Since all authorised GB uses of 'Vibrance SB' and 'Wakil XL' products are on seeds which are direct drilled in the field, these products do not comply with the restriction and therefore could not be renewed under Article 43 of Regulation No 1107/2009. HSE notes that no authorisation for 'Vibrance SB' or 'Wakil XL' is sought within this Article 7 amendment application. Therefore, HSE has only considered the information presented in the draft registration reports that relate to metalaxyl-M. For a future GB authorisation of these products a separate application would be required with a full evaluation of the data and information for all active substances present in the formulation. Note that as of 1st January 2024, The Retained EU Law (Revocation and Reform) Act 2023 has taken effect and retained EU law are now known as assimilated law. As this assessment has been prepared prior to the Retained EU Law Act taking effect, assessment may still refer to "retained" regulation as opposed to "assimilated".

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6 Mammalian Toxicology (KCP 7)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	**HSE Chemicals Regulation Division (CRD), UK**
Reviewer's comments	Toxicology: The product Wakil XL (A9873C) has been evaluated as a representative use for the Article 7 evaluation of the active substance metalaxyl-M. Wakil XL was previously authorised under an Article 33 Following Zonal application. Where relevant, re-evaluation of data to address human health hazard classification or dermal absorption values have not occurred. Where possible the conclusions from the following zonal evaluation have been confirmed under this Article 7 evaluation. For the groundwater metabolite relvance assessment and dermal absorption values, only the active substance metalaxyl-M has been evaluated. Combined toxicity between active substances present in Wakil XL (A9873C) not been evaluated under the Article 7 evaluation, only the toxicity of metalaxyl-M has been considered.

6.1 Summary

Table 6.1-1: Information on A9873C *

Product name and code	Wakil XL / A9873C
Formulation type	Water dispersible granules (WG)
Active substance(s) (incl. content)	Cymoxanil: 100 g/kg Fludioxonil: 50 g/kg Metalaxyl-M: 169.6 g/kg
Function	Fungicide
Product already evaluated as the 'representative formulation' during the approval of the active substance(s)	No
Product previously evaluated in another MS according to Uniform Principles	Yes

* Information on the detailed composition of A9873C 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 A9873C according to Regulation (EC) No 1272/2008

Hazard class(es), categories:	Reproductive toxicity Category 2 Specific target organ toxicity - repeated exposure Category 2
Hazard pictograms or Code(s) for hazard pictogram(s):	GHS09; GHS08
Signal word:	Warning
Hazard statement(s):	H361fd Suspected of damaging fertility. Suspected of damaging the unborn child. H373 May cause damage to organs through prolonged or repeated exposure.
Precautionary statement(s):	**Prevention:** P201 Obtain special instructions before use. P260 Do not breathe dust/ fume/ gas/ mist/ vapours/ spray. P280 Wear protective gloves/ protective clothing/ eye protection/ face protection. **Response:** P308 + P313 IF exposed or concerned: Get medical advice/ attention. **Disposal:** P391 Collect spillage.
Additional labelling phrases:	To avoid risks to human health and the environment, comply with the instructions for use. [EUH401] Contains cymoxanil. May produce an allergic reaction. [EUH208] Hazardous components which must be listed on the label: 2-cyano-N-[(ethylamino)carbonyl]-2-(methoxyimino)acetamide

Table 6.1-3: Summary of risk assessment for operators, workers, bystanders and residents for A9873C

	Result	PPE / Risk mitigation measures
Operators	Acceptable	Gloves during mixing/loading, calibration and cleaning.
Workers	Acceptable	Gloves while loading hopper
Bystanders	Not applicable	Not applicable
Residents	Not applicable	Not applicable

No unacceptable risk for operators was identified when the product is used as intended and provided that the PPE/ risk mitigation measures stated in Table 6.1-3 are applied. Since A9873C is to be used indoors for the treatment of seeds prior to sowing, exposure to workers, bystanders and residents is not applicable.

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

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	**HSE Chemicals Regulation Division (CRD), UK**
Reviewer's comments	Toxicology: Based on the information available, the formulated product A9873C meets the criteria for classification for the following human health hazard in accordance with Regulation 1272/2008 (CLP): **Reproductive toxicity Category 2 (Fertility and Development) (H361fd)**

Specific Target Organ Toxicity- Repeat Exposure Category 2 (H373)

The following label elements should be used with respect to human health:

Hazard class(es), categories	Repro. Cat. 2, H361fd STOT-RE Cat. 2 H373
Hazard pictograms or Code(s) for hazard picto-gram(s)	GHS08
Signal word	Warning
Hazard statement(s)	Suspected of damaging fertility. Suspected of dam-aging the unborn child. May cause damage to organs through prolonged or repeated exposure
Precautionary Statements triggered by human health hazard classification P280 Wear protective gloves/protective clothing/eye protection/face protection P308 + P313 IF exposed or concerned: Get medical advice/attention. P314: Get medical advice/ attention if you feel unwell.	
EUH208	**‘**Contains Cymoxanil. May produce an allergic reac-tion’

In addition to the human health hazard classifications, the label needs to include the additional labelling EUH208 **‘**Contains Cymoxanil. May produce an allergic reaction’.

No other classification for human health hazards is required based on the submitted in-formation and in accordance with Regulation 1272/2008.

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**	**F, Fn, Fpn G, Gn, Gpn or I ****	**Application**		**Application rate**		**PHI (d)**	**Remarks:** **(e.g. safen-er/synergist (L/ha))** **critical gap for operator, worker, bystander or resi-dent exposure based on [Exposure model]**	**Acceptability of exposure as-sessment**			
			Method / Kind **(incl. applica-tion technique *****	**Max. num-ber (min. interval between applications)** **a) per use** **b) per crop/ season**	**Max. applica-tion rate kg as/tonne seed** **a) Metalaxyl-M** **b) Fludioxonil** **c) Cymoxanil**	**Dilution factor**			**Operator**	**Worker**	**Bystander**	**Residents**
1	Combining Peas	I	Commercial Seed treatment	1 ; 1	a) 0.339 b) 0.100 c) 0.200	Not appli-cable: concentrate is used as worst case	n/a	Operator [Seed-TROPEX/study]				

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

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

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

Explanation for column 10 "Acceptability of exposure assessment"

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

Data gaps

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

Noticed data gaps are: None

6.2 Toxicological Information on Active Substances

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 substances

	Metalaxyl-M	**Fludioxonil**	**Cymoxanil**
Common Name	Metalaxyl-M	Fludioxonil	Cymoxanil
CAS-No.	70630-17-0	131341-86-1	57966-95-7
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 Category 4 Serious eye damage Category 1 **Code(s) for hazard pictogram(s):** GHS05, GHS07 **Signal word:** Danger **Hazard statement(s):** H302 Harmful if swallowed. 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 CENTER/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. Imme-	**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	**Hazard classes (s), categories:** Reproductive toxicity, Category 2 Acute toxicity, Category 4 Specific target organ toxicity - repeated exposure, Category 2 Skin sensitisation, Category 1 **Code(s) for hazard pictogram(s):** GHS07 ; GHS08 **Signal word:** Warning **Hazard statement(s):** H361fd Suspected of damaging fertility. Suspected of damaging the unborn child. H373 May cause damage to organs through prolonged or repeated exposure. (Blood, thymus) H302 Harmful if swallowed. H317 May cause an allergic skin reaction. **Precautionary statement(s):** P201 Obtain special instructions before use. P260 Do not breathe dust. P264 Wash skin thoroughly after handling. P270 Do not eat, drink or

	Metalaxyl-M	Fludioxonil	Cymoxanil
	diately call a POISON CENTER/doctor. Disposal: P501 Dispose of contents/ container to an approved waste disposal plant.		smoke when using this product. P272 Contaminated work clothing should not be allowed out of the workplace. P280 Wear protective gloves. P308 + P313 IF exposed or concerned: Get medical advice/ attention. P301 + P312 IF SWALLOWED: Call a POISON CENTER or doctor/ physician if you feel unwell. P333 + P313 If skin irritation or rash occurs: Get medical advice/ attention. P501 Dispose of contents to an approved incineration plant in accordance with local, regional and national legislations. P501 Dispose of container to a waste disposal plant in accordance with local, regional and national legislations.
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.	n/a	This substance is not considered to be 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 80 % oral absorption)	0.59 mg/kg bw/d (corrected for 80 % oral absorption)	0.01 mg/kg bw/d (corrected for 75% oral absorption)
Reference	EFSA Journal 2015;13(3):3999	*EFSA Scientific Report* (2007) 110, 1-85, Conclusion on the peer review of fludioxonil	*EFSA Scientific Report* (2008) 167, 1-116 Conclusion on the peer review of cymoxanil
Conditions to take into account/critical areas of concern with regard to toxicology			
Review 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*	The risk to fish and aquatic invertebrates is high and risk mitigation measures are required for the foliar use in vine. • Based on the available information, soil photolysis metabolites CGA 339833 and CGA 192155 (relevant for foliar spray use only) have the potential to leach to groundwater above the trigger	None

	Metalaxyl-M	Fludioxonil	Cymoxanil
	shown to be potentially clastogenic and that was not tested at appropriate levels in the toxicological studies." An on-going 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. 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 been fully addressed and full summaries of these studies are described in detail in Appendix 2 (**Error! Reference source not found.** and 0). **Reference** KCA 5.4.2, [redacted], 2015, VV-411540 KCA 5.4.2, [redacted], 2017, VV-468462	of 0.1 µg/L under vulnerable conditions (to be confirmed by new modelling). A full assessment of the toxicological relevance of these metabolites has not been performed in line with the Guidance document.	

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	**HSE Chemicals Regulation Division (CRD)**
Reviewer's comments	Toxicology: **Toxicological information on active substances contained within A9873C**

Metalaxyl-M

The information relating to the human health hazard classification of the active substance metalaxyl-M, as presented in Table 6.2-1 is correct in accordance with the GB mandatory classification of metalaxyl-M[1] and Annex VI of CLP.

The information presented in Table 6.2-1 with regards to toxicological reference values is correct in accordance with the agreed values for metalaxyl-M (EFSA Journal 2015;13(3):3999).

For the sake of clarity, the correct classification and agreed reference values for metalaxyl-M are as follows:

Metalaxyl-M (17% in product) (EFSA Journal 2015;13(3):3999)

Classification	Acute oral toxicity Category 4; H302, Serious eye damage Category 1; H318; EU CLH and GB MCL (mandatory classification)'				
AOEL	0.08 mg/kg bw/d	NOAEL = 8 mg/kg bw/d	AF= 100	Dog RDT studies (90-day, 6-month, 1 &2-years)	Increases in liver weight and AP and ALT levels; anaemia
ADI	0.08 mg/kg bw/d	NOAEL = 8 mg/kg bw/d	AF= 100	Dog RDT studies (90-day, 6-month, 1 &2-years)	Increases in liver weight and AP and ALT levels; anaemia
ARfD	0.5 mg/kg bw	NOAEL = 50 mg/kg bw/d	AF= 100	Rat Develop-mental study	Mortality, clinical signs and decrease in bw gain
AAOEL	N/A	N/A	N/A	N/A	N/A

[1] The retained CLP Regulation (EU) No. 1272/2008 as amended for Great Britain.

Cymoxanil

The information relating to the human health hazard classification of the active substance cymoxanil, as presented in Table 6.2-1 is correct in accordance with the GB mandatory classification [1] and Annex VI of CLP.

The information presented in Table 6.2-1 with regards to toxicological reference values is correct in accordance with the agreed values for cymoxanil (EFSA Scientific Report (2008) 167, 1-116).

For the sake of clarity, the correct classification and agreed reference values for cymoxanil are as follows:

Cymoxanil (10% in product) (EFSA Scientific Report (2008) 167, 1-116)

Classification	Repro. 2; H361fd, Acute Tox.4; H302, STOT RE 2; H373 (blood, thymus), Skin Sens. 1; H317; EU CLH and GB MCL (mandatory classification)				
AOEL	0.01 mg/kg bw/d	NOAEL = 1.3 mg/kg bw/d	AF= 100 +*75%	Dog 1-year study	Testes (organ weight, macroscopic and microscopic changes), epididym-

ADI	0.013 mg/kg bw/d	NOAEL = 1.3 mg/kg bw/d	AF= 100	Dog 1-year study	ides (macroscopic and microscopic changes), liver (organ weight, histology), kidney (organ weight) and thymus (histology)
ARfD	0.08 mg/kg bw	NOAEL = 8 mg/kg bw/d	AF= 100	Rabbit Teratogenicity stucy	Increased incidences of skeletal malformations, hydrocephaly and cleft palates; increased incidences of visceral malformations
AAOEL	N/A	N/A	N/A	N/A	N/A

[1] The retained CLP Regulation (EU) No. 1272/2008 as amended for Great Britain.

Fludioxonil

The information relating to the human health hazard classification of the active substance fludioxonil, as presented in Table 6.2-1 is correct in accordance with the GB mandatory classification of fludioxonil[1] and Annex VI of CLP.

The information presented in Table 6.2-1 with regards to toxicological reference values is correct in accordance with the agreed values for fludioxonil (EFSA Journal 2015;13(3):3999).

For the sake of clarity, the correct classification and agreed reference values for fludioxonil are as follows:

Fludioxonil (5% in product) (EFSA Scientific Report (2007) 110, 1-85, Conclusion on the peer review of fludioxonil)

Classification	Not classified; EU CLH and GB MCL (mandatory classification)				
AOEL	0.59 mg/kg bw/d	NOAEL = 58.5 mg/kg bw/d	AF= 100	Dog RDT studies (90-day)	Liver; increased weight, hepatocyte hypertrophy, bile duct proliferation
ADI	0.37 mg/kg bw/d	NOAEL = 37 mg/kg bw/d	AF= 100	Rat 2-years	Liver; increased weight, hepatocyte hypertrophy, bile duct proliferation Kidney; increased weight, nephropathy
ARfD	N/A	N/A	N/A	N/A	N/A
AAOEL	N/A	N/A	N/A	N/A	N/A

[1] The retained CLP Regulation (EU) No. 1272/2008 as amended for Great Britain.

6.3 Toxicological Evaluation of Plant Protection Product

A summary of the toxicological evaluation for A9873C 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.

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	**HSE Chemicals Regulation Division (CRD), UK**
Reviewer's comments	Toxicology: The applicant proposes to meet the data requirements for acute toxicity (oral, dermal), skin and eye irritation and skin sensitisation using studies previously evaluated by HSE. The studies were accepted and evaluated during the following zonal application. Summaries of the studies and confirmation of the conclusions from their evaluation can be found in Appendix 2 of this document. For acute inhalation toxicity, no data has been provided, the applicant has produced a waiver for generating data. HSE concludes that the waiver is acceptable (see Appendix 2 for details). Based on the information available, the formulated product A9873C does not meet the criteria for classification for any acute human health hazard in accordance with Regulation 1272/2008 (CLP). The product contains the active cymoxanil. Cymoxanil is classified in Category 2 for reproductive toxicity (developmental and fertility) and specific target organ toxicity- repeat exposure in accordance with the GB MCL. Cymoxanil is present at 100 g/L or 10 % w/w in the product. In accordance with Regulation 1272/2008 (CLP), the generic concentration limits are ≥ 3% and ≥ 10%, respectively; **therefore the product should be classified for Repro. Cat. 2, H361fd and STOT-RE Cat. 2 H373**.

Table 6.3-1: Summary of evaluation of the studies on acute toxicity including irritancy and skin sensitisation for A9873C

Type of test, species, model system (Guideline)	Results	ATE & Additivity Calculation Result	Acceptability	Classification [1] (acc. to the criteria in Reg. 1272/2008)	Reference
LD_{50} oral, rat (OECD 401)	> 2000 mg/kg bw (not classified)	1672.36 mg/kg Category 4 (SDS Wakil XL >2000 mg/kg, not classified)	Yes	None	[redacted], 1998, VV-376066
LD_{50} dermal, rat (OECD 402)	> 2000 mg/kg bw	>2000 mg/kg Not classified (SDS Wakil XL >2000 mg/kg, not classified)	Yes	None	[redacted], 1998, VV-376067
LC_{50} inhalation, rat	Not submitted, not necessary. Justification presented in	1.43 mg/L Category 4 (SDS Wakil XL, >2.29 mg/L MAC, not classified)	Yes	None	n/a

	Appendix 2				
Skin irritation, rabbit (OECD 404)	Non- irritating (not classified)	Not irritant Not classified (SDS Wakil XL Not irritant, not classified)	Yes	None	[redacted], 1998, VV-376068
Eye irritation, rabbit (OECD 405)	No irreversible damage.	Eye irritant Category 1 (SDS Wakil XL, Not irritant, not classified)	Yes	None	[redacted], 1998, VV-376069
Skin sensitisation, guinea pig (OECD 406, M&K)	Non-sensitising	Skin sensitizer Category 1 (SDS Wakil X, not classified)	Yes	None	[redacted], 1998, VV-376070
Supplementary studies for combinations of plant protection products	No data – not required				

[1] Proposed acute toxicity classifications are based on A9873C study results.

Although the classification of this A9873C formulation has been performed using the additivity calculation as indicated in the CLP Guidance to Regulation (EC) No 1272/2008, that ATE calculations result in a more conservative approach. However, Syngenta has also conducted acute toxicity studies on this formulation as at the time of the initial registration these studies were required for registration in the EU. 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.

Table 6.3-2: Additional toxicological information relevant for classification/labelling of A9873C

	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 statements Acute Tox.4; H302 Eye Dam.1; H318	Reg. (EC) 1272/2008 MSDS**	Hazard statement(s) H361fd Suspected of damaging fertility. Suspected of damaging the unborn child. H373 May cause damage to organs (blood, thymus) through prolonged or repeated exposure.
	Fludioxonil (>= 2.5 - < 10% w/w))	Hazard statements n/a		
	2-cyano-N-[(ethylamino)carbonyl]2-(methoxyimino)acetamide (>= 10 - < 20% (w/w))	Hazard statements Acute Tox.4; H302 Skin Sens.1; H317 Repr.2; H361fd STOT RE2; H373		
Toxicological properties of non-	Citric acid (CAS No, 77-92-9, >= 1 - <	Hazard statements Eye Irrit. 2; H319		

	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)
active substance(s) (relevant for classification of product)	10% (w/w))*			
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

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	**HSE Chemicals Regulation Division (CRD), UK**
Reviewer's comments	Toxicology: Three metabolites of metalaxyl-M are predicted to occur in groundwater at concentrations above 0.1 µg/L: SYN546520, NOA409045 and CGA67868. Metabolite CGA67868 is predicted to occur between 0.1 and 0.75 µg/L. The two other metabolites are predicted to occur in groundwater at levels above 0.75 µg/L. Toxicological data relating to these groundwater metabolites (as summarised below) have previously been evaluated at the EU level and have not been considered further under this application. A critical area of concern was raised concerning the genotoxic potential of NOA409045, based on a positive in vitro chromosome aberration assay. The applicant provided two in vivo micronucleus assays as an appropriate follow up to address the outstanding concerns regarding the clastogenic potential of NOA409045 (SANTE/11112/2019 Rev 5, 2020). The studies were conducted on NOA409045 and the racemic mixture CGA62826 (50% NOA409045 and 50% NOA436575) ([redacted] 2015b and 2014, respectively). HSE concluded the study on the racemic mixture was not required to determine the clastogenic potential of NOA409045. HSE evaluated the study on the metabolite (see Appendix 2 for details), the result of the study was negative. Therefore, the clastogenic potential of NOA409045 can be dismissed. Assessment of the relevance of these metabolites according to the stepwise procedure of the guidance document SANCO 221/2000 Rev 11; 21/10/2021 is reported in dRR Part B 10. No metabolites were found to be relevant.

