REGISTRATION REPORT

Part B

Section 9

Ecotoxicology

Detailed summary of the risk assessment

Product code: 19202

Product name: **KINVARA**

Chemical active substances:

MPCA, 233 g/L
Fluroxypyr (acid), 50 g/L
Clopyralid, 28 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(formulation renewal)

Applicant: XXXX

Submission date: 31/01/2024

Evaluation date: October 2024

MS Finalisation date: March 2025 / February 2026

Version history

When	What
31/01/2024	Art.43 Kinvara
October 2024	Conclusions of zRMS
February 2025	Applicant updates
March 2025	Final version by zRMS
December 2025	Final applicant updates – point 9.13 (confidential data removal)
December 2025	zRMS updated version
February 2026	Last zRMS correction following Applicant's comment

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9 Ecotoxicology (KCP 10)

This document reviews the eco-toxicological studies for the product Kinvara, a micro emulsion formulation containing the active substances MCPA 233 g/L, fluroxypyr 50 g/L and clopyralid 28 g/L for use on winter and spring cereals, grassland (professional and amenity) and amenity turf. Kinvara is currently registered for use in the Central Zone and this authorisation was granted under current/modern EU protocols. The need to renew the formulation was triggered by EU renewal of the active substance clopyralid on October 1, 2021 and therefore the only significant changes to this document over the previous authorisation report pertain to where the EU changed the active substance endpoints for clopyralid.

Fluroxypyr was included into Annex I under transitional arrangements of Directive 91/414 and Regulation 1107/2009 (Commission Directive 2000/10/EC) as amended by Reg. 736/2011 on 1St January 2012. Clopyralid and MCPA active substances were included into Annex I under Directive 91/414 on 1st May 2007 (Commission Directive 06/64/EC) and 1st October 2021 (Commission Directive 2021/1191/EC) respectively, as amended under Regulation (EU) No. 540/2011.

A full risk assessment according to Uniform Principles is provided which demonstrates that the product is safe for the environment.

Where appropriate this document refers to the conclusions of the EU review for MCPA, fluroxypyr and clopyralid. This will be where:

- the active substance data is relied upon in the risk assessment of the formulation; or when
- the EU review concluded that additional data/information should be considered at national re-registration.

It is proposed that Annex II data requirements for the active substances are addressed as follows.

XXXX have access to a full Annex II data package from the notifier Nufarm in respect of the active substance MCPA. Please refer to the enclosed LoA.

XXXX has successfully obtained Annex I listing of their technical fluroxypyr under Regulation 1107/2009 and have been considered to have a full Annex II data package on the active substance through Step 1 of the regulatory process (RMS: IE).

XXXX requested access to all relevant data submitted by DOW for clopyralid during the Community review programme under Council Directive 91/414/EEC. Data submitted under Annex II is no longer considered to be under data protection.

A full technical equivalence assessment was conducted for each active substance in-line with appropriate SANCO guidelines and can be provided to other Member State regulatory authorities if required.

This product was not the representative formulation. The product has not been previously evaluated in the United Kingdom according to Uniform Principles.

To address Annex III requirements, studies have been provided for Kinvara and all relevant risk assessments are reported within this dossier and are considered adequate.

The SANCO report and EFSA final conclusions, were applicable, for the active substances MCPA (*SANCO/4062/2001-final 11 July 2008*), fluroxypyr (*SANCO/11019/2011 rev 3 17 June 2011) and EFSA final conclusions (EFSA journal 2011 9 (3):2091*) and clopyralid (*EFSA journal 2018; 16(7):5389*) are considered to provide the relevant review information or a reference to where such information can be found.

The Annex I Inclusion Directive for MCPA (Commission Directive 2005/57/EC), fluroxypyr (Reg (EU) 736/2011) and clopyralid (Commission Directive 06/64/EC) provide specific provisions under Part B which need to be considered by the applicant in the preparation of their submission and by the MS prior to granting an authorisation.

For the implementation of the uniform principles of Annex VI, the conclusions of the review reports on the active substances MCPA, fluroxypyr and clopyralid, and in particular Appendices I and II therein, as finalised in the Standing Committee on the Food Chain and Animal Health on the 21st September 2005, 26th July 2011 and 1th October 2021 respectively, shall be taken into account. In this overall assessment:

For the active substance MCPA, Member States must pay particular attention to the:

- *potential for groundwater contamination, when the active substance is applied in regions with vulnerable soil and/or climatic conditions. Conditions of authorisation should include risk mitigation measures, where appropriate*
- *protection of aquatic organisms and must ensure that the conditions of authorisation include risk mitigation measures, where appropriate, such as buffer zones*

For the active substance fluroxypyr, Member States must pay particular attention to the:

- *pay particular attention to the potential contamination of groundwater by metabolite fluroxypyr pyridinol, when the active substance is applied in regions with alkaline or vulnerable soil and/or with vulnerable climatic conditions;*
- *pay particular attention to the risk to aquatic organisms.*

For the active substance clopyralid, Member States must pay particular attention to the:

- *the protection of non target plants and groundwater under vulnerable conditions. Conditions of authorisation should include risk mitigation measures and monitoring programmes should be initiated to verify potential groundwater contamination in vulnerable zones, where appropriate.*

Appendix 1 of this document contains the list of references included in this document for support of the evaluation.

Appendix 2 of this document contains detailed evaluation of new studies.

Information on the detailed composition of Kinvara can be found in the confidential dossier of this submission (Registration Report – Part C).

9.1 Critical GAP and overall conclusions

Table 9.1-1: Table of critical GAPs

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Use-No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	**Application**				**Application rate**			PHI (days)	Remarks: e.g. g safener/ synergist per ha	**Conclusion**						
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms	Non-target plants
Zonal uses (field or outdoor uses, certain types of protected crops)																				
1	AT, IE, NI, BE, RO, CZ, DE, HU, PL	Wheat,Barley, Oats, Rye, Triticale	F	Annual and perennial broadleaf weeds	Foliar spray	BBCH 24 - 39	1	N/A	3	0.7 (MCPA) 0.15 (Fluroxypyr) 0.084 (Clopyralid)	200 – 400		BBCH 25-39 (AT) BE- 3L/ha RO- 2-3L/ha CZ -2-3L/ha HU – 2-3L/ha PL – 2-3L/ha - winter wheat, spring wheat, winter triticale, spring barley, rye, winter oats							
2	AT, IE, NI, BE, CZ, DE	Established Grassland (> 1 yr)	F	Annual and perennial broadleaf weeds	Foliar spray	March – ~~Sept~~ August 15th	1	N/A	~~3~~	~~0.7 (MCPA) 0.15 (Fluroxypyr) 0.084 (Clopyralid)~~	200 – 400	AT, IE, CZ, DE, BE (PHI	IE (1st March-31st Aug) BE – 2.25 or 2.5L/ha							

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
									2.5	0.5833 (MCPA) 0.125 (Fluroxypyr) 0.0667 (Clopyralid)		7d)								
3	BE	New Grassland (< 1 year)	F	Annual and perennial broadleaf weeds	Foliar spray	March – ~~End Sept~~ August 15th (min. BBCH 20)	1	N/A	~~3~~ 2.5	~~0.7 (MCPA) 0.15 (Fluroxypyr) 0.084 (Clopyralid)~~ 0.5833 (MCPA) 0.125 (Fluroxypyr) 0.0667 (Clopyralid)	200 – 400		BE – 2.25/2.5L/ha							

* 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

Explanation for column 15 – 21 "Conclusion"

A	Acceptable, Safe use
R	Further refinement and/or risk mitigation measures required
C	To be confirmed by cMS
N	No safe use

Remarks table:

(1) Numeration necessary to allow references
(2) Use official codes/nomenclatures of EU
(3) For crops, the EU and Codex classifications (both) should be used; where relevant, the use situation should be described (*e.g.* fumigation of a structure)
(4) 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
(5) Scientific names and EPPO-Codes of target pests/diseases/ weeds or when relevant the common names of the pest groups (e.g. biting and sucking insects, soil born insects, foliar fungi, weeds) and the developmental stages of the pests and pest groups at the moment of application must be named
(6) Method, e.g. high volume spraying, low volume spraying, spreading, dusting, drench
Kind, e.g. overall, broadcast, aerial spraying, row, individual plant, between the plants - type of equipment used must be indicated
(7) Growth stage at first and last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application
(8) The maximum number of application possible under practical conditions of use must be provided
(9) Minimum interval (in days) between applications of the same product.
(10) For specific uses other specifications might be possible, e.g.: g/m³ in case of fumigation of empty rooms. See also EPPO-Guideline PP 1/239 Dose expression for plant protection products
(11) The dimension (g, kg) must be clearly specified. (Maximum) dose of a.s. per treatment (usually g, kg or L product / ha).
(12) If water volume range depends on application equipments (e.g. ULVA or LVA) it should be mentioned under “application: method/kind”.
(13) PHI - minimum pre-harvest interval
(14) Remarks may include: Extent of use/economic impotance/restrictions