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	[redacted], 1997*
Gene mutation test in chinese hamster ovary [CGA62826] (92/69/EEC B.17)	non-genotoxic	Yes	[redacted], 1998*
Gene mutation in mammalian Cells [CGA62826] (OECD 476)	non-genotoxic	Yes	[redacted], 2006*
Acute Oral Toxicity [CGA62826] (92/69/EEC B.1)	LD_{50} >2000 mg/kg	Yes	[redacted], 1996*
Acute Dermal Toxicity [CGA62826] (92/69/EEC B.3)	LD_{50} >2000 mg/kg	Yes	[redacted], 1996a*
28 Day Oral Gavage [CGA62826] (96/54/EEC B.7)	NOAEL = 1000 mg/kg/day	Yes	[redacted], 1997*
In vitro cytogenetic test [NOA409045] (OECD 473)	Positive - clastogenic	Yes	[redacted], 2014*
In vivo mouse micronucleus assay [CGA62826] (OECD 474)	Negative – non genotoxic	No	[redacted], 2014, VV-410510
In vivo mouse micronucleus assay [NOA409045] (OECD 474)	Negative – non genotoxic	Yes	[redacted], 2015, VV-28599

* indicates that a study was reviewed at EU level

SYN546520

An overview of the results of the accepted toxicological studies for groundwater metabolite SYN546520 (tested as the R/S racemate CGA108906) is given in the following table.

Table 6.4-2: Summary of the results of toxicity studies for CGA108906

Type of test, species (Guideline)	Result	Acceptability	Reference*
Ames test (OECD 471)	non-genotoxic	Yes	[redacted], 1997*
In vitro cytogenetic test (92/69/EEC B.17)	non-genotoxic	Yes	[redacted], 1998*
In vitro cytogenetic test (OECD 473)	non-genotoxic	Yes	[redacted], 2001*
Gene mutation in mammalian cells (OECD 476)	non-genotoxic	Yes	[redacted], 2001*
Acute Oral Toxicity (92/69/EEC B.1)	LD_{50} >2000 mg/kg	Yes	[redacted], 1994*

Type of test, species (Guideline)	Result	Acceptability	Reference*
Acute Dermal Toxicity (92/69/EEC B.3)	LD_{50} >2000 mg/kg	Yes	[redacted], 1996b*
28 Day Oral Gavage (96/54/EEC B.7)	NOAEL = 1000 mg/kg/day	Yes	[redacted], 1997*

* indicates that a study was reviewed at EU level

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. 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 6.4-3: 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	[redacted], 2012*
In vitro cytogenetic test[CGA92370] (440/2008/EC B10 & OECD 473)	non-genotoxic	Yes	[redacted], 2012*
Gene mutation in mammalian Cells [CGA92370] (OECD 476)	non-genotoxic	Yes	[redacted], 2012*

* indicates that a study was reviewed at EU level

6.4.2 Fludioxonil

There are no relevant metabolites for fludioxonil.

6.4.3 Cymoxanil

All cymoxanil metabolite concentrations IN-U3204, IN-W3595, IN-KQ960 and IN-JX915, relevant to the seed treatment use of A9873C, are predicted to stay below 0.1 µg/L – no groundwater assessment is required.

6.5 Dermal Absorption (KCP 7.3)

A summary of the dermal absorption rates for the active substances in A9873C are presented in the following table.

Table 6.5-1: Dermal absorption rates for active substances in A9873C

	Metalaxyl-M		Fludioxonil		Cymoxanil	
	Value	Reference	Value	Reference	Value	Reference
Concentrate	0.85 %	[redacted], 2015	10 %	Default value	0.3%	[redacted], 2015

6.5.1 Justification for proposed values - metalaxyl-M

<table>
<tr><th colspan="2">EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY</th></tr>
<tr><td>Name of authority</td><td>HSE Chemicals Regulation Division (CRD), UK</td></tr>
<tr><td>Reviewer's comments</td><td>
<u>Toxicology:</u>

Under the Article 7 evaluation of metalaxyl-M, the product Wakil XL (A9873C) has been evaluated as a representative use. As such, only the dermal absorption of metalaxyl-M has been evaluated.

The applicant proposed to meet the data requirements for dermal absorption, with the submission of an in vitro dermal absorption study ([redacted], 2015).

The study "Fludioxonil/Metalaxyl-M/Cymoxanil WG (A9873C) - The In Vitro Percutaneous Absorption of Radiolabelled Metalaxyl-M and Radiolabelled Cymoxanil in a Concentrate Through Human Split-thickness Skin", was in compliance with Good Laboratory Practice (GLP) and followed OECD TG 428. There were no deviations from the test guideline and therefore, the study is acceptable. The details of the full evaluation of the study can be found in Appendix 2 (A 2.10).

The dermal absorption of metalaxyl-M through human skin was calculated to be 0.6 ± 0.27% (mean ± standard deviation) for the concentrate. These data were interpreted in accordance with the EFSA guidance on dermal absorption (2017), including correction for variability by addition of k x SD, resulting in finalised a dermal absorption value of 0.85%, respectively.

<u>Conclusion</u>

Based on the available study, interpreted in accordance with EFSA guidance on dermal absorption (2017), the finalised dermal absorption value to be used for risk assessment for the concentrated formulation is 0.85%.

<table>
<tr><th rowspan="2"></th><th colspan="2">Metalaxyl-M</th></tr>
<tr><th>Value (%)</th><th>Reference</th></tr>
<tr><td>Concentrate</td><td>0.85</td><td>[redacted] and [redacted], 2016</td></tr>
<tr><td>In-use dilutions</td><td>N/A</td><td>Seed treatment only</td></tr>
</table>

<u>Formulated Slurry</u>

In discussion with HSE Operator Exposure specialists, it was confirmed that the formulation Wakil XL (A9873C), whilst not applied as an in-use dilution, was to be diluted into a slurry before application. The slurry consists of 2kg of product / 5L of water. In this diluted slurry, the active substance metalaxyl-M will be present at >5% w/w, therefore the slurry is considered to be a 'concentrate' in accordance with EFSA guidance on dermal absorption (2017). As such, HSE proposes to apply the relevant default dermal absorption value for a 'Water Dispersible Granule' concentrate of 10%, for the dermal absorption of the active in the slurry.
</td></tr>
</table>

Proposed dermal absorption rates for metalaxyl-M are based on a dermal absorption study on A9873C. The study results are summarized in the following table. Full summaries of studies on the dermal absorp-

tion of A9873C that have not previously been evaluated within an EU peer review process are described in detail in Appendix 2.

Table 6.5-2: Summary of the results of submitted dermal absorption study for metalaxyl-M

Test	Concentrate	Formulation in study	Acceptability of study	Justification provided on representativity of study formulation for current product	Acceptability of justification	Reference
In vitro (human)	0.85 %	A9873C	Yes	Yes (see Appendix A 2.10)	Justification accepted. Endpoint can be used for current product / Justification not accepted. Endpoint cannot be used for current product.	[redacted], 2015, VV-414733

6.5.2 Justification for proposed values - cymoxanil

Proposed dermal absorption rates for cymoxanil are based on a dermal absorption study on A9873C. The study results are summarized in the following table. Full summaries of studies on the dermal absorption of A9873C that have not previously been evaluated within an EU peer review process are described in detail in Appendix 2.

Table 6.5-3: Summary of the results of submitted dermal absorption study for cymoxanil

Test	Concentrate	Formulation in study	Acceptability of study	Justification provided on representativity of study formulation for current product	Acceptability of justification	Reference
In vitro (human)	0.3 %	A9873C	Yes / No / Supplementary	Yes (see Appendix A 2.10)	Justification accepted. Endpoint can be used for current product / Justification not accepted. Endpoint cannot be used for current product.	[redacted], 2015, VV-414733

6.5.3 Justification for proposed values - fludioxonil

No data on dermal absorption for fludioxonil in A9873C is available. Justifications for default values according to Guidance on Dermal Absorption (EFSA Journal 2017; 15(6):4873) are presented in the fol-

lowing table.

Table 6.5-4: Default dermal absorption rates for fludioxonil

	Value	Justification for value	Acceptability of justification
Concentrate	10 %	Default value	Justification accepted. Endpoint can be used for current product /Justification not accepted. Endpoint cannot be used for current product.
Dilution	N/A	Seed treatment only	Justification accepted. Endpoint can be used for current product /Justification not accepted. Endpoint cannot be used for current product.

6.6 Exposure Assessment of Plant Protection Product (KCP 7.2)

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

Product name and code	A9873C		
Formulation type	Water dispersible granules (WG)		
Category	Fungicide		
Active substances (incl. content)	**Metalaxyl-M** 169.6 g/kg	**Fludioxonil** 50 g/kg	**Cymoxanil** 100 g/kg
AOEL systemic	0.08 mg/kg bw/d	0.59 mg/kg bw/d	0.01 mg/kg bw/d
Inhalation absorption	100%	100%	100%
Oral absorption	80%	100%	75%
Dermal absorption	Concentrate: 0.85% (Based on product (A9873C)) Concentrate is used as worst case	Concentrate: 10% (Default) Concentrate is used as worst case	Concentrate: 0.33% (Based on product (A9873C)) Concentrate is used as worst case

6.6.1 Selection of critical uses and justification

The critical GAP used for the exposure assessment of the plant protection product is shown in No unacceptable risk for operators was identified when the product is used as intended and provided that the PPE/ risk mitigation measures stated in Table 6.1-3 are applied. Since A9873C is to be used indoors for the treatment of seeds prior to sowing, exposure to workers, bystanders and residents is not applicable.

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

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	**HSE Chemicals Regulation Division (CRD), UK**
Reviewer's comments	Toxicology:

Based on the information available, the formulated product A9873C meets the criteria for classification for the following human health hazard in accordance with Regulation 1272/2008 (CLP):

Reproductive toxicity Category 2 (Fertility and Development) (H361fd)
Specific Target Organ Toxicity- Repeat Exposure Category 2 (H373)

The following label elements should be used with respect to human health:

Hazard class(es), categories	Repro. Cat. 2, H361fd STOT-RE Cat. 2 H373
Hazard pictograms or Code(s) for hazard picto-gram(s)	GHS08
Signal word	Warning
Hazard statement(s)	Suspected of damaging fertility. Suspected of dam-aging the unborn child. May cause damage to organs through prolonged or repeated exposure
Precautionary Statements triggered by human health hazard classification P280 Wear protective gloves/protective clothing/eye protection/face protection P308 + P313 IF exposed or concerned: Get medical advice/attention. P314: Get medical advice/ attention if you feel unwell.	
EUH208	**‘**Contains Cymoxanil. May produce an allergic reac-tion’

In addition to the human health hazard classifications, the label needs to include the additional labelling EUH208 **‘**Contains Cymoxanil. May produce an allergic reaction’.

No other classification for human health hazards is required based on the submitted in-formation and in accordance with Regulation 1272/2008.

Table 6.1-4. A list of all intended uses within the central zone/ EU is given in Part B, Section 0.

Justification

A9873C is to be applied to large seeds (peas/beans) and various small seeds.

For large seeds there is only one proposed application rate, 2 kg product/tonne seed. Furthermore, the amount of seed treated per day is expected to be 75 tonnes for all seeds. Therefore, application to peas (Use No. 1) represents the critical GAP for large seeds.

6.6.2 Operator exposure (KCP 7.2.1)

6.6.2.1 Estimation of operator exposure

A summary of the exposure models used for estimation of operator exposure to the active substances dur-ing application of A9873C according to the critical uses is presented in Table 6.6-2.

Table 6.6-2: Exposure models for intended uses

Critical use	Pea (max. 2 kg product/tonne)
Model	SeedTROPEX [[redacted]., [redacted], [redacted], Worker Exposure During Seed Treatment and Sowing of Treated Seed in the UK and France: An Overview. Zeneca Agrochemicals, Fernhurst, Haslemere. Report No. TMF 4896.]

Industrial seed treatment - Large seeds

Operator exposure is estimated using the "Seed-TReatment OPerator EXposure" data (Seed-TROPEX). Seed-TROPEX is an exposure data base submitted to UK-PSD in 1996 for national registrations by an Industry Task Force and contains results from studies performed in the UK and France. The Seed-TROPEX data base submitted in 1996 consists of two parts: Exposure values for operators involved in seed treatment activities and exposure values for operators loading and sowing treated seed.

Data from two Seed-TROPEX studies carried out in 1993 have been used, one study in the UK monitored operators' exposure to 'Baytan' containing triadimenol, applied at 370 g/tonne seed[2] and one study in France monitored the exposure of operators to 'Germinate Double' containing anthraquinone[3], applied at 500 g/tonne seed. In the studies, operator exposure was assessed separately for the activities of equipment calibration, slurry preparation ("mixing and loading"), bagging of treated seed and cleaning of the equipment.

Data from both these studies have been combined to form a generic database that can be used to calculate potential exposure to other seed treatment products. The overview[4] summarises the UK and French data and provides guidance on how to calculate exposure to a seed treatment product using the generic data in the form of a worked example.

For all tasks, except for bagging, it is assumed that operator exposure is a result of contact with the (neat or diluted) seed dressing liquid. Therefore, the generic exposure figures are expressed in mL/operation so that the respective concentration of active substance present in the neat formulation or in the diluted seed dressing liquid is taken into account. For bagging, a constant generic exposure figure – expressed as mg/hr – is used, meaning that the amount of product applied to the seeds is not taken into account.

Since the delivery, some of the generic exposure values have been revised and the values currently being used are presented in Table 6.6-3. Although A9873C is a solid, operator exposure has been estimated using the generic exposure values to a liquid. Exposure during the handling of a solid is likely to be lower than exposure during the handling of a liquid, so these values are expected to be precautionary. Multi-activity exposure was calculated as the cumulative exposure from the calibration, mixing/loading (premix), bagging and cleaning tasks. Operators generally wore gloves for calibration, mixing/loading and cleaning, so this level of PPE has been assumed as standard.

[2] [redacted], [redacted]., [redacted] Worker Exposure During Treatment of Seed with 'Baytan'. Report No. RJ 1621B. 12th December 1994.

[3] [redacted], [redacted], [redacted] Worker Exposure During Treatment of Wheat Seed With 'Germinate Double'. Report No. 93002 HI 557/037/95.

[4] [redacted]., [redacted], [redacted], Worker Exposure During Seed Treatment and Sowing of Treated Seed in the UK and France: An Overview. Zeneca Agrochemicals, Fernhurst, Haslemere. Report No. TMF 4896. The data are property of the Seed-TROPEX Group of which Syngenta is a member.

Table 6.6-3: Generic Seed-TROPEX (UK Data) exposure values for seed treatment activities (geometric mean values)

TASK	Data normalisation	Estimated Actual Dermal Exposure	Inhalation Exposure[(a)]
Calibration	[mL/operation]	0.014	0.001
Mixing / Loading (pre-mix)	[mL/operation]	0.001	0.0001
Mixing / Loading (fast-coupling)[(b)]	[mL/operation]	0.005	0.0001
Bagging (25 kg bags)	[mg/hour]	0.698	0.0054
Cleaning	[mL/operation]	0.083	0.016

(a) Based on an average ventilation rate of 29 L/min
(b) Baytan in 10L bags-in-boxes was used in the original Seed-TROPEX studies performed in the UK. These bags were directly linked to the treater. This system did not have a high level of operator protection built in, and potential dermal exposure in mL/operator was the same for both loading systems, pre-mix and fast-couple. The 10L bags-in-boxes have now been re-placed by more sophisticated packaging designs. The Seed-TROPEX data are therefore of limited relevance for the use of more modern fast-coupling systems.

For the treatment of peas, a 20 litre (i.e. 20 kg) container has been selected as the worst case scenario for a medium sized industrial seed treatment facility. It has been assumed that 75 tonnes of seeds are treated per day.

In the 1993 Seed-TROPEX studies the operators wore a long-sleeved work jacket and long trousers as usual work wear during all tasks and in addition gloves when handling formulated product and treated seeds and cleaning machinery.

Outcome of the estimations are presented in Table 6.6-4. Detailed calculations are in A 0.708A 2.11.4. At this time, no acute AOEL has been set for any of the active substances. Consequently, no acute risk assessment has been provided.

Table 6.6-4: Estimated operator exposure during seed treatment

		Metalaxyl-M		Fludioxonil	
Model data	**Level of PPE[a]**	**Total absorbed dose (mg/kg/day)**	**% of systemic AOEL**	**Total absorbed dose (mg/kg/day)**	**% of systemic AOEL**
Industrial Seed Treatment (150 kg product/day) Container: 20 kg Mixing/loading: 8 operations/day Bagging duration: 8 hr/day Throughput: 75 tonnes peas treated/day					
Application rate:		339.2 g a.s./tonne		100 g a.s./tonne	
SeedTROPEX (Geometric mean) Multi Activity Task[b] Body weight: 60 kg	Gloves during all steps except bagging	0.0569	71	0.0370	6
	Gloves during all steps except bagging + RPE for cleaning	0.0162	20	0.0250	4
SeedTROPEX (Geometric mean) Multi Activity Task[b] Body weight: 70 kg	Gloves during all steps except bagging	0.0488	61	0.0317	5
	Gloves during all steps except bagging + RPE for cleaning	0.0139	17	0.0214	4

		Cymoxanil	
Model data	**Level of PPE[a]**	**Total absorbed dose (mg/kg/day)**	**% of systemic AOEL**
Industrial Seed Treatment (150 kg product/day) Container: 20 kg Mixing/loading: 8 operations/day Bagging duration: 8 hr/day Throughput: 75 tonnes peas treated/day			
Application rate:		200 g a.s./tonne	
SeedTROPEX (Geometric mean) Multi Activity Task[b] Body weight: 60 kg	Gloves during all steps except bagging	0.0325	325
	Gloves during all steps except bagging + RPE for cleaning	0.0085	85
SeedTROPEX (Geometric mean) Multi Activity Task[b] Body weight: 70 kg	Gloves during all steps except bagging	0.0278	278
	Gloves during all steps except bagging + RPE for cleaning	0.0073	73

(a) Seed-TROPEX Model: Operator wearing long sleeved jacket and long trousers (standard work clothing).
(b) Sum of absorbed doses and AOELs for a single operator performing calibration, fast couple mixing/loading, bagging and cleaning.

Mobile treaters

The Seed-TROPEX model does not contain data for the assessment of exposure of operators treating seeds on mobile equipment.

For the following reasons exposure to operators treating seed on mobile equipment is considered to be in the same range or less than the exposure to operators working in static plants:

- Treatment on mobile equipment is usually done outside. This will most likely lead to lower levels of dust in the vicinity of the operators compared to working in a closed environment.
- Treatment capacities are estimated to be lower (0.5 to 2 tonnes/hour) on mobile equipment compared to static industrial equipment (estimated to be in the range of 2 to 9 tonnes/hour).
- Exposure time is likely to be shorter than in static plants because part of the working day is used for movement of the treatment equipment to the farms or between farms.

On-farm treatment

The Seed-TROPEX model does not contain data for the assessment of exposure of operators treating seeds using on-farm treatment equipment.

For the following reasons exposure to operators treating seed on-farm is considered to be in the same range or less than the exposure to operators working in static plants:

- Treatment on-farm is usually done outside. This will most likely lead to lower levels of dust in the vicinity of the operators compared to working in a closed environment.
- Treatment capacities are estimated to be lower (0.5 to 2 tonnes/hour) with on-farm equipment compared to static industrial equipment (estimated to be in the range of 2 to 9 tonnes/hour).

- Exposure time is likely to be shorter than in static plants because the operator will only treat sufficient seed for planting on the farm.

6.6.2.2 Measurement of operator exposure

Operator exposure estimations for the treatment of seeds indicate that the acceptable operator exposure level (AOEL) for cymoxanil can only be achieved if RPE is worn. Therefore, a higher tier risk assessment has been performed to confirm levels of exposure for workers treating seeds with A9873C will be within acceptable levels, without the requirement of RPE. This higher tier assessment is based on a study which measures operator exposure to prochloraz and fluquinconazole during the bagging and cleaning tasks. Given the age of the studies in the SeedTROPEX model, this modern study better reflects the equipment and work practices now found in seed treatment plants and is therefore expected to provide more realistic exposure measurements for these individual tasks. For the detailed evaluation of this study please refer to Appendix 4.