9.1.1 Overall conclusions

Risk assessments for all required target organism groups in all relevant scenarios can be resolved for applications of Kinvara to spring and winter cereals and grassland. No-spray buffer strips are necessary to mitigate the risk of Kinvara to non-target plants. For non-target plants a 20 m no-spray buffer is required to protection off-field plants.

9.1.1.1 Effects on terrestrial vertebrates other than birds (KCP 10.1.2), Effects on other terrestrial vertebrate wildlife (reptiles and amphibians) (KCP 10.1.3)

A standard Tier 1 risk assessment was conducted according the EFSA (2009) Birds and Mammals guidance. At Tier 1 a potential risk was identified to large herbivorous birds and small omnivorous mammals within the chronic risk assessment. Residue decline data on MCPA was used to refine the assessments and resolve the potential risk. Overall, neither the formulation Kinvara nor any of its active substances pose a risk to birds and mammals when applied to winter or spring cereals.

A safe risk from the mixture toxicity assessment is concluded for all species except voles. The risk to voles in grassland cannot be resolved using the standard EU TER thresholds. ~~This is no different from the previous registration of Kinvara in Central Zone, as neither the endpoints nor the EU guidance has changed meaning the calculations are identical. Previous registrations were granted accepting the full rate and resolving the assessment following German standard guidance. The EU definition of the vole is vastly over-conservative for application in the Central Zone. This has long been recognized in Germany and resolved by reducing the TER criteria for the vole to 5 for the acute assessment and 2 for the chronic assessment. This has long been accepted as a more realistic representation of the risk to small mammals and allows the assessment to be fully resolved.~~

~~No safe use for grasslands (use No 2 i 3) was concluded.~~

For lower application rate of 2.5 L/ha the safe use in grasslands was confirmed.

9.1.1.2 Effects on aquatic organisms (KCP 10.2)

Active substance and formulation risk assessments result in PEC/RAC values above the trigger value of 1 for all scenarios using either Step 1, Step 2 or Step 3 FOCUS PECsw. MCPA and the metabolite PCOC result in PEC/RAC >1 for D1 and D2 scenarios, but these are not relevant for the Central Zone. Therefore it can be concluded that applications of Kinvara to cereals and grassland do not pose a risk to aquatic organisms.

9.1.1.3 Effects on bees (KCP 10.3.1)

Neither the Kinvara formulation nor any of the relevant active substances pose an acute risk to bees according to the standard acute risk assessment. A higher-tier field study on the formulation indicates that Kinvara does not pose a chronic and/or non-lethal risk bees.

9.1.1.4 Effects on arthropods other than bees (KCP 10.3.2)

A standard Tier-1 non-target arthropod assessment did not resolve the risk of the Kinvara formulation following applications to cereals. New extended laboratory experiments have been submitted which allow the assessment to be refined and indicate the formulation does not pose a risk to NTAs.

9.1.1.5 Effects on non-target soil meso- and macrofauna (KCP 10.4), Effects on soil microbial activity (KCP 10.5)

The standard Tier-1 exposure assessment on non-target soil meso- and macrofauna indicates the Kinvara formulation does not pose a risk following application to cereals and grassland. One of the metabolites, methoxypyridine, must be considered at higher-tier, but this same assessment has already been agreed at the EU level during the EFSA (2011) evaluation of fluroxypyr.

9.1.1.6 Effects on non-target terrestrial plants (KCP 10.6)

Kinvara is a herbicide so an in-field non-target terrestrial plant assessment is not required. The formulation does pose a risk to off-field terrestrial plants which must be managed via the introduction of a non-spray mitigation buffer strip. A minimum of a 20m no-spray strip is required to protect non-target plants or 10 m with 50% drift reducing nozzle or 5 m with 75% drift reducing nozzle.

9.1.1.7 Effects on other terrestrial organisms (flora and fauna) (KCP 10.7)

9.1.2 Grouping of intended uses for risk assessment

Not relevant.

9.1.3 Consideration of metabolites

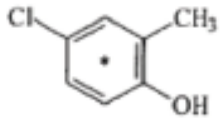
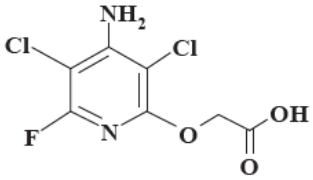
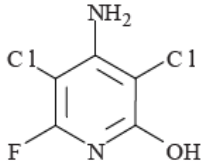
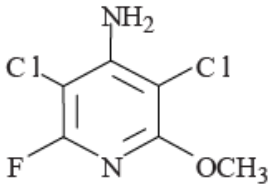
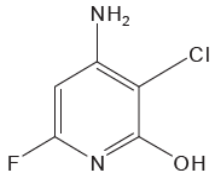
The occurrence and risk from potentially ecotoxicologically relevant metabolites from all active substances has been considered, detailed discussion was provided in the EU review for MCPA, fluroxypyr and clopyralid and in Part B, Section 5. EFSA (2018) concluded that clopyralid has no potentially relevant metabolites.

It is noted that toxicity data are publicly available for PCOC, a metabolite of MCPA, in UNEP (1998). These data were not assessed at the EU level nor was a risk assessment performed for the metabolite in the DAR for MCPA, but the UNEP data have been used to provide an assessment of PCOC here.

Fluroxypyr (acid) forms in the environments following rapid hydrolysis of fluroxypyr-MHE which is the technical material contained with the Kinvara formulation. This is consistent with the EFSA (2011) peer review which considered fluroxypyr-MHE as a relevant precursors substance and fluroxypyr (acid) was treated as a toxicologically relevant metabolite of fluroxypyr-meptyl. The same approach has been taken here assuming applications occur as fluroxypyr-metpyl which is then converted in fluroxypyr (acid).

Pyridinol is a major metabolite of fluroxypyr and its sorption in soil is strongly pH dependent. The EFSA (2011) peer review considered the behaviour of pyridinol in both acidic and alkaline soils but clearly demonstrated the risk of pyridinol is far higher in alkaline soils due to a much lower soil sorption potential. Therefore, to achieve a concise risk assessment all values reported here assume alkaline soils as their value clearly cover the risk of both alkaline and acidic soils. As a terminal metabolite, using conservative endpoints for pyridinol does not alter the risk assessment of the other metabolites.

Table 9.1-2 Metabolites of covered in this assessment

Name	Molar mass	Chemical structure	Maximum observed occurrence in compartments	Exposure assessment required due to
PCOC	142.6		Soil: 55 % Water/Sediment: 11.6 %	Yes, aquatic organisms
Fluroxypyr (acid)	255		Assumed 100% conversion from fluroxypyr-meptyl	Yes, all organisms
Pyridinol	197.0		Soil 23.9% Water 55.5%	Yes, aquatic and soil organisms
Methoxypyridine	211.1		Soil 38.2%	Yes, aquatic and soil organisms
3-CP metabolite IV, monochloropyridinol	162.0		Water 25.2%	Yes, aquatic organisms