The proposed application rate of A9873C is 2 kg/tonne seed, which is equivalent to 0.339 g metalaxyl-M/ kg seed, 0.100 g fludioxonil/kg seed and 0.200 g cymoxanil/kg seed. The application rates of prochloraz and fluquinconazole in the study were 0.128-0.140 g/kg seed and 0.681-0.752 g/kg seed, respectively. Although the application rates of metalaxyl-M and cymoxanil are higher than the application rate of prochloraz, as the prochloraz exposure measurements are higher than the corresponding fluquinconazole measurements, the prochloraz data have been used to conduct the higher tier risk assessment. The bagging exposure data are normalized (mg/kg a.s./handled) to reflect the actual application rates of metalaxyl-M, fludioxonil and cymoxanil/kg seed. The cleaning data are not normalized and reflect a worker performing a single task.

Whilst nine subjects were monitored during mixing and loading, the four using the dry-couple (closed-transfer) procedure for transferring the product from the product container to the seed treater had significantly lower levels of exposure than the five who used a pre-mix procedure. Therefore, these data cannot be combined into a single dataset. In addition, inhalation exposure was not measured for all operators. In order to obtain estimates for operator exposure during seed treatment, predicted exposures from the SeedTROPEX model for the mixing/loading and calibration tasks have been added to the exposure study measurements for bagging and cleaning to give a combined exposure for the four activities.

The EFSA opinion[5] recommends, for longer term exposure assessment, the realistic upper estimate of daily exposure should be taken as the higher of a) the 75th percentile calculated from the empirical dataset or b) a statistical estimate of the 75th percentile for a theoretical population of measurements from which the empirical dataset was derived. The EFSA opinion concludes "it is expected that using the 75th percentile provides a realistic upper estimate (for longer term exposure) that will very rarely, if ever, be exceeded". Following this approach empirical and parametric 75th percentile values have been calculated from the fluquinconazole exposure study for total systemic exposure. This is carried out with the assumption that the population has a log-normal distribution using the following formula:

$$\exp\left[\bar{x} + t_{n-1,a} * S * \sqrt{\left(1 + \frac{1}{n}\right)}\right]$$

where '$\bar{x}$' is the mean of the natural logarithms of the sample measurements, 'S' is the standard deviation of the logarithms of the sample measurements, 't_{n-1}' is a t statistic with 'n 1' degrees of freedom (n being the number of measurements in the sample), and 'a' is the relevant centile. Statistical analysis shows the data within the study are log normally distributed.

The predicted total systemic exposure values during the bagging and cleaning tasks are given in Table 6.6-5. The higher of the respective empirical and parametric values are used in the risk assessment for

[5]EFSA Panel on Plant Protection Products and their Residues (PPR); Scientific Opinion on Preparation of a Guidance Document on Pesticide Exposure Assessment for Workers, Operators, Bystanders and Residents. EFSA Journal 2010;8(2):1501. [65 pp.].. doi:10.2903/j.efsa.2010.1501. Available online: www.efsa.europa.eu

each active substance. The predicted exposures for all tasks, based on a 60 kg and 70 body weight are given in Table 6.6-6.

Table 6.6-5: Measured values used to calculate operator exposure during seed treatment

			Estimated Total Systemic Exposure[a] (mg/kg bw/day)			
Active substance	**TASK**	**PPE (gloves)**	**60 kg body weight**		**70 kg body weight**	
			Empirical[b]	**Parametric[b]**	**Empirical[b]**	**Parametric[b]**
Metalaxyl-M	Bagging (25 kg bags)	No	0.00036	**0.00046**	0.00031	**0.00039**
	Cleaning	Yes	0.00015	**0.00016**	0.00013	**0.00014**
Fludioxonil	Bagging (25 kg bags)	No	0.00037	**0.00045**	0.0003	**0.0004**
	Cleaning	Yes	**0.00028**	0.00027	**0.00024**	0.00023
Cymoxanil	Bagging (25 kg bags)	No	0.00021	**0.00022**	0.00018	**0.00019**
	Cleaning	Yes	0.00014	**0.00015**	0.00012	**0.00013**

(a) Inhalation exposure values from prochloraz study have been adjusted to 21 L/min.
(b) Prochloraz study values (75th percentile).

Table 6.6-6: Estimated operator exposure during seed treatment using higher tier study data - Gloves for calibration, mixing/loading and cleaning

Model data	**Level of PPE[(a)]**	**Metalaxyl-M**		**Fludioxonil**	
		Total absorbed dose (mg/kg/day)	**% of systemic AOEL**	**Total absorbed dose (mg/kg/day)**	**% of systemic AOEL**
SeedTROPEX (Geometric mean) Calibration and Mixing/loading **Prochloraz study** (75th percentile) Bagging and Cleaning Body weight: 60 kg Container: 20 kg					
Calibration	Gloves	0.0042	5.3	0.0023	0.4
Mixing/loading –fast-coupling	Gloves	0.0039	4.9	0.0043	0.7
Bagging (25 kg bags)	Standard Work Clothing	0.0005	0.6	0.0005	0.1
Cleaning	Gloves	0.0002	0.2	0.0003	0.05
Multi Activity Task[(b)]	As above	0.0088	11	0.0074	1.3
SeedTROPEX (Geometric mean) Calibration and Mixing/loading **Prochloraz study** (75th percentile) Bagging and Cleaning Body weight: 70 kg Container: 20 kg					
Calibration	Gloves	0.0036	4.5	0.0020	0.3
Mixing/loading –fast-coupling	Gloves	0.0033	4.2	0.0037	0.6
Bagging (25 kg bags)	Standard Work Clothing	0.0004	0.5	0.0004	0.1
Cleaning	Gloves	0.0001	0.2	0.0002	0.04
Multi Activity Task[(b)]	As above	0.0074	9.4	0.0063	1.0

Model data	Level of PPE[a]	Cymoxanil	
		Total absorbed dose (mg/kg/day)	% of systemic AOEL
SeedTROPEX (Geometric mean) Calibration and Mixing/loading **Prochloraz study** (75th percentile) Bagging and Cleaning Body weight: 60 kg Container: 20 kg			
Calibration	Gloves	0.0024	24
Mixing/loading –fast-coupling	Gloves	0.0019	19
Bagging (25 kg bags)	Standard Work Clothing	0.0002	2.2
Cleaning	Gloves	0.0002	1.5
Multi Activity Task[b]	As above	0.0047	46.7
SeedTROPEX (Geometric mean) Calibration and Mixing/loading **Prochloraz study** (75th percentile) Bagging and Cleaning Body weight: 70 kg Container: 20 kg			
Calibration	Gloves	0.0020	20
Mixing/loading –fast-coupling	Gloves	0.0017	17
Bagging (25 kg bags)	Standard Work Clothing	0.0002	1.9
Cleaning	Gloves	0.0001	1.3
Multi Activity Task[b]	As above	0.004	40.2

(a) No PPE: Operator wearing long sleeved jacket and long trousers (standard work clothing).
(b) Sum of absorbed doses and AOELs for a single operator performing calibration, fast couple mixing/loading, bagging and cleaning.

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY

HSE Chemicals Regulation Division (CRD), UK

Reviewer's comments:

Toxicological endpoints to be used in the exposure assessment for 'Wakil XL'

The table below summaries the toxicological endpoints for metalaxyl-M to be used in the operator exposure risk assessment.

Table 1. Summary of toxicological endpoints

	Metalaxyl-M
AOEL	0.08 mg/kg bw/day
Dermal absorption	Concentrate: 0.85%* Dilution: 10%**

* Based on in-vitro study ([redacted], 2015)
**See note on dermal absorption below for further information

HSE notes that under the Article 7 evaluation of metalaxyl-M, the product 'Wakil XL' (A9873C) has been evaluated as a representative use. Therefore, only non-dietary exposure to the active substance metalaxyl-M has been evaluated below. The product 'Wakil XL' has not been considered fully nor is able to be author-ised for use from the assessment below.

Note on dermal absorption

It is noted that the toxicological assessor for this Article 7 assessment initially concluded concentrate value

of 0.85% for metalaxyl-M, whilst no in-use dilution value was given. As the product formulation is water dispersible granule, the product requires dilution into a slurry in order for it to be used as a seed treatment. In the draft product label (submitted previously), the mixing and loading instructions were to dilute 2 kg of 'Wakil XL' into a total slurry volume of 5 L water per tonne of seed to be treated. Therefore, the use of a 0.85% dermal absorption value for the in-use dilution is not appropriate. Furthermore, the dermal absorption value of 0.85% for the concentrate was generated in a study ([redacted], 2015) where only a single concentration (representative of the formulated concentrate) was tested. Thus no other value is possible to be derived from that study.

As no other value could be derived from the applicants submitted dermal absorption study, according to the EFSA guidance on dermal absorption (EFSA Journal 2017;15(6):4873), a default dermal absorption value of 50% may be applied for the (in use) dilutions of water-based/dispersed or solid formulation types. However, the HSE toxicological assessor for this Article 7 assessment considered the use of a 50% dermal absorption value for the in-use dilution to be overly precautionary, given the dermal absorption for the concentrate was 0.85% as determined in the dermal absorption study.

In-use slurry concentrations of active substance metalaxyl-M

The previous draft product label for 'Wakil XL' stated the following mixing instructions:

MIXING AND SPRAYING

WAKIL XL should be applied through continuous flow seed treaters. Adequate seed coverage is obtained if WAKIL XL is added at the start of the conveyor or mixing process.

WAKIL XL should be made into a slurry by pouring gradually into water and mixing at the rate of 2kg product in water to reach a final volume of 5 litres of slurry. It should be applied to seed using a volume of 5 litres of slurry per tonne of seed.

Continuous flow seed treaters should be calibrated using WAKIL XL before use.

Seed must only be treated by means which incorporate engineering controls for workers' protection together with means of accurately dispensing the dose

MIXING PROCEDURE

1. Measure the required quantity of water into the mixing tank (0.96 litres water per 1 kg WAKIL XL).
2. Slowly add the required amount of WAKIL XL to the tank without agitation.
3. Leave for 10 minutes.
4. Add further water as required to make 5 litres slurry per tonne of seed treated (based on amount WAKIL XL used).
5. Agitate the mixture to form a homogenous slurry.
6. Continue agitation during use, but stop agitation if a vortex develops as volume remaining reduces.

Do not leave slurry for long periods without agitation or overnight.

Based on these instructions, 'Wakil XL' is diluted in a two-step process as follows:

- First dilution step - mix a ratio of 1 kg 'Wakil XL' to 0.96 L water, so to treat 1 tonne seeds this is equivalent to 2 kg product in 1.92 L water. The concentration of metalaxyl-M active substance in this first step would be 176.7 g a.s./L or 18%.
- Second dilution step – to make up to a 5 L slurry to treat 1 tonne seed.

In the second dilution step, the exact water volume is not known but it is estimated that approximately 3 litres would be added to the 1.92 L slurry to make up to a total of 5 L slurry to treat 1 tonne seed. In this final dilution, the concentration of the metalaxyl-M active substance would be approximately 110 g a.s./L or 11%.

As a worst case it is assumed that, to treat 1 tonne seed, 2 kg product is dissolved in maximum of 5 L water to make a 5 L slurry. From the perspective of metalaxyl-M, 2 kg product is equivalent to 0.34 kg metalaxyl-M (rounded). When considering the in-use concentration of metalaxyl-M:

Worst-case concentration in the slurry

- 0.34 kg / 5 L water = 0.068 kg.

As a percentage per 1 L of slurry

- (0.068 / 1) * 100 = **6.8%**

It can therefore be demonstrated that the minimal amount of active substance in the slurry is more than 5%.

Dermal absorption value of slurry dilution of 'Wakil XL'

As demonstrated above, the minimal amount of metalaxyl-M active substance in the slurry is more than 5%. The 2017 EFSA dermal absorption guidance states that active substances that are present in a product >5% are classified as concentrates (which has a default dermal absorption value of 10%).

When considering all available evidence, HSE considers that it is appropriate to use the default dermal absorption value of 10% (for concentrated water dispersed or solid formulations) as a worst case for the exposure risk assessment rather than a default dermal absorption value of 50% (for dilutions). As the product is not to be authorised, and as such has no proposed label, the studies have not been strictly evaluated in relation to the in use concentration. Thus, the dermal absorption value concluded for this Article 7 assessment may be revisited for a future product authorisation.

Operator Exposure

HSE does not agree with the applicants operator exposure calculations using the seed-TROPEX model in Table 6.6-4 for the proposed uses of 'Wakil XL' on combing and vining pea seeds. As stated above, HSE considers that it is appropriate to use the default dermal absorption value of 10% (for concentrated water dispersed or solid formulations) as a worst case for the operator exposure risk assessment rather than a default dermal absorption value of 50% (for dilutions). At the first tier, HSE considers that a worst-case scenario assessment using the UK version of the Seed-TROPEX Model according to the uses outlined in the Part A GAP table should be performed. Thus, HSE has conducted a standalone assessment below considering the proposed uses of 'Wakil XL' on combining and vining pea seeds. This assessment uses the UK default values of a treatment capacity of 75 tonnes of seed/day, a dilution factor of 2.5, 8 hour bagging duration and a realistic container size of 10 L. These values are summarised in the table below.

Table 2. Scenario variables assumed for operator exposure during seed treatment

Seed treated	Combining and vining pea
Amount of seed treatment capacity	75 tonnes seed/day
Cleaning tasks performed	1
Mixing/loading tasks performed	15 (based on realistic worst-case container size of 10 L)
Calibration task performed	1
Bagging performed	8 h/day
Maximum application rate	2 kg product/tonne
Amount of active substance in product	169.6 g/kg metalaxyl-M
Dilution factor	2.5 (2 kg product made up to 5 L slurry, 1:1.5 ratio)
Dermal absorption for the dilution	**Metalaxyl-M:** 10%
Inhalation absorption	100%
Operator clothing (ADE)	• Protective clothing (coveralls) and gloves when handling the concentrate, contaminated surfaces or handling treated seed. • Protective clothing (coveralls) when bagging treated seed.

The generic exposure values for this version of the Seed-TROPEX exposure model are given below.

Table 3. Task-related generic exposure values (geometric mean) for seed treatment plant operatives

Task		Total Potential Dermal Exposure (ml/operation)*	Estimated Actual Dermal Exposure (ml/operation)*	Inhalation Exposure (ml/operation) *
Calibration		0.033	0.014	0.001
Mixing / Loading	Fast-Couple	0.0052	0.005	0.0001
	Pre-mix	0.0047	0.001	0.0001
Bagging (mg/hr)	all data	1.84	0.698	**0.0054
	worst			**0.054
Cleaning		0.872	0.083	0.0160

* exposure during bagging in mg/hour
**these values are based on a combination of Seed-TROPEX data and UK HSE data

The following table shows a summary of the calculations, with full details provided in Appendix 3 (Calculation 1).

Note: In the Seed TROPEX studies, operators wore coveralls and gloves for all tasks except for bagging when only coveralls were worn (it was considered impractical for gloves to be worn during bagging operations due to the need to manipulate bag labels etc.). Therefore, the estimated actual dermal exposure values reflect this level of PPE.

Operator exposure estimate – Seed-TROPEX – Diluted product (2.5 dilution factor)

TASK	Total potential dermal exposure (mg/op)*	Estimated actual dermal exposure (mg/op)*	Inhalation exposure (mg/op)*	Frequency of operation**/ day	Total potential dermal exposure (mg/day)	Estimated actual dermal exposure (mg/day)	Inhalation exposure (mg/day)
Calibration	5.52	2.41	0.170	1	5.52	2.41	0.170
Mixing / loading	0.7961	0.194	0.017	15	11.94	2.90	0.260
Bagging (mg/hour)	1.84	0.70	0.0054	8	14.7	5.58	0.0432
Cleaning	59	5.66	1.08544	1	59	5.66	1.08544
Total route specific exposure (mg/person/day)					**91.323**	**16.556**	**1.558**
Dermal absorption undiluted product					n/a	0.85%	
Dermal absorption diluted product						10.00%	
Inhalation absorption							100%
Route specific absorbed dose (mg/kg bw/day)						**0.01949**	**0.0260**
Total absorbed dose (mg/kg bw/day)						**0.0454**	
% of AOEL						**57%**	

* exposure during bagging in mg/hour

** duration of bagging in hours/day

VARIABLES		
a.s. concentration	***169.6***	g/kg
Dilution factor	***2.5***	
Bodyweight	***60***	kg
Dermal absorption	***10%***	
AOEL	***0.08***	mg/kg bw

Based on the estimates using the UK version of Seed-TROPEX (based on geometric mean values), the systemic exposure to an operator using diluted product is calculated to be 0.0455 mg/kg bw/day, equivalent to 57% of the AOEL of metalaxyl-M. The predicted operator exposure is within acceptable limits and no further refinement is necessary.

Operators not directly involved in the seed-treatment process

The Seed-TROPEX model contains data which allows estimation of exposure of people working in the seed treatment plant, but who are not directly involved in the seed treatment process. The model contains exposure data for three forklift truck drivers operating in cereal seed treatment plants. Based on data for these

forklift truck drivers, the geometric mean levels of potential dermal exposure and potential inhalation exposure were equivalent to 7.66 x 10^{-4} ml formulation/h and 8.74 x 10^{-6} ml formulation/h, respectively.

Exposure to the diluted product is estimated assuming a dermal absorption value of 10%, a duration of exposure of 8 hours, a bystander body weight of 60 kg and no protection provided by normal work wear, systemic exposure resulting from the proposed use of 'Wakil XL' is calculated for metalaxyl-M as follows:

$$\frac{(0.000766 \text{ ml/h} \times 169.9 \text{ mg/ml} \times 8 \text{ h} \times 10\%) + (0.00000874 \text{ ml/h} \times 169.9 \text{ mg/ml} \times 8 \text{ h})}{60}$$

= 0.0019 mg/kg bw/day, equivalent to 2% of the AOEL of metalaxyl-M.

The systemic exposure to metalaxyl-M is calculated to be 0.0019 mg/kg bw/day which is equivalent to 2% of the AOEL. This is within acceptable limits.

Operator exposure in mobile treaters and during on-farm treatment

The applicant has presented a case proposing to use 'Wakil XL' as a treatment for vining and combining pea seeds in mobile treaters and as an on-farm treatment. As the Seed-TROPEX model does not contain data for the assessment of operator exposure to operators treating seeds via mobile equipment, this is assessed on a case-by-case basis.

The applicant has proposed that the use of 'Wakil XL' on vining and combining pea seeds is acceptable due to the following reasons.

Mobile plants:

- Treatment on mobile equipment is usually done outside. This will most likely lead to lower levels of dust in the vicinity of the operators compared to working in a closed environment.
- Treatment capacities are estimated to be lower (0.5 to 2 tonnes/hour) on mobile equipment compared to static industrial equipment (estimated to be in the range of 2 to 9 tonnes/hour).
- Exposure time is likely to be shorter than in static plants because part of the working day is used for movement of the treatment equipment to the farms or between farms.

On-farm treatment:

- Exposure to operators treating seed on-farm is considered to be in the same range or less than the exposure to operators working in static plants:
- Treatment on-farm is usually done outside. This will most likely lead to lower levels of dust in the vicinity of the operators compared to working in a closed environment.
- Treatment capacities are estimated to be lower (0.5 to 2 tonnes/hour) with on-farm equipment compared to static industrial equipment (estimated to be in the range of 2 to 9 tonnes/hour).
- Exposure time is likely to be shorter than in static plants because the operator will only treat sufficient seed for planting on the farm.

HSE agrees with the applicants case. The operator exposure assessment conducted above is considered to be a worst-case exposure estimate. HSE agrees that treatment in mobile plants or on-farm has a much lower treatment capacity and is usually performed outdoors, ensuring that exposure is within the risk envelope of what has been assessed above. Therefore, exposure to metalaxyl-M from the use of 'Wakil XL' on vining and combining beet seeds in mobile plants and on-farm treatment is within the risk envelope of what is assessed above.

Operator Protection Phrases

'Wakil XL' is classified with respect to human health. The classification and resulting PPE requirements are listed in the following table.

H Phrase	PPE
H361fd: Suspected of damaging fertility. Suspected of damaging the unborn child.	No PPE. Effect considered in setting of AOEL
H373: May cause damage to organs through prolonged or repeated exposure	No PPE. Effect considered in setting of AOEL

Considering the classification of 'Wakil XL' and the operator exposure risk assessment (diluted product), the following operator protection phrases are required on the product label (if the product underwent a full assessment):

- Operators must wear suitable protective clothing (coveralls) and suitable protective gloves when handling the concentrate, contaminated surfaces or handling treated seed.
- Operators must wear suitable protective clothing (coveralls) when bagging treated seed.