9.2 Effects on birds (KCP 10.1.1)

zRMS Comments:	The submitted screening step and first tier assessment of the acute and long-term risk for birds, respectively, due to the use of Kinvara in proposed uses were accepted. The used endpoints for all active substances and their metabolites were agreed at the EU level or accepted at zonal level (MCPA). **MCPA**. The LD_{50} = 220 mg a.s/kg bw was accepted at the zonal level and in this evaluation. TER_A values are above the trigger value of 10 at first-tier assessment indicating an acceptable acute risk for birds due to use of Kinvara in cereals and grasslands. The long-term risk assessment is based on calculated endpoint LD_{50}/10; this approach was accepted. The TER_{LT} values for long-term risk are below the trigger value of 5 at first tier step for large herbivorous bird, goose. The further refinement was required. The submitted refinement based on residue decline was accepted (geometric mean of DT_{50} of 2.4 d and the Ftwa of 0.16 (calculated) were accepted). In higher tier assessment the TER_{LT} values for long-term risk are above the trigger value of 5 are indicating an acceptable long-term risk for birds. No further refinement is required. **Fluroxypyr (acid)** as representing a worse case**.** The TER_A values for small omnivorous, granivorous and insectivorous birds are above the trigger value of 10 at first-tier assessment indicating an acceptable acute risk for birds. The TER_{LT} values for long-term risk are above the trigger value of 5 at first tier assessment indicating an acceptable long-term risk for birds. No further refinement is required. **Clopyralid**. The TER_A values for small omnivorous, granivorous and insectivorous birds are above the trigger value of 10 at first-tier assessment indicating an acceptable acute risk for birds. The TER_{LT} values for long-term risk are above the trigger value of 5 at first tier assessment indicating an acceptable long-term risk for birds. No further refinement is required. **Combined risk assessment**. The MCPA was identified as a driver in the mixture toxicity assessment. The submitted risk assessment was accepted. The risk assessment for birds was amended by additional assessment due to fluroxypyr-meptyl following applications of Kinvara to cereals and grassland. The submitted risk assessment was accepted. **Drinking water exposure**. The submitted exposure assessment via drinking water, the puddle scenario, was accepted. The justification concerning the leaf scenario was accepted. **Secondary poisoning**. Risk assessment for earthworm-eating birds and for fish-eating birds via secondary poisoning was accepted. The risk to birds following application of Kinvara in accordance with the proposed pattern use is acceptable.

9.2.1 Toxicity data

Agreed endpoints from avian toxicity studies performed with each of the active substances are provided in the peer review reports on MCPA (Review Report[1]), fluroxypyr (EFSA Conclusions[2]) and clopyralid (EFSA Conclusions[3]) and are presented in Table 9.2-1 below.

The provision of further data on the formulation is not considered essential, because Kinvara is applied to cereal crops and grassland. Since birds will avoid the disturbance caused by the spraying activity, direct exposure of birds to the formulated product is unlikely to occur. Moreover, the acute toxicity of the formulated product may adequately be extrapolated from toxicity data on MPCA. In accordance with the Birds and Mammals Guidance, the ratio of 'tox per fraction (a.s.) and tox per fraction (mix)' of MCPA within Kinvara deviates by <10 % indicating the risk assessment can be performed on the basis of this most toxic substance. The reproductive toxicity of the formulation can be conservatively estimated based on the most toxic substance as outlined in EFSA (2009). Consequently, further studies of the acute oral toxicity of Kinvara to birds and the concomitant expenditure of vertebrate test organisms are unnecessary.

Table 9.2-1: Endpoints and effect values relevant for the risk assessment for birds

Species	Test substance	Endpoint	Value	Reference
Bobwhite quail	MCPA	Acute oral toxicity	LD_{50}=220 mg as/kg bw (converted from 270 mg MCPA-DMA)	MCPA - Review Report
Bobwhite quail	Fluroxypyr-meptyl	Acute oral toxicity	LD_{50}>2000	Fluroxypyr - EFSA Final Conclusions
Bobwhite quail	Fluroxypyr acid	Acute oral toxicity	LD_{50}>2000	Fluroxypyr - EFSA Final Conclusions
Mallard duck	Clopyralid	Acute oral toxicity	LD_{50}=1465 mg a.s./kg bw	Clopyralid - EFSA Final Conclusion
Bobwhite quail	MCPA	Long-term toxicity and reproduction	LD_{50}/10=22 mg as/kg bw*	
Mallard duck	Fluroxypyr-meptyl	Long-term toxicity and reproduction	NOEL= 57.8	Fluroxypyr - EFSA Final Conclusions
Mallard duck	Fluroxypyr acid	Long-term toxicity and reproduction	NOEL= 40.1	Fluroxypyr - EFSA Final Conclusions
Mallard duck	Clopyralid	Long-term toxicity and reproduction	NOEL=118 mg as/kg bw/day	Clopyralid - EFSA Final Conclusion

*The measured endpoint in the MCPA review report is a NOEL of 93.2 mg as/kg bw/day. As this values is greater than the actual endpoint divided by 10 the more conservative 22 mg a.s./kg bw/day (one tenth of the acute LD_{50} value) has been used in the risk assessment, as required by EFSA (2009).