11.1.1 Worker exposure (KCP 7.2.3)

11.1.1.1 Estimation of worker exposure

A summary of the exposure model used for estimation of worker exposure to the active substance during the loading and sowing of seeds treated with A9873C according to the critical uses is presented in Table 6.6-1. The outcome of the estimation is presented in Table 6.6-3 (longer term exposure). Detailed calculations are in A 0.708A 2.11.4.

At this time, no acute AOEL has been set for any of the active substances. Consequently, no acute risk assessment has been provided.

Table 6.6-1: Exposure models for intended uses

Critical use	Loading and sowing seeds
Model	SeedTROPEX [[redacted]., [redacted], [redacted], Worker Exposure During Seed Treatment and Sowing of Treated Seed in the UK and France: An Overview. Zeneca Agrochemicals, Fernhurst, Haslemere. Report No. TMF 4896.]

Loading and sowing of treated seeds- large seeds

Worker exposure is estimated using the "Seed-TReatment OPerator EXposure" data (Seed-TROPEX). Seed-TROPEX is an exposure data base submitted to UK-PSD in 1996 for national registrations by an Industry Task Force and contains results from studies performed in the UK and France.

In order to estimate the likely exposure to operators involved in the loading and sowing of treated seed data combined from two worker exposure studies carried out in 1993 the UK and France have been used as a source of generic exposure data (Seed-TROPEX Studies).

The UK study monitored the sowing of wheat seed treated with 'Baytan' and measured exposure to the triadimenol component of the formulation[6]. The French study measured exposure to anthraquinone during a day of sowing of wheat seed treated with 'Germinate Double'[7].

The generic Seed-TROPEX exposure figures to estimate dermal and inhalation exposure of the operator cover exposure during both activities, loading and sowing of treated seed. These exposure figures are normalised to mg/hour and accordingly do not take into account the amount of product applied to the seed. The generic Seed-TROPEX exposure values for loading and sowing treated seeds are given in Table 6.6-2.

Table 6.6-2: Generic Seed-TROPEX exposure values for loading and sowing treated seeds (geometric mean values)

TASK	Unit of exposure	Estimated Actual Dermal Exposure	Inhalation Exposure[a]
Loading and sowing seeds	[mg/person/hour]	0.733	0.020

a) Based on an average ventilation rate of 29 L/min.

As for bagging during seed treatment, for loading and sowing activities dust is likely to be the main source of exposure, whereby it is reasonable to conclude that the contents of active substance in the dust is related to the loading of active substances on the seed. Thus, for all active substances applied to seed in lower doses than those used in the 1993 Seed-TROPEX studies (i.e. UK-study: 370 g a.s./tonne, French-study: 500 g a.s./tonne) a pure time dependent exposure figure is likely to result in a significant overestimation of operator exposure.

Exposure by inhalation of operators loading and sowing of treated seed is based on an average ventilation rate of 29 L/min, which is in accordance with the value applied in the 1993 Seed-TROPEX studies for this activity. Although loading of the hopper may be physically demanding where manual handling of seed bags is involved, this activity is usually of short duration compared to the actual sowing of the seed. During the latter the operator is driving the tractor, possibly leaving it once in a while to verify the drilling depth or to check and equalise remaining amount of seeds in the hopper. An average ventilation rate of 29 L/min for the combined loading and sowing task is therefore considered conservative.

[6] [redacted], [redacted] - Worker Exposure During Sowing of Treated Seed with 'Baytan'. Report No. WER001, issued 3 February 1995.

[7] [redacted] - Worker Exposure During Drilling of Wheat Seed Treated With Germinate Double. Report No. 93003 HI 5/42, issued March 1995.

Table 6.6-3: Estimated operator exposure during the loading and sowing of treated seeds

Model data	Level of PPE[(a)]	Metalaxyl-M		Fludioxonil	
		Total absorbed dose (mg/kg/day)	% of systemic AOEL	Total absorbed dose (mg/kg/day)	% of systemic AOEL
Loading and Sowing Treated Seed Work rate:10 hr/day					
SeedTROPEX (Geometric mean) Body weight: 60 kg	Gloves while loading hopper	0.0044	5.5	0.0156	2.6
SeedTROPEX (Geometric mean) Body weight: 70 kg	Gloves while loading hopper	0.0037	4.7	0.0133	2.3

Model data	Level of PPE[(a)]	Cymoxanil	
		Total absorbed dose (mg/kg/day)	% of systemic AOEL
Loading and Sowing Treated Seed Work rate:10 hr/day			
SeedTROPEX (Geometric mean) Body weight: 60 kg	Gloves while loading hopper	0.0037	37
SeedTROPEX (Geometric mean) Body weight: 70 kg	Gloves while loading hopper	0.0032	32

(a) Seed-TROPEX Model: Operator wearing long sleeved jacket and long trousers (standard work clothing).

11.1.1.2 Refinement of generic DFR value (KCP 7.2)

Not applicable for seed treatment products.

11.1.1.3 Measurement of worker exposure

Not required.

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY

HSE Chemicals Regulation Division (CRD), UK

Reviewer's comments:

HSE disagrees with the applicants estimates of worker exposure presented in Table 6.6-6. The applicant has presented a worker exposure estimate that considers workers to be wearing gloves while loading treated seed. As stated above, HSE considers that it is appropriate to use the default dermal absorption value of 10% (for concentrated water dispersed or solid formulations) as a worst case for the worker exposure risk assessment rather than a default dermal absorption value of 50% (for dilutions). At the first tier, HSE considers worker exposure for a worker loading/sowing treated seed without protection from clothing or PPE. Thus, HSE has conducted a worst-case worker exposure assessment using the UK version of the Seed-TROPEX Model assuming workers are not wearing PPE.

Estimated worker exposure (longer term exposure)

Model: UK SeedTROPEX model

Worker exposure drilling treated seed - Geometric mean values				
SOWING SEED				
		Total Potential Dermal exposure	Estimated Actual Dermal exposure	Inhalation exposure
Exposure during loading/sowing (mg/person/10 hour day)		14.787261	7.330926	0.2000000
Absorbed dose (mg/kg bw/day)		0.024645	0.012218	0.003333
Systemic exposure (mg/kg bw/day)		0.027979	0.015552	
% of AOEL		35%	19%	
Dermal absorption	10%			
AOEL	0.08	mg/kg bw/day		

The systemic longer term potential exposure is calculated to be equivalent to 35% of the AOEL of metalaxyl-M for a worker loading/sowing treated seed without protection from clothing or PPE. This is within acceptable limits and no further refinement is necessary.

11.1.2 Bystander and resident exposure (KCP 7.2.2)

In industrial seed treatment facilities the incidental presence of bystanders can be excluded by technical management measures. If occurring, exposure of bystanders would be of short duration and normally lower than that of seed treatment operators who are occupationally exposed all day long. The same applies for seed loading and sowing activities. Therefore, it is reasonable to assume that there will be no undue risk to persons being incidentally exposed to seed treatment or seed sowing operations.

11.1.2.1 Estimation of bystander and resident exposure

Not applicable.

11.1.2.2 Measurement of bystander and/or resident exposure

Not applicable.

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY

HSE Chemicals Regulation Division (CRD), UK

Reviewer's comments:

The treatment of vining and combing pea seeds is usually performed in professional plants where access is restricted to people working at the plant. Therefore, it is considered that bystanders and residents will not be exposed to 'Wakil XL' during the seed treatment process. Therefore, no resident/bystander exposure risk is expected. No further assessment is necessary.

11.1.3 Combined exposure

The product is a mixture of three active substances.

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	**HSE Chemicals Regulation Division (CRD), UK**
Reviewer's comments	Toxicology: Under the Article 7 evaluation of metalaxyl-M, the product Wakil XL (A9873C) has been evaluated as a representative use. As such, only the toxicity of metalaxyl-M has been considered, therefore combined toxicity between active substances present in Wakil XL (A9873C) has not been evaluated under the Article 7 evaluation.

11.1.3.1 Exposure Assessment of cymoxanil, fludioxonil, metalaxyl-M

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 converted to decimal. The Hazard Index (HI) is the sum of the individual HQs.

Table 6.6-4: Risk assessment from combined exposure – assuming 60 kg bw

Application scenario	Active Ingredient	Estimated exposure / AOEL (HQ)
Operators (60kg) – treating seeds Higher tier assesment based on prochloraz exposure study Gloves for calibration, mixing/loading and cleaning	Metalaxyl-M	0.11
	Fludioxonil	0.01
	Cymoxanil	0.47
	Cumulative risk Operators	**0.59**
Workers (60 kg) – loading and sowing treated seed [Gloves while loading hopper]	Metalaxyl-M	0.06
	Fludioxonil	0.03
	Cymoxanil	0.37
	Cumulative risk Operators	**0.46**
Bystander	Not applicable	
Resident	Not applicable	

Table 6.6-5: Risk assessment from combined exposure – assuming 70 kg bw

Application scenario	Active Ingredient	Estimated exposure / AOEL (HQ)
Operators (70kg) – treating seeds Higher tier assesment based on	Metalaxyl-M	0.09
	Fludioxonil	0.01

Application scenario	Active Ingredient	Estimated exposure / AOEL (HQ)
prochloraz exposure study Gloves for calibration, mixing/loading and cleaning	Cymoxanil	0.40
	Cumulative risk Operators	**0.50**
Workers (70 kg) – loading and sowing treated seed [Gloves while loading hopper]	Metalaxyl-M	0.05
	Fludioxonil	0.02
	Cymoxanil	0.32
	Cumulative risk Operators	**0.39**
Bystander	Not applicable	
Resident	Not applicable	

The Hazard Index is < 1. Thus, combined exposure to all active substances in A9873C is not expected to present a risk for operators, workers, residents and bystanders.

No further refinement of the assessment is required.

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY

HSE Chemicals Regulation Division (CRD), UK

Reviewer's comments:

HSE notes that under the Article 7 evaluation of metalaxyl-M, the product 'Wakil XL' (A9873C) has been evaluated as a representative use. Therefore, only non-dietary exposure to the active substance metalaxyl-M has been evaluated. Thus, a combined exposure assessment for the proposed uses of 'Wakil XL' has not been considered.

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	[redacted]	31/07/1998	Acute oral toxicity in the rat. Report No. 983060 Document No. VV-376066 , CGA173506/1166 Test Facility [redacted] GLP Unpublished	Y	SYN
KCP 7.1.2	[redacted]	04/08/1998	Acute dermal toxicity in the rat. Report No. 983061 Document No. VV-376067 , CGA173506/1167 Test Facility [redacted] GLP Unpublished	Y	SYN
KCP 7.1.4	[redacted]	04/08/1998	Acute dermal irritation/corrosion in the rabbit. Report No. 983062 Document No. VV-376068 , CGA173506/1168 Test Facility [redacted] GLP Unpublished	Y	SYN
KCP 7.1.5	[redacted]	31/07/1998	Acute eye irritation/corrosion in the rabbit. Report No. 983063 Document No. VV-376069 , CGA173506/1169 Test Facility [redacted] 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 7.1.6	[redacted]	31/07/1998	Skin sensitization in the Guinea pig. Report No. 983064 Document No. VV-376070 , CGA173506/1170 Test Facility [redacted] GLP Unpublished	Y	SYN
KCP 7.2.1.1	[redacted]	23/02/2009	Fluquinconazole and Prochloraz: Determination of Operator Exposure During Cereal Seed Treatment with "Jockey" Fungicide in Germany, United Kingdom and France. Report No. ACI07-006 Document No. VV-393832 , ASF827_10000 Test Facility Agrochemex International Ltd. GLP Unpublished	N	Seed Tropex Group (SYN access)
KCP 7.2.1.1	[redacted]	21/09/2006	Determination of Operator Exposure to Imidacloprid during Treatment of Sugar Beet Seeds with IMPRIMO in France Report No. 04B033 HI Document No. VV-379857 , ASF654/0001 Test Facility RHODIA Recherches et Technologies GLP Unpublished	N	Seed Tropex Group (SYN access)
KCP 7.2.1.1 / 02	[redacted]	2007	Determination of operator exposure to imidacloprid during loading/sowing of GAUCHO treated maize seeds under realistic field conditions in Germany and Italy Syngenta Crop Protection AG, Basel, Switzerland , IF-05/00328969 GLP not published Syngenta File No ASF654/0002	N	Seed Tropex Group (SYN access)
KCP 7.3	[redacted]	22/10/2015	Fludioxonil/Metalaxyl-M/Cymoxanil WG (A9873C) - The In Vitro Percutaneous Absorption of Radiolabelled Metalaxyl-M and Radiolabelled Cymoxanil in a Concentrate Through Human Split thickness Skin	N	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
			Report No. 36500 Document No. VV-414733 , A9873C_10365 Test Facility Charles River Laboratories GLP Unpublished		
KCA1 5.2.1	[redacted]	2013	Validation of Multi-Residue Method DFG S19 (LC-MS/MS module) for the Determination of Residues of Cymoxanil in Tomato, Grapes, Oilseed Rape and Wheat Grain. DuPont-35769. DuPont Report No. 35769 Eurofins Agroscience Services Chem GmbH (EAS Chem) GLP Unpublished	N	Du Pont (SYN access)
KCA1 5.2.1	[redacted]	2013	Independent Laboratory Validation of Multi-Residue Method DFG S19 for the Determination of Residues of Cymoxanil in Tomato, Grapes, Oilseed Rape and Wheat Grain using LC-MS/MS - DuPont-35770. Výzkumný ústav organických syntéz a.s. (Research Institute for Organic Syntheses, Inc.) GLP Unpublished	N	Du Pont (SYN access)
KCA1 5.4.2	[redacted]	08/06/2015	NOA409045 - Oral (Gavage) Mouse Micronucleus Test Report No. [redacted] Document No. VV-28599 , NOA409045_10012 Test Facility [redacted] GLP Unpublished	Y	SYN
KCA1 5.4.2	[redacted]	15/09/2017	CGA226048 - Oral (Gavage) Mouse Micronucleus Test Report No. [redacted] Document No. VV-468462 , CGA226048_10000 Test Facility [redacted] 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

The following tables are to be completed by MS

List of data submitted by the applicant and not relied on

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Owner**
KCP XX	Author	YYYY	Title Company Report N Source GLP/non GLP/GEP/non GEP Published/Unpublished	Y/N	Owner
KCA1 5.4.2	[redacted]	12/08/2014	CGA62826 - Oral (Gavage) Mouse Micronucleus Test Report No. [redacted] Document No. VV-410510 , CGA062826_10006 Test Facility [redacted] GLP Unpublished	Y	SYN
KCA1 5.4.2	[redacted]	27/01/2015	Metalaxyl-M - Oral (Gavage) Mouse Micronucleus Test Report No. [redacted] Document No. VV-411540 , CGA329351_11683 Test Facility [redacted] GLP Unpublished	Y	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**
KCP XX	Author	YYYY	Title Company Report N Source GLP/non GLP/GEP/non GEP Published/Unpublished	Y/N	Owner

Appendix 2 Detailed evaluation of the studies relied upon

A 2.1 Statement on bridging possibilities

A 2.2 Acute oral toxicity (KCP 7.1.1)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	**HSE Chemicals Regulation Division (CRD), UK**
Reviewer's comments	Toxicology: The applicant proposes to meet the data requirement for acute oral toxicity using a study previously evaluated by HSE. The study was evaluated during the following zonal application. Based on the available study, HSE concluded that the acute oral LD_{50} was > 2000 mg/kg bw. Conclusion Based on the available study, the acute oral LD_{50} was > 2000 mg/kg bw, therefore the product does not meet the criteria for classification for acute oral toxicity in accordance with Regulation 1272/2008 (CLP).

Reference:	KCP 7.1.1
Report	[redacted], 1998 CGA173506 + CGA329351 + Cymoxanil (5+17.5+10) WG 5/17.5/10 (A9873C): Acute Oral Toxicity Study In The Rat (Limit Test). 983060 CGA173506/1166 - VV-376066
Guideline(s):	OECD 401 (1987) 92/69/EEC, B.1 (1992)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test material (Lot/Batch No.)	A9873C (P.803003)
Species	Rat, HanIbm:WIST

No. of animals (group size)	10 rats/5 male,5 female
Dose(s)	2000 mg/kg bw
Exposure	Once by gavage
Vehicle/Dilution	Distilled water
Post exposure observation period	14 days
Remarks	None

Results and discussions

Table A 1: Results of acute oral toxicity study in rats of A9873C

Dose (mg/kg bw)	Toxicological results *	Duration of signs	Time of death	LD50 (mg/kg bw) (14 days)
Male rats				
2000	0/5/5	-	Day 14	> 2000
Female rats				
2000	1/5/5	Day 1	5 hrs after dosing/ 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 A9873C

Mortality:	One moribund female was killed for humane reasons 5 hours after dosing. All other animals survived to termination of the study.
Clinical signs:	On the day of dosing, ventral recumbency and hypoactivity were seen in all animals and piloerection and hunched posture were seen in all males and four females.
Body weight:	Body weight gain was considered to be normal.
Macroscopic examination:	Necropsy examinations revealed a reddish small intestine in one male rat.

Conclusion

Under the experimental conditions, the oral LD50 of A9873C is higher than 2000 mg/kg bw in rats.

Thus, no classification is required according to Regulation (EC) No. 1272/2008.

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

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	**HSE Chemicals Regulation Division (CRD), UK**
Reviewer's comments	Toxicology: The applicant proposes to meet the data requirement for acute dermal toxicity using a study previously evaluated by HSE. The study was evaluated during the following zonal application. Based on the available study, HSE concluded that the acute dermal LD_{50} was > 2000 mg/kg bw. Conclusion Based on the available study, the acute dermal LD_{50} was > 2000 mg/kg bw, therefore the product does not meet the criteria for classification for acute dermal toxicity in accordance with Regulation 1272/2008 (CLP).

Reference:	KCP 7.1.2
Report	[redacted], 1998 CGA173506 + CGA329351 + Cymoxanil (5+17.5+10) WG 5/17.5/10 (A9873C): Acute Dermal Toxicity Study In The Rat (Limit Test). 983061 CGA173506/1167 -VV-376067
Guideline(s):	OECD 402
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test material (Lot/Batch No.)	A9873C (P.803003)
Species	Rat, HanIbm:WIST
No. of animals (group size)	10 rats/5 female, 5 male
Dose(s)	2000 mg/kg bw
Exposure	24 hours (dermal, semi-occlusive)
Vehicle/Dilution	Distilled water
Post exposure observation period	14 days
Remarks	None

Results and discussions

Table A 3: Results of acute dermal toxicity study in rats of A9873C

Dose (mg/kg bw)	Toxicological results *	Duration of signs	Time of death	LD50 (mg/kg bw) (14 days)
Male rats				
2000	0/0/5	-	Day 14	> 2000
Female rats				
2000	0/0/5	-	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 A9873C

Mortality:	No mortality occurred.
Clinical signs:	No clinical signs of toxicity were observed.
Body weight:	A slight loss of bodyweight was recorded in three female rats during the first week after treatment.
Macroscopic examination:	Necropsy examinations revealed reddened skin at the application site in one female rat.

Conclusion

Under the experimental conditions, the dermal LD_{50} of A9873C is higher than 2000 mg/kg bw in rats.

Thus, no classification is required according to Regulation (EC) No. 1272/2008.

A 2.4 Acute inhalation toxicity (KCP 7.1.3)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	**HSE Chemicals Regulation Division (CRD), UK**
Reviewer's comments	Toxicology: The applicant proposes to present a waiver for the data requirements for acute inhalation toxicity. The waiver is acceptable. Conclusion The product does not meet the criteria for classification for acute inhalation toxicity in accordance with Regulation 1272/2008 (CLP).

According to Commission Directive 283/2013, the placing of plant protection products on the market, an acute inhalation test must be carried out where the plant protection product:

- the active substance has a vapour pressure > 1×10^{-2} Pa at 20 °C;

- the active substance is a powder containing a significant proportion of particles of a diameter < 50 µm (> 1 % on weight basis);
- the active substance is included in products that are powders or are applied by spraying.
- The head/nose only exposure shall be used, unless whole body exposure can be justified.

The active ingredient, metalaxyl-M (CGA 329351) has a vapour pressure of $3.3 \cdot 10^{-3}$ at 25°C (trigger value: $1 \cdot 10^{-2}$ Pa). The formulation is a liquid. The application technique for treatment of seed (continuous flow/closed system) will not lead to the formation of inhalable particles. Handling the treated seed (as sowing) may lead to the generation of dust. However, model calculations as well as exposure studies show that the inhalative exposure route is of minor relevance only (see below).

As the use of metalaxyl-M 350ES (A9873G) will not result in any significant inhalative exposure, no acute inhalative toxicity testing is required. Considering the favourable acute toxicity profile, the inhalative hazard of the product is expected to be low.

A 2.5 Skin irritation (KCP 7.1.4)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	**HSE Chemicals Regulation Division (CRD), UK**
Reviewer's comments	Toxicology: The applicant proposes to meet the data requirement for skin irritation using a study previously evaluated by HSE. The study was evaluated during the following zonal application. Based on the available study, HSE concluded that the mean erythema and oedema scores for 24-72 hours were 0 and (0.67in one animal), with inflammation reversed after 3 days . Conclusion Based on the available study, the criteria for classification in Category 2 for skin irritation were not met. Therefore, the product does not meet the criteria for classification for skin irritation in accordance with Regulation 1272/2008 (CLP).

Reference:	KCP 7.1.4
Report	[redacted], 1998 CGA173506 + CGA329351 + Cymoxanil (5+17.5+10) WG 5/17.5/10 (A9873C): Acute Dermal Irritation/Corrosion In The Rabbit. 983062 CGA173506/1168 - VV-376068
Guideline(s):	OECD 404 (2002)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test material (Lot/Batch No.)	A9873C (P.803003)
Species	Rabbit, New Zealand White
No. of animals (group size)	3 females, 3 males
Initial test using one animal	No
Exposure	0.5 mL (4 hours, semi-occlusive)
Vehicle/Dilution	Test and control patches were moistened with distilled water to improve contact.
Post exposure observation period	72 hrs
Remarks	None

Results and discussions

Table A 5: Skin irritation of A9873C

Animal No.		Scores after treatment *				Mean scores (24-72 h)	Reversible (day)
		1 h	24 h	48 h	72 h		
1	Erythema Oedema	0 0	0 0	0 0	0 0	0 0	- -
2	Erythema Oedema	0 0	0 0	0 0	0 0	0 0	- -
3	Erythema Oedema	0 0	0 0	0 0	0 0	0 0	- -
4	Erythema Oedema	0 0	0 0	0 0	0 0	0 0	- -
5	Erythema Oedema	0 0	1 0	1 0	0 0	0.67 0	3 -
6	Erythema Oedema	0 0	0 0	0 0	0 0	0 0	- -

* scores in the range of 0 to 1

Clinical signs:	No clinical signs of toxicity were observed.

Conclusion

Under the experimental conditions, A9873C 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)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	**HSE Chemicals Regulation Division (CRD), UK**
Reviewer's comments	Toxicology: The applicant proposes to meet the data requirement for eye irritation using a study previously evaluated by HSE. The study was evaluated during the following zonal application. Based on the available study, HSE concluded that the cornea opacity, iritis, conjunctive redness or oedema mean scores for 24-72 hours were 0.4, 0.4, 1.3 and 0.6, respectively. All inflammation was reversed by 7 days. Conclusion Based on the available study, the criteria for classification in Category 2 for eye irritation were not met. Therefore, the product does not meet the criteria for classification for eye irritation in accordance with Regulation 1272/2008 (CLP).

Reference:	KCP 7.1.5
Report	[redacted], 1998 CGA173506 + CGA329351 + Cymoxanil (5+17.5+10) WG 5/17.5/10 (A9873C): Acute Eye Irritation/Corrosion In The Rabbit. 983063 CGA173506/1169 - VV-376069
Guideline(s):	OECD 405 (2002)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test material (Lot/Batch No.)	A9873C (P.803003)
Species	Rabbit, New Zealand White
No. of animals (group size)	3 females, 3 male
Initial test using one animal	No
Exposure	0.1 mL (single instillation in conjunctival sac)
Irrigation (time point)	No
Vehicle/Dilution	None

Post exposure observation period	7 Days
Remarks	None

Results and discussions

Table A 6: Eye irritation of A9873C

Animal No.		Scores after treatment *				Mean scores (24-72 h)	Reversible (day)
		1 h	24 h	48 h	72 h		
076	Corneal opacity	1	1	0	0	0.4	2
	Iritis	0	1	0	0	0.4	2
	Redness conjunctivae	1	2	2	1	1.3	7
	Chemosis conjunctivae	1	1	1	1	0.6	7
166	Corneal opacity	1	1	1	1	0.4	7
	Iritis	0	1	1	1	0.4	7
	Redness conjunctivae	1	2	2	1	1.3	7
	Chemosis conjunctivae	2	1	1	1	0.6	7
026	Corneal opacity	1	0	0	0	0.4	2
	Iritis	0	0	0	0	0.4	-
	Redness conjunctivae	1	1	0	0	1.3	2
	Chemosis conjunctivae	0	0	0	0	0.6	-
764	Corneal opacity	1	1	1	0	0.4	3
	Iritis	0	1	1	0	0.4	3
	Redness conjunctivae	1	2	2	1	1.3	7
	Chemosis conjunctivae	2	1	1	0	0.6	3
734	Corneal opacity	1	1	0	0	0.4	2
	Iritis	0	0	0	0	0.4	-
	Redness conjunctivae	1	1	1	1	1.3	7
	Chemosis conjunctivae	1	1	0	0	0.6	2
860	Corneal opacity	1	1	0	0	0.4	2
	Iritis	0	1	0	0	0.4	2
	Redness conjunctivae	1	2	1	1	1.3	7
	Chemosis conjunctivae	2	1	0	0	0.6	2

* scores in the range of 0 to 4 for cornea opacity and chemosis, 0 to 3 for redness of conjunctivae and 0 to 1 for iritis

Clinical signs:	No clinical signs of toxicity were observed.

Conclusion

Under the experimental conditions, A9873C is not an eye irritant.

Thus, no classification is required according to Regulation (EC) No. 1272/2008.

A 2.7 Skin sensitisation (KCP 7.1.6)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	**HSE Chemicals Regulation Division (CRD), UK**
Reviewer's comments	Toxicology: The applicant proposes to meet the data requirement for skin sensitisation using a study previously evaluated by HSE. The study evaluated during the following zonal application. Based on the available study, HSE concluded that 1/20 had a reaction after the test application. Conclusion Based on the available study, the criteria for classification for skin sensitisation were not met. Therefore, the product does not meet the criteria for classification for skin sensitisation in accordance with Regulation 1272/2008 (CLP).

Reference:	KCP 7.1.6
Report	[redacted], 1998 CGA173506 + CGA329351 + Cymoxanil (5+17.5+10) WG 5/17.5/10 (A9873C): Skin Sensitisation In The Guinea Pig (Maximisation Test). 983064 CGA173506/1170 - VV-376070
Guideline(s):	OECD 406 (1992)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test material (Lot/Batch No.)	A9873C (P.803003)
Species	Guinea pig, Himalayan Spotted (GOHI)
No. of animals (group size)	Test substance group: 10 male guinea pigs / 10 female guinea pigs Vehicle control goup: 5 male guinea pigs / 5 female guinea pigs
Range finding:	No
Exposure (concentration(s), no. of applications)	Intradermal induction 1% in saline Topical induction 70% in saline Challenge70% in saline
Vehicle	saline solution
Pretreatment prior to topical application	Yes (sodium lauryl sulfate)

Reliability check	None
Remarks	None

Results and discussions

Vehicle Flank

	24 hours	**48 hours**	**Total number of animals affected**
	After challenge		
A9873C	0/20*	0/20*	0/20
Test Vehicle Control Group	0/10*	0/10*	0/10

* Number of animals with positive dermal response (scores of 1-3) /number of animals in dose group

Test Flank

	24 hours	**48 hours**	**Total number of animals affected**
	After challenge		
A9873C	1/20*	0/20*	1/20
Test Vehicle Control Group	0/10*	0/10*	0/10

* Number of animals with positive dermal response (scores of 1-3) /number of animals in dose group

Clinical signs:	No clinical signs of toxicity were observed.

Conclusion

Under the experimental conditions, A9873C is not a skin sensitiser. Thus, no classification is required according to Regulation (EC) No. 1272/2008.

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

Not required.

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)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	**HSE Chemicals Regulation Division (CRD), UK**
Reviewer's comments	Toxicology: The applicant proposes to meet the data requirements for dermal absorption, with the submission of an *in vitro* dermal absorption study ([redacted], 2015). Evaluation The study "Fludioxonil/Metalaxyl-M/Cymoxanil WG (A9873C) - The *In Vitro* Percutaneous Absorption of Radiolabelled Metalaxyl-M and Radiolabelled Cymoxanil in a Concentrate Through Human Split-thickness Skin.", was in compliance with Good Laboratory Practice (GLP) and followed OECD TG 428. There were no deviations from the test guideline and the study is acceptable. HSE has evaluated the study in accordance with the most recent EFSA guidance on dermal absorption 2017. Human skin was exposed *in vitro* to [^{14}C]- Metalaxyl-M formulated as the WG formulation A9873C for six hours under non-occluded conditions in eight static cells. The WG formulation was dissolved into a 1:1 saline solution before application to skin, to mimic exposure with sweating. The test was performed using a single concentrate of 175 g/L (87.5 g/L). The doses were applied at 10 µL/cm^2 and left non-occluded for an experimental period of 24 h, with an interim wash at 6 h post-application. *Concentrate* Cell 7 was excluded from the data set based on statistical grounds and a plausible cause of misdose resulting in a very low total recovery (mass balance) of 12.33. The exclusion of Cell 7 in the results leads to a more conservative estimate of dermal absorption. HSE agrees with the exclusion of this cell data in accordance with EFSA 2017 guidance. Results More than 75% of the total absorption (material in the receptor fluid at the end of the study) occurred within the first half of the study for all tested concentrations. Therefore, all tape strips were excluded from absorption calculations as recommended in the EFSA (2017) Guidance on Dermal Absorption. The mean recovery was 108.89%, and is acceptable. The dermal absorption of metalaxyl-M through human skin was calculated to be 0.6 ± 0.27% (mean ± standard deviation) for the concentrate. These data were interpreted in accordance with the EFSA guidance on dermal absorption (2017), including correction for variability by addition of k x SD, resulting in finalised a dermal absorption value of 0.85%, respectively. Conclusion **Based on the EFSA guidance on dermal absorption (2017), the dermal absorption value to be used for risk assessment for the concentrated formulation is 0.85%.**

Reference	KCP 7.3
Report	[redacted], 2015 Fludioxonil/Metalaxyl-M/Cymoxanil WG (A9873C) - The *In Vitro* Percutaneous Absorption of Radiolabelled Metalaxyl-M and Radiolabelled Cymoxanil in a Concentrate Through Human Split-thickness Skin. 36500 VV-414733
Guideline(s)	Yes (OECD 428)
Deviations	No
GLP	Yes
Acceptability	Yes
Duplication (if vertebrate study)	N/A

Materials and methods

Test materials	**Name (Batch No.)**	[phenyl-U-14C]-CGA329351 (RDR-XX-92)
	Test preparation	Radioformulation
	Specific activity	87.8 µCi/mg
	Radiochemical purity	98.6 %
	Name (Batch No.)	[Acetyl-2-14C]-Cymoxanil (CFQ42309)
	Test preparation	Radioformulation
	Specific activity	71.1 µCi/mg
	Radiochemical purity	99.9 %
	Name (Batch No.)	Metalaxyl-M Technical (678767)
	Product Code	CGA329351
	Name (Batch No.)	Cymoxanil Technical (LS1207012)
	Product Code	DPX-T3217-266
Product	**Name (Batch No.)**	Metalaxyl-M/Cymoxanil/Fludioxonil WG A9873C (KWL0K111)
	Concentration a.s.	175 g/kg Metalaxyl-M/
		100 g/kg Cymoxanil
	Formulation type	WG (water dispersible granule)
Blank product	**Name (Batch No.)**	A9873C Blank without Mefenoxam (SSN001-047-002)
		A9873C Blank without Cymoxanil (SSN001-047-003)
	Concentration a.s.	0 g/L

Test system		
Diffusion cell	Cell type	Static diffusion cell
	(if dynamic) Flow rate	N/A
	Exposed skin area	0.64 cm²
	Cover	Unoccluded
Membrane	Skin type	Dermatomed
	Skin thickness range	330-400 µm
	Skin donors age	18-62 Y
	Skin donors sex	Female/Male
	Location	Abdomen, arm, breast
	Source	*Ex vivo*
	Integrity test	Electrical resistance
Receptor	Receptor medium	Phosphate buffered saline containing polyoxyethylene 20 oleyl ether (PEG, ca 6%, w/v),

		sodium azide (ca 0.01%, w/v), streptomycin (0.1 mg/mL) and penicillin G (100 units/mL) (pH 7.4).
	Solubility in receptor medium	Yes
Sample Time	Exposure time	6 h
	Observation time	24 h
Sampling	Sample intervals	2, 4, 6, 8 and 12 h post dose
Washing		Post exposure and 24 hours
Final Procedure	Tape stripping	Yes
	TS1-2 analysed separately	Yes
Remarks: Cell 7 was rejected due to a suspected misdose		

Tested doses	**Metalaxyl-M Formulation Concentrate**	**Cymoxanil Formulation Concentrate**
Target concentration [g/kg]	87.5	50
Area dose [mg/cm²]	10	10
Total dose [mg/cell]	6.4	6.4
Specific activity [µCi/mg]	1.92	3.27
No. of donors	5	4
No. of valid/used cells	7/8	8/8

Results and discussions

Table A 7: *In-vitro* dermal penetration of Metalaxyl-M and Cymoxanil in a mixture of Fludioxonil/Metalaxyl-M/Cymoxanil WG (A9873C) through human dermatomed skin - Recovery data

Dose group	Metalaxyl-M Formulation Concentrate		Cymoxanil Formulation Concentrate	
Mean actual applied dose [g/kg]	88.94		53.01	
	Recovery [%]			
	Mean	S.D.	Mean	S.D.
Dislodgeable dose	**108.26**	**2.63**	**100.53**	**7.15**
e.g. Skin washing after 6 h	108.09	2.54	100.08	7.29
e.g. Skin washing after 24 h	0.15	0.11	0.36	0.19
Donor chamber wash	0.03	0.02	0.08	0.16
Dose associated to skin	**0.07**	**0.06**	**0.18**	**0.20**
Tape strips: 1 – 2	0.01	0.01	0.13	0.16
Tape strips: 3 - 20	0.02	0.02	0.02	0.03
Exposed skin	0.04	0.03	0.02	0.01
Unexposed skin	0.00	0.00	0.00	0.00
Absorbed dose	**0.57**	**0.25**	**0.23**	**0.08**
Receptor fluid	0.54	0.23	0.22	0.08
Receptor chamber wash	0.03	0.02	0.01	0.01
Total recovery[1]	**108.90**	**2.88**	**100.94**	**7.03**
Absorption essentially complete at end of study (>75% absorption within half the study duration) [%Absorption at $t_{0.5}$]	Yes [88.0% absorbed at 12 h]		Yes [78.4% absorbed at 12 h]	
If no: Absorption estimates = absorbed dose + skin preparation + tape strips 3-20)[2]	N/A		N/A	
If yes: Absorption estimates	0.61	0.27	0.25	0.09

= absorbed dose + exposed skin				
Absorption estimate normalised[3]	No		No	
Multiplication factor added to the SD (k)	0.92		0.84	
SD * k	0.25		0.08	
Relevant absorption estimate[4]	0.85%		0.33%	
Absorption estimates used for risk assessment[5]	**0.85%**		**0.33%**	

[1] Values may not calculate exactly due to rounding of figures

[2] In accordance with the EFSA Guidance on Dermal Absorption (EFSA 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 2017 EFSA Guidance on Dermal Absorption the appropriate multiplication factor (k) has been included as a multiple to the standard deviation (s) prior to the addition of the based on the number of samples analysed was added to the mean i.e. mean + ks.

[5] Relevant absorption estimate was rounded to the required number of significant figures.

N/A: not applicable

Remarks:

Cell 7 was rejected from the mean and SD due to a suspected misdose.

Conclusion/endpoint:

The dermal penetration of Metalaxyl-M and Cymoxanil formulated as Fludioxonil/Metalaxyl-M/Cymoxanil WG (A9873C) through human dermatomed skin was determined *in vitro*. The amount of applied dose penetrating within 24 hours was determined to be 0.57 ± 0.25% and 0.23± 0.08% for Metalaxyl-M and Cymoxanil concentrates respectively, as measured in the receptor fluid and the receptor chamber wash. The dermal penetration estimates to be used for risk assessment were set as 0.85% and 0.33% for Metalaxyl-M and Cymoxanil concentrates respectively, based on the EFSA guidance criteria.

A 2.11 Other/Special Studies

A 2.11.4 CGA62826: Oral (Gavage) Mouse Micronucleus Test

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	**HSE Chemicals Regulation Division (CRD), UK**
Reviewer's comments	<u>Toxicology:</u> The applicant submitted an in vivo micronucleus assay in the mouse to demonstrate the clastogenic potential of the metalaxyl-M metabolite NOA409045 R/S racemate CGA62826 ([redacted] 2014). This study was concluded not to be required for HSE regulative decision on the clastogenic potential of metalaxyl-M metabolite NOA409045. The study has therefore not been evaluated.

Report author	[redacted]
Report year	2014
Report title	CGA62826 – Oral (Gavage) Mouse Micronucleus Test.

Report No	[redacted]
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 [redacted], 2014 Report No. [redacted] Syngenta File No. CGA062826_10006 / VV-410510
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 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:** 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	[redacted]
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 sam-

ples 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 8: 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 2.11.4 NOA409045: Oral (Gavage) Mouse Micronucleus Test

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	**HSE Chemicals Regulation Division (CRD), UK**
Reviewer's comments	Toxicology: The applicant submitted an in vivo micronucleus assay in the mouse to demonstrate the clastogenic potential of the metalaxyl-M metabolite NOA409045 ([redacted] 2015b). Evaluation The study "NOA409045- Oral (Gavage) Mouse Micronucleus Test", was in compliance with Good Laboratory Practice (GLP) and followed OECD TG 474 (1997 version). There were no deviations from the test guideline and the study is acceptable. A range finding study consisting of 3 males and 6 female mice established the maximum tolerated dose at 2000 mg/kg bw/d, proof of bone marrow exposure was also confirmed. The doses for the main study were spaced by a factor of 2 resulting in doses of 0, 500, 1000, and 2000 mg/kg bw/d. Groups of six male mice were administered a dose twice oraly, spaced 24 hours apart, with a control group receiving the vehicle (1% carbox-ymethylcellulose with 0.1% Tween 80) and a positive control group administered 4 mg/kg bw mitomycin C. Animals were sacrificed 24 hours post the final dose and bone marrow samples were collected and scored via stained slides. 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. Results 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. A slight dose-related increase in MN-PCE can be seen, this trend was not analysed in the study report by an appropriate trend test. However, the frequencies of micronucleated PCEs for all test groups fell within the range of the historical control data for the negative control. Therefore, in accordance with the OECD test guideline, the criteria for a positive result have not been met. Conclusion During an GLP and OECD compliant study under the described experimental conditions reported, the test item did not induce micronuclei up to the maximum tolerated dose as determined by the micronucleus test in the bone marrow cells of the mouse. Therefore, NOA409045 is considered to be non-genotoxic in this bone marrow micronucleus assay.

Report author	[redacted]
Report year	2015
Report title	NOA409045 – Oral (Gavage) Mouse Micronucleus Test.