9.2.1.1 Justification for new endpoints

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[1] Review report for the active substance **MCPA,** SANCO/4062/2001-final 11 July 2008
[2] Conclusion on the peer review of the pesticide risk assessment of the active substance fluroxypyr (evaluated variant fluroxypyr-meptyl), EFSA Journal 2011;9(3):2091
[3] *EFSA Journal* (2018) 16(7):5389,28, 1–65, Peer review of the pesticide risk assessment of the active substance clopyralid.

9.2.2 Risk assessment for spray applications

The risk assessment is based on the methods presented in the Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438; hereafter referred to as EFSA/2009/1438).

First-tier assessment (screening/generic focal species)

The results of the acute and reproductive first-tier risk assessments for the individual active substances are summarised in Table 9.2-2 to

. A first-tier combination toxicity acute risk assessment is not required as MCPA accounts for >90% of the estimated formulation toxicity (Table 9.2-10). A first-tier combination long term risk assessment based on the EFSA (2009) worst-case approach is provided in Table 9.2-11.

Table 9.2-2: First-tier assessment of the acute and long-term/reproductive risk for birds due to MCPA following applications of Kinvara to cereals and grassland

Intended use		**Kinvara**				
Active substance/product		**MCPA**				
Application rate (g/ha)		**1 × 700 g a.s./ha**				
Acute toxicity (mg/kg bw)		**220**				
TER criterion		**10**				
Crop scenario	**Growth stage**	**Generic focal species**	**SV_{90th}**	**MAF**	**DDD (mg/kg bw/d)**	**TER_a**
Cereals	Early (shoots) autumn-winter BBCH 10-29	Large herbivorous bird "goose"	30.5	1	21.4	10.3
	BBCH 10-29	Small omnivorous bird “lark”	24.0	1	16.8	13.1
	BBCH 30-39	Small omnivorous bird “lark”	12.0	1	8.4	26.2
Grassland	New sown grass seeds	Small granivorous bird "sparrow"	20.4	1	14.3	15.4
	Late season (seed heads)	Small granivorous bird "finch"	24.7	1	17.3	12.7
	Growing shoots	Large herbivorous bird "goose"	30.5	1	21.4	10.3
	Growing shoots	Small insectivorous bird “wagtail”	26.8	1	18.8	11.7
Reproductive toxicity (mg/kg bw)		**22**				
TER criterion		**5**				
Crop scenario	**Growth stage**	**Generic focal species**	**SV_{mean}**	**MAF x fTWA**	**DDD (mg/kg bw/d)**	**TER_{lt}**
Cereals	Early (shoots) autumn-winter BBCH 10-29	Large herbivorous bird "goose"	16.2	0.53	6.0	**3.7**
	BBCH 10-29	Small omnivorous bird “lark”	10.9	0.53	4.0	5.4
	BBCH 30-39	Small omnivorous bird “lark”	5.4	0.53	2.0	11.0
Grassland	New sown grass seeds	Small granivorous bird "sparrow"	9.4	0.53	3.5	6.3
	Late season (seed heads)	Small granivorous bird "finch"	11.4	0.53	4.2	5.2
	Growing shoots	Large herbivorous bird "goose"	16.2	0.53	6.0	**3.7**
	Growing shoots	Small insectivorous bird “wagtail”	11.3	0.53	4.2	5.2

Table 9.2-3: First-tier assessment of the acute and long-term/reproductive risk for birds due to fluroxypyr (acid) following applications of Kinvara to cereals and grassland