Report No	[redacted]
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 [redacted], 2015 Report No. [redacted] Syngenta File No. NOA409045_10012 / VV-28599
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	[redacted]
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 9: 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 Con-trol 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

([redacted] 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 2.11.4 Metalaxyl-M – Oral (Gavage) Mouse Micronucleus Test

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	**HSE Chemicals Regulation Division (CRD), UK**
Reviewer's comments	Toxicology: The applicant submitted an in vivo micronucleus assay in the mouse to demonstrate the clastogenic potential of metalaxyl-M. This study was concluded not to be required for HSE regulative decision on the clastogenic potential of metalaxyl-M impurity CGA226048. The study has therefore not been evaluated.

Report author	[redacted]
Report year	2015
Report title	Metalaxyl-M – Oral (Gavage) Mouse Micronucleus Test.
Report No	[redacted]
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 5.4.2/03
Report	Metalaxyl-M – Oral (Gavage) Mouse Micronucleus Test. [redacted], 2015 Report No. [redacted] Syngenta File No. CGA329351_11683 - VV-411540
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 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:** 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	[redacted]
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 10: 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

([redacted] 2014)

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 2.11.4 CGA226048 - Oral (Gavage) Mouse Micronucleus Test

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	**HSE Chemicals Regulation Division (CRD), UK**
Reviewer's	Toxicology:

comments	The applicant submitted an in vivo micronucleus assay in the mouse to demonstrate the clastogenic potential of the metalaxyl-M impurity CGA226048 ([redacted] 2017). Evaluation The study " CGA226048- Oral (Gavage) Mouse Micronucleus Test ", was in compliance with Good Laboratory Practice (GLP) and followed OECD TG 474 (1997 version). There were no deviations from the test guideline and the study is acceptable. A range finding study consisting of 3 male and 3 female mice established the maximum tolerated dose at 2000 mg/kg bw/d, proof of bone marrow exposure was also confirmed. The doses for the main study were spaced by a factor of 2 resulting in doses of 0, 500, 1000, and 2000 mg/kg bw/d. Groups of six male mice were administered a dose twice oraly, spaced 24 hours apart, with a control group receiving the vehicle (0.5% HMPC) and a positive control group administered 1 mg/kg bw mitomycin C. Animals were sacrificed 24 hours post the final dose and bone marrow samples were collected and scored via stained slides. 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. Results There were no statistically significant increases in micronucleus frequency in male mice treated at any dose level of CGA226048, compared with the negative control group. The frequencies of micronucleated PCEs for all test groups fell within the range of the historical control data for the negative control. Therefore, in accordance with the OECD test guideline, the criteria for a positive result have not been met. Conclusion During an GLP and OECD compliant study under the described experimental conditions reported, the test item did not induce micronuclei up to the maximum tolerated dose as determined by the micronucleus test in the bone marrow cells of the mouse. Therefore, CGA226048 considered to be non-genotoxic in this bone marrow micronucleus assay.

Report author	[redacted]
Report year	2017
Report title	CGA226048 - Oral (Gavage) Mouse Micronucleus Test
Report No	[redacted]
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. [redacted] (2017) Report No. [redacted], Syngenta File No. CGA226048_10000 / VV-468462
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 % hydroxypropylmethylcellulose (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	[redacted]
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 11 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 12: Input parameters considered for the estimation of operator exposure during industrial seed treatment – large seeds

Formulation type:	WG		**Application technique:**	Industrial scale seed treatment	
Application rate (AR):	33.92	g a.s./100 kg seed	**AOEL**	0.08	mg/kg bw/d
Seed treated per day:	75	tonnes/d	**Amount of a.s. applied:**	25.44	kg a.s./d
Bag size	25	kg	**Amount of product used:**	150	kg/d
Dermal absorption (DA):	0.85	%	**Dilution factor:**	1: undiluted product taken as the worst case scenario	
			Cleaning tasks performed:	1	per day
Inhalation absorption (IA):	100	%	**Mixing/loading tasks performed:**	8	per day (20 L container)
Body weight (BW):	60 and 70	kg/person	**Calibration tasks performed**	1	per day
			Duration of bagging	8	Hours

Table A 13: Estimation of operator exposure towards metalaxyl-M using the Seed-TROPEX model - with RPE for cleaning

60 kg bw

TASK	Total Potential Dermal Exposure (mg/op)*	Estimated Actual Dermal Exposure (mg/op)*	Inhalation Exposure (mg/op)*	*Frequency of operation ** / day*	*PPE# Use of additional RPE yes/no*	Total Potential Dermal Exposure (mg/day)	Estimated Actual Dermal Exposure (mg/day)	Inhalation Exposure (mg/day)
Calibration	5.52	2.41	0.234	*1*	*no*	5.5214	2.4129	0.2342
Mixing / Loading	0.8805	0.881	0.022	*8*	*no*	7.0443	7.0443	0.1737
Bagging (mg/hr)	1.84	0.698	0.0054	*8*	*no*	14.7200	5.5840	0.0432
Cleaning	148	14.14	2.71344	*1*	*yes*	147.8412	14.1377	0.2713
Dermal absorption/Inhaltion absorption								
			Calibration			n/a	0.85%	100%
			Mixing/loading			n/a	0.85%	100%
			Bagging			n/a	0.85%	100%
			Cleaning			n/a	0.85%	100%
Task specific absorbed dose (mg/kg bw/day)								
			Calibration				**0.00034**	**0.00390**
			Mixing/loading				0.00100	0.00289
			Bagging				**0.00079**	**0.00072**
			Cleaning				**0.00200**	**0.00452**
Total absorbed dose (mg/kg bw/day)							**0.0162**	

standard clothing of the operators is one layer of work clothing during all tasks and in addition protective gloves except for bagging

* exposure during bagging mg/hour

** frequency during bagging in hours/day

% of AOEL	20.22	***0.0800*** mg/kg bw/day

70 kg bw

TASK	Total Potential Dermal Exposure (mg/op)*	Estimated Actual Dermal Exposure (mg/op)*	Inhalation Exposure (mg/op)*	*Frequency of operation ** / day*	*PPE# Use of additional RPE yes/no*	Total Potential Dermal Exposure (mg/day)	Estimated Actual Dermal Exposure (mg/day)	Inhalation Exposure (mg/day)
Calibration	5.52	2.41	0.234	*1*	*no*	5.5214	2.4129	0.2342
Mixing / Loading	0.8805	0.881	0.022	*8*	*no*	7.0443	7.0443	0.1737
Bagging (mg/hr)	1.84	0.698	0.0054	*8*	*no*	14.7200	5.5840	0.0432
Cleaning	148	14.14	2.71344	*1*	*yes*	147.8412	14.1377	0.2713
Dermal absorption/Inhaltion absorption								
			Calibration			n/a	0.85%	100%
			Mixing/loading			n/a	0.85%	100%
			Bagging			n/a	0.85%	100%
			Cleaning			n/a	0.85%	100%
Task specific absorbed dose (mg/kg bw/day)								
			Calibration				**0.00029**	**0.00335**
			Mixing/loading				0.00086	0.00248
			Bagging				**0.00068**	**0.00062**
			Cleaning				**0.00172**	**0.00388**
Total absorbed dose (mg/kg bw/day)							**0.0139**	

standard clothing of the operators is one layer of work clothing during all tasks and in addition protective gloves except for bagging

* exposure during bagging mg/hour

** frequency during bagging in hours/day

% of AOEL	17.33	***0.0800*** mg/kg bw/day

A 3.1.2 Calculations for fludioxonil

Table A 14: Input parameters considered for the estimation of operator exposure during industrial seed treatment – large seeds

Formulation type:	WG		**Application technique:**	Industrial scale seed treatment	
Application rate (AR):	10	g a.s./100 kg seed	**AOEL**	0.59	mg/kg bw/d
Seed treated per day:	75	tonnes/d	**Amount of a.s. applied:**	7.5	kg a.s./d
Bag size	25	kg	**Amount of product used:**	150	kg/d
Dermal absorption (DA):	10	%	**Dilution factor:**	1: undiluted product taken as the worst case scenario	
			Cleaning tasks performed:	1	per day
Inhalation absorption (IA):	100	%	**Mixing/loading tasks performed:**	8	per day (20 L container)
Body weight (BW):	60 and 70	kg/person	**Calibration tasks performed**	1	per day
			Duration of bagging	8	hours

Table A 15: Estimation of operator exposure towards fludioxonil using the SeedTROPEX model - with RPE for cleaning

60 kg bw

TASK	Total Potential Dermal Exposure (mg/op)*	Estimated Actual Dermal Exposure (mg/op)*	Inhalation Exposure (mg/op)*	*Frequency of operation ** / day*	*PPE# Use of additional RPE yes/no*	Total Potential Dermal Exposure (mg/day)	Estimated Actual Dermal Exposure (mg/day)	Inhalation Exposure (mg/day)
Calibration	1.63	0.71	0.069	*1*	*no*	1.6279	0.7114	0.0690
Mixing / Loading	0.2596	0.260	0.006	*8*	*no*	2.0769	2.0769	0.0512
Bagging (mg/hr)	1.84	0.698	0.0054	*8*	*no*	14.7200	5.5840	0.0432
Cleaning	44	4.17	0.8	*1*	*yes*	43.5878	4.1682	0.0800
Dermal absorption/Inhaltion absorption								
			Calibration			n/a	10.00%	100%
			Mixing/loading			n/a	10.00%	100%
			Bagging			n/a	10.00%	100%
			Cleaning			n/a	10.00%	100%
Task specific absorbed dose (mg/kg bw/day)								
			Calibration				**0.00119**	**0.00115**
			Mixing/loading				0.00346	0.00085
			Bagging				**0.00931**	**0.00072**
			Cleaning				**0.00695**	**0.00133**
Total absorbed dose (mg/kg bw/day)							**0.0250**	

standard clothing of the operators is one layer of work clothing during all tasks and in addition protective gloves except for bagging
* exposure during bagging mg/hour
** frequency during bagging in hours/day

% of AOEL	4.23	***0.5900***	mg/kg bw/day

70 kg bw

TASK	Total Potential Dermal Exposure (mg/op)*	Estimated Actual Dermal Exposure (mg/op)*	Inhalation Exposure (mg/op)*	*Frequency of operation ** / day*	*PPE# Use of additional RPE yes/no*	Total Potential Dermal Exposure (mg/day)	Estimated Actual Dermal Exposure (mg/day)	Inhalation Exposure (mg/day)
Calibration	1.63	0.71	0.069	*1*	*no*	1.6279	0.7114	0.0690
Mixing / Loading	0.2596	0.260	0.006	*8*	*no*	2.0769	2.0769	0.0512
Bagging (mg/hr)	1.84	0.698	0.0054	*8*	*no*	14.7200	5.5840	0.0432
Cleaning	44	4.17	0.8	*1*	*yes*	43.5878	4.1682	0.0800
Dermal absorption/Inhaltion absorption								
			Calibration			n/a	10.00%	100%
			Mixing/loading			n/a	10.00%	100%
			Bagging			n/a	10.00%	100%
			Cleaning			n/a	10.00%	100%
Task specific absorbed dose (mg/kg bw/day)								
			Calibration				**0.00102**	**0.00099**
			Mixing/loading				0.00297	0.00073
			Bagging				**0.00798**	**0.00062**
			Cleaning				**0.00595**	**0.00114**
Total absorbed dose (mg/kg bw/day)							**0.0214**	

standard clothing of the operators is one layer of work clothing during all tasks and in addition protective gloves except for bagging
* exposure during bagging mg/hour
** frequency during bagging in hours/day

% of AOEL	3.63	***0.5900***	mg/kg bw/day

A 3.1.3 Calculations for cymoxanil

Table A 16: Input parameters considered for the estimation of operator exposure during industrial seed treatment – large seeds

Formulation type:	WG		**Application technique:**	Industrial scale seed treatment	
Application rate (AR):	20	g a.s./100 kg seed	**AOEL**	0.01	mg/kg bw/d
Seed treated per day:	75	tonnes/d	**Amount of a.s. applied:**	15	kg a.s./d
Bag size	25	kg	**Amount of product used:**	150	kg/d
Dermal absorption (DA):	0.33	%	**Dilution factor:**	1: undiluted product taken as the worst case scenario	
			Cleaning tasks performed:	1	per day
Inhalation absorption (IA):	100	%	**Mixing/loading tasks performed:**	8	per day (20 L container)
Body weight (BW):	60 and 70	kg/person	**Calibration tasks performed**	1	per day
			Duration of bagging	8	Hours

Table A 17: Estimation of operator exposure towards cymoxanil using the SeedTROPEX model - with RPE for cleaning

60 kg bw

TASK	Total Potential Dermal Exposure (mg/op)*	Estimated Actual Dermal Exposure (mg/op)*	Inhalation Exposure (mg/op)*	*Frequency of operation ** / day*	*PPE# Use of additional RPE yes/no*	Total Potential Dermal Exposure (mg/day)	Estimated Actual Dermal Exposure (mg/day)	Inhalation Exposure (mg/day)
Calibration	3.26	1.42	0.138	*1*	*no*	3.2557	1.4228	0.1381
Mixing / Loading	0.5192	0.519	0.013	*8*	*no*	4.1537	4.1537	0.1024
Bagging (mg/hr)	1.84	0.698	0.0054	*8*	*no*	14.7200	5.5840	0.0432
Cleaning	87	8.34	1.6	*1*	*yes*	87.1757	8.3364	0.1600
Dermal absorption/Inhaltion absorption								
			Calibration			n/a	0.33%	100%
			Mixing/loading			n/a	0.33%	100%
			Bagging			n/a	0.33%	100%
			Cleaning			n/a	0.33%	100%
Task specific absorbed dose (mg/kg bw/day)								
			Calibration				**0.00008**	**0.00230**
			Mixing/loading				0.00023	0.00171
			Bagging				**0.00031**	**0.00072**
			Cleaning				**0.00046**	**0.00267**
Total absorbed dose (mg/kg bw/day)							**0.0085**	

standard clothing of the operators is one layer of work clothing during all tasks and in addition protective gloves except for bagging
* exposure during bagging mg/hour
** frequency during bagging in hours/day

% of AOEL	84.67	***0.0100***	mg/kg bw/day

70 kg bw

TASK	Total Potential Dermal Exposure (mg/op)*	Estimated Actual Dermal Exposure (mg/op)*	Inhalation Exposure (mg/op)*	*Frequency of operation ** / day*	*PPE# Use of additional RPE yes/no*	Total Potential Dermal Exposure (mg/day)	Estimated Actual Dermal Exposure (mg/day)	Inhalation Exposure (mg/day)
Calibration	3.26	1.42	0.138	*1*	*no*	3.2557	1.4228	0.1381
Mixing / Loading	0.5192	0.519	0.013	*8*	*no*	4.1537	4.1537	0.1024
Bagging (mg/hr)	1.84	0.698	0.0054	*8*	*no*	14.7200	5.5840	0.0432
Cleaning	87	8.34	1.6	*1*	*yes*	87.1757	8.3364	0.1600
Dermal absorption/Inhaltion absorption								
			Calibration			n/a	0.33%	100%
			Mixing/loading			n/a	0.33%	100%
			Bagging			n/a	0.33%	100%
			Cleaning			n/a	0.33%	100%
Task specific absorbed dose (mg/kg bw/day)								
			Calibration				**0.00007**	**0.00197**
			Mixing/loading				0.00020	0.00146
			Bagging				**0.00026**	**0.00062**
			Cleaning				**0.00039**	**0.00229**
Total absorbed dose (mg/kg bw/day)							**0.0073**	

standard clothing of the operators is one layer of work clothing during all tasks and in addition protective gloves except for bagging
* exposure during bagging mg/hour
** frequency during bagging in hours/day

% of AOEL	72.58	***0.0100***	mg/kg bw/day

A 3.2 Worker exposure calculations (KCP 7.2.3.1)

A 3.2.1 Calculations for metalaxyl-M

Table A 18: Input parameters considered for the estimation of worker exposure during loading and sowing of treated seed

Formulation type:	WG		**Application technique:**	loading and sowing of treated seeds	
Dermal absorption (DA):	0.85	%			
Inhalation absorption (IA):	100	%	**AOEL**	0.08	mg/kg bw/d
Body weight (BW):	60 and 70	kg/person	**Duration of sowing**	10	hours

Table A 19: Estimated worker exposure to metalaxyl-M during loading and sowing of treated seed - Based on 1993 Seed-TROPEX data (geometric mean values), with PPE

60 kg bw
SOWING SEED

		Total Potential Dermal exposure	Estimated Actual Dermal exposure	Inhalation exposure	
Loading / Sowing (x10 hrs)	(mg/day)	14.79	7.33	0.200	
Total exposure	(mg/kg bw/day)	0.25	0.122	0.003	
			Dermal	Inhalation	Total
Systemic Exposure (mg/kg bw/day)			0.0010	0.0033	0.0044
%AOEL	5.5				

70 kg bw
SOWING SEED

		Total Potential Dermal exposure	Estimated Actual Dermal exposure	Inhalation exposure	
Loading / Sowing (x10 hrs)	(mg/day)	14.79	7.33	0.200	
Total exposure	(mg/kg bw/day)	0.21	0.105	0.003	
			Dermal	Inhalation	Total
Systemic Exposure (mg/kg bw/day)			0.0009	0.0029	0.0037
%AOEL	4.7				

A 3.2.2 Calculations for fludioxonil

Table A 20: Input parameters considered for the estimation of operator exposure during loading and sowing of treated seed

Formulation type:	WG		**Application technique:**	loading and sowing of treated seeds	
Dermal absorption (DA):	10	%			
Inhalation absorption (IA):	100	%	**AOEL**	0.59	mg/kg bw/d
Body weight (BW):	60 and 70	kg/person	**Duration of sowing**	10	hours

Table A 21: Estimated operator exposure to fludioxonil during loading and sowing of treated seed - Based on 1993 Seed-TROPEX data (geometric mean values), with PPE

60 kg bw

SOWING SEED

		Total Potential Dermal exposure	Estimated Actual Dermal exposure	Inhalation exposure	
Loading / Sowing (x10 hrs)	(mg/day)	14.79	7.33	0.200	
Total exposure	(mg/kg bw/day)	0.25	0.122	0.003	
			Dermal	Inhalation	Total
Systemic Exposure (mg/kg bw/day)			0.0122	0.0033	0.0156
%AOEL	**2.6**				

70 kg bw

SOWING SEED

		Total Potential Dermal exposure	Estimated Actual Dermal exposure	Inhalation exposure	
Loading / Sowing (x10 hrs)	(mg/day)	14.79	7.33	0.200	
Total exposure	(mg/kg bw/day)	0.21	0.105	0.003	
			Dermal	Inhalation	Total
Systemic Exposure (mg/kg bw/day)			0.0105	0.0029	0.0133
%AOEL	**2.3**				

A 3.2.3 Calculations for cymoxanil

Table A 22: Input parameters considered for the estimation of operator exposure during loading and sowing of treated seed

Formulation type:	WG		**Application technique:**	loading and sowing of treated seeds	
Dermal absorption (DA):	0.33	%			
Inhalation absorption (IA):	100	%	**AOEL**	0.01	mg/kg bw/d
Body weight (BW):	60 and 70	kg/person	**Duration of sowing**	10	hours

Table A 23: Estimated operator exposure to cymoxanil during loading and sowing of treated seed - Based on 1993 Seed-TROPEX data (geometric mean values), with PPE

60 kg bw

SOWING SEED					
		Total Potential Dermal exposure	Estimated Actual Dermal exposure	Inhalation exposure	
Loading / Sowing (x10 hrs)	(mg/day)	14.79	7.33	0.200	
Total exposure	(mg/kg bw/day)	0.25	0.122	0.003	
			Dermal	Inhalation	Total
Systemic Exposure (mg/kg bw/day)			0.0004	0.0033	0.0037
%AOEL	37.4				

70 kg bw

SOWING SEED					
		Total Potential Dermal exposure	Estimated Actual Dermal exposure	Inhalation exposure	
Loading / Sowing (x10 hrs)	(mg/day)	14.79	7.33	0.200	
Total exposure	(mg/kg bw/day)	0.21	0.105	0.003	
			Dermal	Inhalation	Total
Systemic Exposure (mg/kg bw/day)			0.0003	0.0029	0.0032
%AOEL	32.0				

A 3.3 Bystander and resident exposure calculations (KCP 7.2.2.1)

The only intended use of A9873C is treatment of seed prior to sowing. Consequently, no bystander/resident scenario is given.

A 3.4 Combined exposure calculations for metalaxyl-M, fludioxonil and cymoxanil

See section 6.6.5.

Appendix 4 Detailed evaluation of exposure and/or DFR studies relied upon (KCP 7.2, KCP 7.2.1.1, KCP 7.2.2.1, KCP 7.2.3.1)

Report:	KCP 7.2.1.1 [redacted] (2006) Determination of operator exposure to imidacloprid during treatment of sugar beet seeds with IMPRIMO® in France. Amended Final Report 04B033 HI, Rhodia Recherches et Technologies, Laboratoire d'Hygiène Industrielle, F-69162 Saint-Fons Cedex, France. Unpublished. The data are property of the SeedTROPEX Group. Syngenta File No. ASF654/0001 / VV-379857

Guidelines

OECD Series on Principles of GLP and Compliance Monitoring No. 1 (as revised in 1997) "OECD Principles on Good Laboratory Practice", Paris 1998.

OECD Series on Principles of GLP and Compliance Monitoring No. 6 (revised)" The application of GLP-Principles to Field Studies", Paris 1999.

OECD Series on Principles of GLP and Compliance Monitoring No. 13" The application of the OECD Principles of GLP to the Organisation and Management of Multi-Site Studies 2002."

Quality Assurance: OECD Series on Principles of GLP and Compliance Monitoring No. 4 (revised) "Quality Assurance and GLP", Paris 1999.

GLP

Yes (certified laboratory)

Executive Summary

The study was conducted in France in 2004 in two seed treatment plants specially equipped for treatment of sugar beet seeds. The two main application techniques used in sugar beet seed treatment plants were investigated during this study i.e. batch treatment with seed coating and drying operated under negative pressure and coating and drying in fluid bed equipment.

Operators involved in the study were employees of these seed treatment plants. In total 12 replicates (6 per site) were monitored (4 during mixing/loading and 8 during seed treatment operations including supervision, maintenance and cleaning of the equipment). All operators were monitored for a period of a usual working shift.

Potential and actual dermal exposure to imidacloprid was measured by means of whole-body passive dosimetry. The outer dosimeter clothing (long work trousers, long-sleeved shirt and work jacket) corresponded to what workers usually wear at the particular period when sugar beet seeds are treated. Operators also wore a Tyvek® coverall over outer dosimeter clothing following the working rules of the plant. The inner dosimeters (representing the skin) consisted of long-sleeved and long-legged cotton undergarments. Head exposure was measured by performing face/neck wipes, potential and actual hand exposure was determined by performing hand and glove washes. Potential inhalation exposure was measured by means of personal air sampling pumps and an IOM sampler which was positioned in the breathing zone of the operators.

All dosimeter specimens were analysed for imidacloprid.

Materials

Test Item:	IMPRIMO® (containing 400 g/L imidacloprid and 17.8 g/L tefluthrin)

Description:	Water-based seed dressing liquid, formulated as a flowable concentrate (FS)
Lot/Batch #:	Various (commercial product)
Purity:	400 g/L imidacloprid, 17.8 g/L tefluthrin (nominal contents)
Stability of test compound:	Commercial product within shelf-life

Study parameters

Application rate:	0.225 L product/unit seed, corresponding to 90 g imidacloprid and 4.0 g tefluthrin per unit (1 unit seeds = 100'000 seeds).
Seed treatment equipment:	Drum treater under negative pressure (Mereville); fluidized bed treaters (Nerac).
Monitoring times:	Mixing/loading: 78 – 97 minutes (average: 88 minutes); Seed treatment / maintenance / cleaning: 270 – 437 minutes (average: 379 minutes).
Amount seed treated:	482 – 1218 units (average: 921 units).
Amount product used:	Mixing/loading: 181 – 627 kg (average: 383 kg); Seed treatment / maintenance / cleaning: 129 – 325 kg (average: 246 kg)
Number of replicates:	Mixing/loading: 4 (2 in Mereville and 2 in Nerac); Seed treatment / maintenance / cleaning: 8 (4 in Mereville and 4 in Nerac).

Description of mixing / loading

The product was supplied in 25 litre containers.

At Mereville, the mixing/loading operation was performed in a specific area at the opposite side of the seed treatment area. The task consisted in manually loading the components of the mixture containing IMPRIMO® into a vessel for around 30 minutes, stirring the mixture for around 45 minutes and then gravity transferring the mixture into a 1000 L container.

At Nerac, the mixing/loading operation was performed in a specific area closed to the seed treatment area. IMPRIMO® was pumped directly into a storage container using a plunger, which was manually transferred from one container to the other one. For that purpose, all the containers were first opened. IMPRIMO® was pumped. Containers were rinsed with water one by one. Rinsing water was then transferred into the vessel used for mixture preparation. After that, IMPRIMO® containers were re-plugged.

Description of seed treatment activities (supervision, maintenance, cleaning)

At Mereville one operator per shift conducted treatment operations. Coating was performed in two drums which ran in parallel. Around 300 units of seeds were treated per batch in each drum. Coating of each batch lasted around 2 hours. A cleaning cycle was conducted after two treatment cycles. Cleaning was partially automated. The drum was automatically washed with water, however, operators needed to finish drum cleaning using high-pressure water. They also had to unload unused mixture and manually clean the discharge hopper and filters. Some parts of the equipment were removed and washed in a sink.

At Nerac, two operators per shift conducted the seed treatment. Coating was simultaneously performed in 10 fluidized bed treaters. Per batch, 10 units of seeds were treated in each fluidized bed system. At the end of a coating cycle, either a new cycle or a cleaning cycle began. Cleaning was done manually with water and a sponge. Some parts of the equipment were removed and washed in a sink.

Summarised study results

Table A 24: Operator exposure to imidacloprid during mixing/loading

	OP 02	OP 05	OP 07	OP 12	arithm. mean	geom. mean	70th perc.	90th perc.
Total potential dermal exposure (TPDE) 1)								
µg a.s./task	234940	24207	85917	192216	134320	98445	196488	222123
Total actual dermal exposure (TADE) 2)								
µg a.s./task	1013	254	373	1095	684	569	1021	1070
µL IMPRIMO/task	2.53	0.634	0.933	2.74	1.71	1.42	2.55	2.68
µg a.s./hr	627	164	270	842	476	391	648	777
µg a.s./kg b.w. & task	12.1	3.02	4.97	13.5	8.39	7.03	12.2	13.1
µg a.s./kg a.s. & task	16.7	4.17	2.05	5.19	7.02	5.22	6.34	13.2
Potential inhalation exposure (IHL) 3)								
µg a.s./task	8.23	4.51	4.84	21.8	9.84	7.91	9.59	17.7
µL IMPRIMO/task	0.021	0.011	0.012	0.054	0.025	0.020	0.024	0.044
µg a.s./hr	5.09	2.91	3.50	16.7	7.06	5.43	6.25	13.2
µg a.s./kg b.w. & task	0.098	0.054	0.065	0.269	0.121	0.098	0.115	0.217
µg a.s./kg a.s. & task	0.135	0.074	0.027	0.103	0.085	0.072	0.106	0.126

1) Sum of residues on outer dosimeters (work trousers and work jacket, shirt, Tyvek® coverall where worn), inner dosimeters (representing the skin), face/neck wipes, hand wash solutions, gloves. Values for individual operators have been taken from Table 13 of the Amended Final Report.
2) Sum of residues on inner dosimeters (representing the skin), face/neck wipes, hand wash solutions.
3) Based on an average ventilation rate of 14 L/min.

Table A 25: Operator exposure to imidacloprid during seed treatment (supervision / maintenance / cleaning)

	OP 01	OP 03	OP 04	OP 06	OP 08	OP 09	OP 10	OP 11	arith m. mean	geom. mean.	70th perc.	90th perc.
	Total potential dermal exposure (TPDE) 1)											
µg a.s./task	128027	137653	31594	20252	74322	39043	54903	59059	68107	56651	72796	130915
	Total actual dermal exposure (TADE) 2)											
µg a.s./task	1794	1110	1046	1014	4283	1890	5266	7022	2928	2239	4044	5793
µg a.s./hr	246	173	152	153	695	304	829	1561	514	358	656	1049
µg a.s./kg b.w. & task	32.6	13.7	19.0	12.5	57.1	22.4	53.5	86.7	37.2	29.8	51.4	66.0
µg a.s./kg a.s. & task	16.4	11.3	10.2	9.60	63.4	28.0	77.9	161.7	47.3	28.3	59.9	103
	Potential inhalation exposure (IHL) 3)											
µg a.s./task	132	63.1	28.2	95.5	14.9	13.6	8.82	54.6	51.3	34.9	62.3	106
µg a.s./hr	18.1	9.8	4.08	14.4	2.42	1.93	1.26	12.1	8.02	5.42	11.9	15.5
µg a.s./kg b.w. & task	2.39	0.779	0.512	1.18	0.199	0.161	0.090	0.674	0.748	0.465	0.769	1.54
µg a.s./kg a.s. & task	1.21	0.643	0.276	0.907	0.221	0.201	0.131	1.26	0.605	0.440	0.881	1.22

1) Sum of residues on outer dosimeters (work trousers and work jacket, shirt, Tyvek® coverall where worn), inner dosimeters (representing the skin), face/neck wipes, hand wash solutions, gloves. Values for individual operators were taken from Table 14 of the Amended Final Report.
2) Sum of residues on inner dosimeters (representing the skin), face/neck wipes, hand wash solutions.
3) Based on an average ventilation rate of 14 L/min.

Conclusions

The study is considered to provide suitable data for the estimation of operator exposure during treatment of sugar beet seeds by means of drum coaters and fluidized bed treaters ([redacted] 2006).

Report:	KCP 7.2.1.2 [redacted], 2009 Fluquinconazole and Prochloraz: Determination of Operator Exposure During Cereal Seed Treatment With "Jockey" Fungicide in Germany, United Kingdom and France. ACI07-006 Syngenta file No. ASF827_10000 / VV-393832

Guidelines:

OCDE/GD(97)148 Series on Testing and Assessment No. 9, Guidance Document for the Conduct of Studies of Occupational Exposure to Pesticides During Agricultural Application, Organisation for Economic Cooperation and Development, Paris.

Deviations: none

GLP

Yes (certified laboratory)

Executive Summary

In 2007, a Good Laboratory Practice (GLP) operator exposure study was conducted with thirty-nine operators in Germany, United Kingdom and France. The study was performed to monitor potential dermal and inhalation exposure to fluquinconazole and prochloraz during a typical days' activities associated with mixing/loading, bagging of treated seed and cleaning of seed treatment equipment. Twenty two operators were monitored for exposure during procedures associated with bagging only. Eight operators were monitored for exposure during procedures associated with the cleaning of the treatment chamber. Nine operators were monitored for the exposure during procedures associated with mixing/loading and when performed calibration.

Bagging

The bagging activities were performed as closely as possible to normal practices whilst using commercial equipment in commercial seed treatment facilities.

The type of seed bagged were small grain cereals (wheat). The seed treatment was performed at 0.681 to 0.752 g/kg seed (fluquinconazole) and 0.128 to 0.140 g/kg seed (prochloraz) using 'Jockey Plus AB' containing 167 g/L fluquinconazole (nominal) and 31.2 g/L prochloraz (nominal). In some cases, the test item was diluted with water prior to treatment (either in the slurry tank, or directly at the treatment chamber). The duration of each bagging activity was 2.30 to 7.72 hours (average: 5.30 hours excluding any routine breaks) and the quantity of seed actually bagged was 25.05 to 86.00 tonnes (average: 54.1 tonnes) for each bagging line. One to three operators worked on the same bagging line. The total amount of fluquinconazole handled for each bagging line was 17.07 to 64.63 kg (average: 42.23 kg). The total amount of prochloraz handled for each bagging line was 3.189 to 12.08 kg (average: 7.907 kg).

Cleaning

The cleaning activity was performed as closely as possible to normal practices using commercial equipment in commercial seed treatment facilities. Cleaning was monitored at four locations in Germany, three locations in UK and one location in France.

Cleaning involved cleaning of the treatment chamber. Cleaning was conducted on either continuous flow or batch treatment chambers. The duration of each cleaning activity was between 0.12 to 0.55 hours (average: 17 min). The cleaning of the treatment chamber was performed by one operator (working alone).

Mixing/loading/calibration

Mixing/loading/calibration was monitored in four locations in Germany and one in France. The procedure involved either suction transfer from 200L drums, two locations in Germany, or a transfer into a mixing tank in two locations in Germany and the single location in France. Manual calibration was performed in two locations in Germany. Automatic calibration occurred in two locations in Germany and the location in France.

In the United Kingdom, mixing/loading was monitored in four locations. The procedure always involved dry-coupling and calibration was automatic.

Materials

Test Material:	'Jockey' (called Jockey Plus AB in France)
Description:	A flowable suspension for seed treatment
Lot/Batch Number:	1159541, 1556013, 1239029, 1970163, 1816396, 1460359, 1859936, 1387219, 1816396, 1443159, 1816393, 1816396
Purity:	Nominal 167 g/L fluquinconazole and 31.2 g/L prochloraz
Stability of test compound:	Stable for the duration of the study

Study Design and Methods

Field Phase dates:	23 August 2007 to 14 September 2007
Experimental dates:	23 August 2007 to 19 December 2007

Study Description

39 operators were monitored between 23 August 2007 and 14 September 2007.

The purpose of this study was to generate operator exposure data during the mixing/loading/calibration, bagging of treated seed and cleaning of seed treatment equipment at static sites in Germany (6 sites), United Kingdom (4 sites) and France (1 site) following treatment with a fungicide nominally containing 167 g/L fluquinconazole and 31.2 g/L prochloraz (34 g/L as copper chloride complex) using batch or continuous flow seed treatment equipment. The recommended use rate of the product is 4.5 L per tonne of seed, equivalent to 751.5 g fluquinconazole and 140.4 g prochloraz per tonne of seed.

The three main phases of seed treatment were followed in this study, namely the mixing/loading/calibration, bagging of treated seed and cleaning of seed treatment equipment.

Dermal exposure was measured by operators wearing standardised whole-body outer and inner dosimeters. For the bagging activities, each operator wore dosimeters consisting of a long sleeved jacket and long trousers (100% cotton), long sleeved vest and long-johns (100% cotton). The nitrile gloves were made available for the operators (worn at the discretion of the operator when touching contaminated surfaces). For the cleaning activities, each operator wore the same dosimeters as the bagging activities in addition to an impermeable coverall ('Tyvek') and impermeable gloves (nitrile), which were worn throughout the cleaning activities.

Head exposure was measured by face/neck wipes.

Actual hand exposure was measured by the handwash procedure. Protective gloves, worn in accordance with label recommendations, were analysed for the determination of potential hand exposure.

Inhalation exposure was measured by means of personal air sampling pumps connected to an IOM sampling cassette with glass fibre filter located in the operator's breathing zone.

All samples collected were analysed for residues of fluquinconazole and prochloraz.

Inner and outer dosimeters, Tyvek, face/neck wipes and nitrile gloves were cut into small pieces and placed into glass vessels and extracted with methanol. Air sampling filters were extracted with acetone. All extracts were diluted for the determination of fluquinconazole and prochloraz by HPLC-MS/MS.

Hand wash solutions were directly analysed by HPLC-MS/MS.

Results - Prochloraz

Since all mean field fortification recoveries for prochloraz were within the range 92 to 106% operator exposure results have not been corrected. Where a residue below the limit of quantification (LOQ) has been found a value of 0.5 × LOQ has been reported and used in summary calculations. The following table gives a summary of the residues of test item on each dosimeter for each operator. Actual dermal exposure is calculated by summing residues from inner dosimeters, hand wash and face/neck wipe specimens. Potential inhalation exposure is the residues measured in the breathing zone based upon a ventilation rate of 14 L/min for tasks. All field fortified recovery samples for prochloraz gave recoveries ≥ 92%.

Table A 26: Determined Residues of prochloraz during bagging (all values in µg/sample)

Operator Number	6	7	4	14	15	16	17	19	20	21	22
Body Weight (kg)	75.00	83.70	84.00	88.70	109.0	97.30	76.20	100.0	105.2	105.1	100.7
Exposure time (min)	284.0	426.0	403.0	408.0	398.0	458.0	265.0	460.0	402.0	285.0	285.0
Outer Dosimeter – cotton work jacket and trousers											
arms	78.90	6.870	45.30	21.75	208.5	175.5	228.0	14.79	5.070	169.5	5.145
legs	50.00	5.120	62.80	11.12	114.8	182.0	136.4	24.76	8.120	125.2	5.000
torso	101.0	13.80	108.0	28.08	206.2	178.8	286.0	52.68	11.67	240.0	20.04
TOTAL	229.9	25.79	216.1	60.95	529.5	536.3	650.4	92.23	24.86	534.7	30.19
Inner dosimeter (representing the skin)											
arms	11.48	0.770	3.150	0.405	9.660	32.55	27.51	2.625	1.358	15.82	1.204
legs	7.792	0.824	2.160	0.629	6.144	33.60	8.800	1.448	0.824	4.304	0.356
torso	20.68	1.515	4.336	1.440	29.22	32.75	13.17	4.981	2.417	11.17	0.751
TOTAL	39.95	3.109	9.646	2.474	45.02	98.90	49.48	9.054	4.599	31.29	2.311
Handwash											
Measured	34.22	5.910	71.50	10.45	94.80	232.6	115.7	20.28	20.15	193.1	23.26
TOTAL	34.22	5.910	71.50	10.45	94.80	232.6	115.7	20.28	20.15	193.1	23.26
Face/neck wipes											
Measured	2.805	0.201	2.819	0.100	2.749	3.421	11.16	1.353	0.261	0.907	0.186
TOTAL	2.805	0.201	2.819	0.100	2.749	3.421	11.16	1.353	0.261	0.907	0.186
Nitrile Gloves											
TOTAL	NA	5.008	NA	NA	2936	616.0	63.60	NA	38.56	NA	NA
Residues in air sampling tubes											
Measured	0.556	0.297	0.390	0.150	0.287	0.544	1.820	0.337	0.262	0.380	0.025
TOTAL	0.556	0.297	0.390	0.150	0.287	0.544	1.820	0.337	0.262	0.380	0.025

Table A 27: Determined Residues of prochloraz during bagging (all values in µg/sample)

Operator Number	23	24	1	3	5	8	10	11	13	18	25
Body Weight (kg)	90.00	118.0	63.20	80.50	63.00	81.00	65.60	90.10	81.30	71.00	97.70
Exposure time (min)	288.0	463.0	177.0	177.0	270.0	226.0	138.0	265.0	267.0	274.0	383.0
Outer Dosimeter – cotton work jacket and trousers											
arms	62.10	52.50	1.065	6.960	8.190	5.895	0.065	15.75	47.55	8.430	10.61
legs	33.36	45.60	1.664	2.656	5.880	9.840	0.122	40.00	59.20	7.360	17.84
torso	117.0	163.5	2.632	3.500	10.34	8.976	0.120	145.9	54.08	20.48	30.20
TOTAL	212.5	261.6	5.361	13.12	24.41	24.71	0.307	201.7	160.8	36.27	58.65
Inner dosimeter (representing the skin)											
arms	19.39	21.98	0.310	0.131	1.281	0.587	0.056	3.031	14.77	1.260	1.547
legs	4.200	19.52	0.189	0.232	0.363	0.277	0.036	1.736	1.368	0.283	1.400
torso	6.172	10.00	0.390	0.255	0.991	1.553	0.084	28.82	14.95	3.097	5.826
TOTAL	29.76	51.50	0.889	0.617	2.635	2.417	0.176	33.59	31.09	4.640	8.773
Handwash											
Measured	406.0	281.4	4.867	2.294	2.688	8.070	0.050	243.9	48.20	3.380	34.20
TOTAL	406.0	281.4	4.867	2.294	2.688	8.070	0.050	243.9	48.20	3.380	34.20
Face/neck wipes											
Measured	3.573	9.512	0.156	0.100	0.112	0.100	n.d.	4.362	4.895	1.949	0.608
TOTAL	3.573	9.512	0.156	0.100	0.112	0.100	n.d.	4.362	4.895	1.949	0.608
Nitrile Gloves											
TOTAL	NA	NA	NA	NA	NA	3.800	NA	NA	NA	NA	NA
Residues in air sampling tubes											
Measured	0.319	5.280	0.014	0.010	0.107	0.058	0.006	0.033	0.050	0.353	0.090
TOTAL	0.319	5.280	0.014	0.010	0.107	0.058	0.006	0.033	0.050	0.353	0.090

Table A 28: Summary of Field Results – prochloraz bagging

Operator Number	6	7	4	14	15	16	17	19	20	21	22
Actual Dermal Exposure (µg/hr)	16.263	1.298	12.506	1.915	21.492	43.87	39.93	4.002	3.732	47.429	5.423
Potential Inhalation Exposure (µg/hr)	0.822	0.293	0.406	0.158	0.311	0.499	2.885	0.308	0.274	0.561	0.036
Active Substance handled (kg/day)	4.914	3.941	10.73	3.941	11.65	9.126	4.914	10.73	3.941	12.08	12.08

Actual Dermal Exposure (ADE) = Sum of residues on inner dosimeter representing the skin, face/neck wipes and hand wash solutions.
Potential Inhalation Exposure (PIE) = Residues measured in the breathing zone expressed as µg/hr (at a breathing rate of 14 L/min).

Table A 29: Summary of Field Results – prochloraz bagging

Operator Number	23	24	1	3	5	8	10	11	13	18	25
Actual Dermal Exposure (µg/hr)	91.525	44.377	2.004	1.021	1.207	2.808	0.099	63.812	18.917	2.183	6.828
Potential Inhalation Exposure (µg/hr)	0.466	4.790	0.033	0.023	0.167	0.109	0.019	0.053	0.079	0.541	0.099
Active Substance handled (kg/day)	12.08	10.73	6.880	6.880	4.423	7.020	3.189	9.316	9.316	4.423	11.65

Actual Dermal Exposure (ADE) = Sum of residues on inner dosimeter representing the skin, face/neck wipes and hand wash solutions.
Potential Inhalation Exposure (PIE) = Residues measured in the breathing zone expressed as µg/hr (at a breathing rate of 14 L/min).

Table A 30: Determined Residues of prochloraz during cleaning (all values in µg/sample)

Operator Number	38	39	45	48	40	43	44	47
Body Weight (kg)	76.20	90.00	109.0	105.2	65.60	81.00	96.80	100.1
Exposure time (min)	33.00	20.00	9.000	15.00	26.00	16.00	7.000	13.00
Inner dosimeter (representing the skin)								
arms	10.64	3.122	1.274	0.105	0.095	0.203	0.395	25.83
legs	2.560	3.296	24.56	0.089	0.242	0.088	0.165	3.952
torso	4.846	2.737	15.56	0.235	0.183	0.378	1.664	2.582
TOTAL	18.05	9.155	41.40	0.429	0.519	0.669	2.223	32.36
Handwash								
Measured	18.90	24.50	138.0	13.80	0.542	0.824	1.090	13.90
TOTAL	18.90	24.50	138.0	13.80	0.542	0.824	1.090	13.90
Face/neck wipes								
Measured	21.00	14.04	8.503	0.155	0.050	0.269	0.725	2.053
TOTAL	21.00	14.04	8.503	0.155	0.050	0.269	0.725	2.053
Residues in air sampling tubes								
Measured	0.696	0.314	0.980	2.864	0.103	0.258	0.084	0.054
TOTAL	0.696	0.314	0.980	2.864	0.103	0.258	0.084	0.054

Table A 31: Summary of field results - prochloraz cleaning

Operator Number	38	39	45	48	40	43	44	47
Actual Dermal Exposure (µg/operation)	57.95	47.69	187.9	14.384	1.111	1.762	4.038	48.32
Potential Inhalation Exposure (µg/operation)	4.872	2.195	6.860	20.05	0.720	1.806	0.588	0.381

Actual Dermal Exposure (ADE) = Sum of residues on inner dosimeter representing the skin, face/neck wipes and hand wash solutions.
Potential Inhalation Exposure (PIE) = Residues measured in the breathing zone expressed as µg/operation (at a breathing rate of 14 L/min).

Table A 32: Determined Residues of prochloraz during mixing/loading/calibration (all values in µg/sample)

Procedure	Pre-mix					Dry-couple			
Operator Number	27	28	33	34	36	31	26	32	35
Body Weight (kg)	84.00	76.20	65.60	105.2	89.10	100.1	96.80	81.00	70.10
Exposure time (min)	10.00	25.00	32.00	32.00	459.0	6.000	3.000	2.000	2.000
Outer Dosimeter – cotton work jacket and trousers									
arms	0.236	3.870	0.094	22.95	253.5	0.459	0.193	0.642	0.169
legs	1.832	18.16	n.d.	6.120	244.8	0.852	0.159	0.404	0.198
torso	14.200	6.960	n.d.	369.0	634.4	0.714	0.120	1.888	0.040
TOTAL	16.268	28.99	0.094	398.1	1133	2.025	0.472	2.934	0.407
Inner dosimeter (representing the skin)									
arms	0.109	2.884	0.050	0.173	21.77	1.624	0.076	0.110	0.103
legs	0.178	1.194	0.026	0.254	12.08	1.034	0.097	0.103	0.129
torso	0.442	1.039	0.077	0.941	51.93	2.206	0.236	1.060	0.264
TOTAL	0.729	5.117	0.153	1.368	85.78	4.864	0.409	1.274	0.496
Handwash									
Measured	6.080	7.310	n.d.	1.490	109.9	5.34	0.538	0.455	0.200
TOTAL	6.080	7.310	n.d.	1.490	109.9	0.534	0.538	0.455	0.200
Face/neck wipes									
Measured	0.364	1.357	n.d.	0.104	15.21	0.500	0.099	0.050	0.050
TOTAL	0.364	1.357	n.d.	0.104	15.21	0.500	0.099	0.050	0.050
Residues in air sampling tubes									
Measured	0.002	0.008	0.005	0.012	0.400	0.001	n.d.	n.d.	n.d.
TOTAL	0.002	0.008	0.005	0.012	0.400	0.001	n.d.	n.d.	n.d.

Table A 33: Summary of Field Results – prochloraz mixing/loading/calibration

Operator Number	27	28	33	34	36	31	26	32	35
Actual Dermal Exposure (µg/operation)	7.173	13.784	0.153	2.962	210.9	10.704	1.046	1.779	0.746
Potential Inhalation Exposure (µg/operation)	0.015	0.057	0.033	0.082	2.800	0.007	n.d.	n.d.	n.d.

Actual Dermal Exposure (ADE) = Sum of residues on inner dosimeter representing the skin, face/neck wipes and hand wash solutions.
Potential Inhalation Exposure (PIE) = Residues measured in the breathing zone expressed as µg/operation (at a breathing rate of 14 L/min).

Conclusions - Prochloraz

The study is considered to provide suitable data for the estimation of operator exposure for the tasks of bagging and equipment cleaning during the treatment of seed.

Results - Fluquinconazole

Since all mean field fortification recoveries for fluquinconazole were greater than 98% operator exposure results have not been corrected. Where a residue below the limit of quantification (LOQ) has been found a value of 0.5 × LOQ has been reported and used in summary calculations.

The following table gives a summary of the residues of test item on each dosimeter for each operator.

Actual dermal exposure is calculated by summing residues from inner dosimeters, hand wash and face/neck wipe specimens. Potential inhalation exposure is the residues measured in the breathing zone based upon a ventilation rate of 14 L/min for tasks.

All field fortified recovery samples for fluquinconazole, gave recoveries greater than 98%.

Table A 34: Determined Residues of fluquinconazole during bagging (all values in µg/sample)

Operator Number	6	7	4	14	15	16	17	19	20	21	22
Body Weight (kg)	75.00	83.70	84.00	88.70	109.00	97.30	76.20	100.00	105.20	105.10	100.70
Exposure time (min)	284.0	426.0	403.0	408.0	398.0	458.0	265.0	460.0	402.0	285.0	285.0
Outer Dosimeter – cotton work jacket and trousers											
arms	66.45	42.75	196.5	113.000	831.00	211.50	333.00	75.60	41.3	289.50	18.00
legs	76.4	36.5	282.4	77.600	397.60	211.6	105.60	105.60	57.2	327.60	14.0
torso	98.36	101.9	529	152.000	636.00	231.20	518.4	270.0	86.32	810.0	71.44
TOTAL	241.2	181	1008	342.60	1864.60	654.3	957	451	185	1427.1	103.5
Inner dosimeter (representing the skin)											
arms	9.520	5.334	7.140	2.198	30.59	48.58	25.90	12.11	10.99	47.67	1.218
legs	5.920	5.624	8.560	3.944	14.56	23.84	7.384	6.352	5.296	8.080	0.7952
torso	11.430	8.480	8.888	7.244	55.54	30.340	22.500	23.300	17.880	24.12	1.2050
TOTAL	26.87	19.44	24.59	13.39	100.69	102.8	55.78	41.76	34.17	79.87	3.218
Handwash											
Measured	35.060	68.100	317.600	87.500	575.000	244.000	191.600	111.600	180.700	873.000	61.970
TOTAL	35.060	68.100	317.600	87.500	575.000	244.000	191.600	111.600	180.700	873.000	61.970
Face/neck wipes											
Measured	2.493	1.34	16.69	0.983	9.988	4.109	30.380	9.512	2.501	3.294	0.250
TOTAL	2.493	1.34	16.69	0.983	9.988	4.109	30.380	9.512	2.501	3.294	0.250
Nitrile Gloves											
TOTAL	NA	37.12	NA	NA	16040	2024	140.8	NA	213.2	NA	NA
Residues in air sampling tubes											
Measured	1.8	1.208	2.000	0.864	1.3	0.397	6.48	1.752	1.44	1.728	0.076
TOTAL	1.8	1.208	2.000	0.864	1.3	0.397	6.48	1.752	1.44	1.728	0.076

Values in italics are < LOQ. Half the LOQ is taken for the calculations

Table A 35: Determined Residues of fluquinconazole during bagging (all values in µg/sample)

Operator Number	23	24	1	3	5	8	10	11	13	18	25
Body Weight (kg)	90.00	118.00	63.20	80.50	63.00	81.00	65.60	90.10	81.30	71.00	97.70
Exposure time (min)	288.0	463.0	177.0	177.0	270.0	226.0	138.0	265.0	267.0	274.0	383.0
Outer Dosimeter – cotton work jacket and trousers											
arms	283.50	307.50	1.191	11.910	42.9	33.600	0.669	61.350	73.050	45.450	36.000
legs	109.60	234.4	2.356	8.280	31.480	44.800	3.600	32.400	69.200	40.400	40.800
torso	466.40	888.00	6.572	9.876	52.960	48.040	2.444	621.600	55.720	106.100	84.400
TOTAL	859.5	1429.9	10.119	30.066	127.340	126.440	6.713	715.350	197.970	191.950	161.200
Inner dosimeter (representing the skin)											
arms	64.89	105.7	0.4580	0.3920	5.859	1.967	0.4330	5.040	1.260	4.088	3.136
legs	7.336	86.40	0.2080	0.3730	1.528	1.280	1.382	1.400	0.544	0.8080	2.712
torso	19.250	47.080	0.6860	0.5930	4.4710	3.3690	1.4070	88.06	2.2360	10.6100	10.380
TOTAL	91.48	239.2	1.352	1.358	11.86	6.616	3.222	94.50	4.040	15.51	16.23
Handwash											
Measured	1868.000	1779.000	19.530	4.534	14.140	67.530	2.370	1222.000	110.000	17.120	56.400
TOTAL	1868.000	1779.000	19.530	4.534	14.140	67.530	2.370	1222.000	110.000	17.120	56.400
Face/neck wipes											
Measured	15.200	63.92	*0.5*	*0.5*	0.675	0.500	0.250	7.187	1.362	15.080	1.044
TOTAL	15.200	63.92	*0.5*	*0.5*	0.675	0.500	0.250	7.187	1.362	15.080	1.044
Nitrile Gloves											
TOTAL	NA	NA	NA	NA	NA	23.44	NA	NA	NA	NA	NA
Residues in air sampling tubes											
Measured	1.36	27.52	0.056	0.038	0.584	0.33	0.035	0.126	0.154	2.008	0.363
TOTAL	1.36	27.52	0.056	0.038	0.584	0.33	0.035	0.126	0.154	2.008	0.363

Values in italics are <LOQ. Half the LOQ is taken for the calculations

Table A 36: Summary of Field Results – fluquinconazole bagging

Operator Number	6	7	4	14	15	16	17	19	20	21	22
Actual Dermal Exposure (µg/hr)	13.610	12.518	53.431	14.981	103.369	45.965	62.890	21.244	32.443	201.298	13.776
Potential Inhalation Exposure (µg/hr)	2.662	1.191	2.084	0.912	1.407	0.364	10.270	1.600	1.504	2.547	0.112
Active Substance handled (kg/day)	26.300	21.11	57.41	21.11	62.370	48.850	26.30	57.410	21.11	64.630	64.630

Actual Dermal Exposure (ADE) = Sum of residues on inner dosimeter representing the skin, face/neck wipes and hand wash solutions.
Potential Inhalation Exposure (PIE) = Residues measured in the breathing zone expressed as µg/hr (at a breathing rate of 14 L/min).

Table A 37: Summary of Field Results – fluquinconazole bagging

Operator Number	23	24	1	3	5	8	10	11	13	18	25
Actual Dermal Exposure (µg/hr)	411.391	269.819	7.248	2.167	5.927	19.818	2.540	299.703	25.933	10.447	11.541
Potential Inhalation Exposure (µg/hr)	1.983	24.964	0.133	0.090	0.908	0.613	0.107	0.200	0.242	3.078	0.398
Active Substance handled (kg/day)	64.63	57.410	36.820	36.82	23.670	37.580	17.070	49.86	49.860	23.670	62.370

Actual Dermal Exposure (ADE) = Sum of residues on inner dosimeter representing the skin, face/neck wipes and hand wash solutions.
Potential Inhalation Exposure (PIE) = Residues measured in the breathing zone expressed as µg/hr (at a breathing rate of 14 L/min).

Table A 38: Determined Residues of fluquinconazole during cleaning (all values in µg/sample)

Operator Number	38	39	45	48	40	43	44	47
Body Weight (kg)	76.20	90.00	109.00	105.20	65.60	81.0	96.80	100.10
Exposure time (min)	33.00	20.00	9.00	15.00	26.00	16.00	7.0	13.00
Inner dosimeter (representing the skin)								
arms	10.57	8.400	6.118	1.127	0.5215	0.7504	1.554	21.91
legs	1.968	9.760	92.00	0.576	1.896	0.4312	0.4448	11.04
torso	7.0980	10.240	35.840	1.9050	1.6130	1.2770	1.49500	2.2830
TOTAL	19.64	28.40	134.0	3.608	4.031	2.459	3.494	35.23
Handwash								
Measured	13.700	53.100	717.000	109.000	3.880	4.630	2.81	51.300
TOTAL	13.700	53.100	717.000	109.000	3.880	4.630	2.81	51.300
Face/neck wipes								
Measured	37.93	75.98	43.040	1.125	0.571	1.008	3.816	8.746
TOTAL	37.93	75.98	43.040	1.125	0.571	1.008	3.816	8.746
Residues in air sampling tubes								
Measured	0.912	1.252	4.8	0.042	0.804	1.06	0.432	0.079
TOTAL	0.912	1.252	4.8	0.042	0.804	1.06	0.432	0.079

Values in italics are < LOQ. Half the LOQ is taken for the calculations

Table A 39: Summary of Field Results – fluquinconazole cleaning

Operator Number	38	39	45	48	40	43	44	47
Actual Dermal Exposure (µg/operation)	71.266	157.480	893.998	113.733	8.482	8.097	10.120	95.279
Potential Inhalation Exposure (µg/operation)	6.38	8.76	33.60	0.29	5.63	7.420	3.02	0.553

Actual Dermal Exposure (ADE) = Sum of residues on inner dosimeter representing the skin, face/neck wipes and hand wash solutions.
Potential Inhalation Exposure (PIE) = Residues measured in the breathing zone expressed as µg/operation (at a breathing rate of 14 L/min).

Table A 40: Determined Residues of fluquinconazole during mixing/loading/calibration (all values in µg/sample)

Procedure	Pre-mix					Dry-couple			
Operator Number	27	28	33	34	36	31	26	32	35
Body Weight (kg)	84.0	76.2	65.6	105.2	89.1	100.1	96.8	81.0	70.1
Exposure time (min)	10	25	32	32	459	6	3	2	2
Outer Dosimeter – cotton work jacket and trousers									
arms	1.002	0.99	0.51	144.20	112.2	0.150	0.150	3.420	1.308
legs	8.56	11.720	4.520	39.48	135.2	1.120	n.d.	2.072	0.200
torso	71.32	3.408	2.412	1782	246.8	0.836	n.d.	14.68	n.d.
TOTAL	80.88	16.12	7.45	1965.7	494.2	2.106	0.150	20.17	1.508
Inner dosimeter (representing the skin)									
arms	0.537	0.262	0.266	1.792	8.470	0.482	*0.035*	0.507	*0.035*
legs	0.968	0.270	1.856	1.704	5.032	0.606	*0.040*	0.429	*0.040*
torso	2.575	0.157	1.061	4.610	21.06	0.899	*0.090*	1.128	0.146
TOTAL	4.080	0.689	3.183	8.106	34.56	1.987	0.165	2.064	0.221
Handwash									
Measured	25.200	0.995	1.300	15.300	103.4	3.860	*0.250*	2.390	*0.250*
TOTAL	25.200	0.995	1.300	15.300	103.4	3.860	*0.250*	2.390	*0.250*
Face/neck wipes									
Measured	1.705	*0.250*	*0.250*	0.900	6.218	*0.250*	*0.250*	*0.250*	n.d.
TOTAL	1.705	*0.250*	*0.250*	0.900	6.218	*0.250*	*0.250*	*0.250*	n.d.
Residues in air sampling tubes									
Measured	*0.005*	*0.005*	*0.062*	0.076	0.147	n.d.	n.d.	n.d.	n.d.
TOTAL	*0.005*	*0.005*	*0.062*	0.076	0.147	n.d.	n.d.	n.d.	n.d.

Values in italics are < LOQ. Half the LOQ is taken for the calculations

Table A 41: Summary of Field Results – fluquinconazole mixing/loading/calibration

Operator Number	27	28	33	34	36	31	26	32	35
Actual Dermal Exposure (µg/operation)	30.985	1.934	4.733	24.306	144.18	6.097	0.665	4.704	0.471
Potential Inhalation Exposure (µg/operation)	0.035	0.035	0.434	0.529	1.029	n.d.	n.d.	n.d.	n.d.

Actual Dermal Exposure (ADE) = Sum of residues on inner dosimeter representing the skin, face/neck wipes and hand wash solutions.
Potential Inhalation Exposure (PIE) = Residues measured in the breathing zone expressed as µg/operation (at a breathing rate of 14 L/min).

Conclusions - Fluquinconazole

The study is considered to provide suitable data for the estimation of operator exposure for the tasks of bagging and equipment cleaning during the treatment of seed.

Overall Conclusions

Dermal and inhalation exposure to prochloraz and fluquinconazole during mixing/loading/calibration, bagging and cleaning was calculated using the 75th percentile of the measured data (Table A 42). During the mixing/loading/calibration tasks, inhalation exposure was not measured for all operators. This left just five data points, which was not suitable for calculating an exposure value.

Table A 42: Summary of Field Results (75th percentile)

Task	Data normalisation	Prochloraz		Fluquinconazole	
		Estimated actual dermal exposure	Inhalation exposure[(a)]	Estimated actual dermal exposure	Inhalation exposure[(a)]
Mixing/loading/ calibration	[mg/operation]	0.0107	not enough data	0.0243	not enough data
Bagging	[mg/hour]	0.0353	0.0010	0.0605	0.0043
Cleaning	[mg/operation]	0.0507	0.0111	0.1247	0.0161

(a) Based on an average ventilation rate of 29 L/min