Intended use		**Kinvara**				
Active substance/product		**Fluroxypyr (acid)**				
Application rate (g/ha)		**1 × 150 g a.s./ha**				
Acute toxicity (mg/kg bw)		**2000**				
TER criterion		**10**				
Crop scenario	**Growth stage**	**Generic focal species**	**SV_{90th}**	**MAF**	**DDD (mg/kg bw/d)**	**TER_a**
Cereals	Early (shoots) autumn-winter BBCH 10-29	Large herbivorous bird "goose"	30.5	1	4.6	437.2
	BBCH 10-29	Small omnivorous bird “lark”	24.0	1	3.6	555.6
	BBCH 30-39	Small omnivorous bird “lark”	12.0	1	1.8	1111.1
Grassland	New sown grass seeds	Small granivorous bird "sparrow"	20.4	1	3.1	653.6
	Late season (seed heads)	Small granivorous bird "finch"	24.7	1	3.7	539.8
	Growing shoots	Large herbivorous bird "goose"	30.5	1	4.6	437.2
	Growing shoots	Small insectivorous bird “wagtail”	26.8	1	4.0	497.5
Reproductive toxicity (mg/kg bw)		**40.1**				
TER criterion		**5**				
Crop scenario	**Growth stage**	**Generic focal species**	**SV_{mean}**	**MAF x fTWA**	**DDD (mg/kg bw/d)**	**TER_{lt}**
Cereals	Early (shoots) autumn-winter BBCH 10-29	Large herbivorous bird "goose"	16.2	0.53	1.3	31.1
	BBCH 10-29	Small omnivorous bird “lark”	10.9	0.53	0.9	46.3
	BBCH 30-39	Small omnivorous bird “lark”	5.4	0.53	0.4	93.4
Grassland	New sown grass seeds	Small granivorous bird "sparrow"	9.4	0.53	0.7	53.7
	Late season (seed heads)	Small granivorous bird "finch"	11.4	0.53	0.9	44.2
	Growing shoots	Large herbivorous bird "goose"	16.2	0.53	1.3	31.1
	Growing shoots	Small insectivorous bird “wagtail”	11.3	0.53	0.9	44.6

Table 9.2-4: First-tier assessment of the acute and long-term/reproductive risk for birds due to fluroxypyr-meptyl following applications of Kinvara to cereals and grassland

Intended use		**Kinvara**				
Active substance/product		**Fluroxypyr meptyl**				
Application rate (g/ha)		**1 × 216.1 g a.s./ha**				
Acute toxicity (mg/kg bw)		**2000**				
TER criterion		**10**				
Crop scenario	**Growth stage**	**Generic focal species**	**SV_{90th}**	**MAF**	**DDD (mg/kg bw/d)**	**TER_a**
Cereals	Early (shoots) autumn-winter BBCH 10-29	Large herbivorous bird "goose"	30.5	1	4.6	303.4
	BBCH 10-29	Small omnivorous bird “lark”	24.0	1	3.6	385.6
	BBCH 30-39	Small omnivorous bird “lark”	12.0	1	1.8	771.2
Grassland	New sown grass seeds	Small granivorous bird "sparrow"	20.4	1	3.1	453.7
	Late season (seed heads)	Small granivorous bird "finch"	24.7	1	3.7	374.7
	Growing shoots	Large herbivorous bird "goose"	30.5	1	4.6	303.4
	Growing shoots	Small insectivorous bird “wagtail”	26.8	1	4.0	345.3
Reproductive toxicity (mg/kg bw)		**40.1**				
TER criterion		**5**				
Crop scenario	**Growth stage**	**Generic focal species**	**SV_{mean}**	**MAF x fTWA**	**DDD (mg/kg bw/d)**	**TER_{lt}**
Cereals	Early (shoots) autumn-winter BBCH 10-29	Large herbivorous bird "goose"	16.2	0.53	1.3	21.6
	BBCH 10-29	Small omnivorous bird “lark”	10.9	0.53	0.9	32.1
	BBCH 30-39	Small omnivorous bird “lark”	5.4	0.53	0.4	64.8
Grassland	New sown grass seeds	Small granivorous bird "sparrow"	9.4	0.53	0.7	37.2
	Late season (seed heads)	Small granivorous bird "finch"	11.4	0.53	0.9	30.7
	Growing shoots	Large herbivorous bird "goose"	16.2	0.53	1.3	21.6
	Growing shoots	Small insectivorous bird “wagtail”	11.3	0.53	0.9	31.0

Table 9.2-5: First-tier assessment of the acute and long-term/reproductive risk for birds due to clopyralid following applications of Kinvara to cereals and grassland

Intended use		**Kinvara**				
Active substance/product		**Clopyralid**				
Application rate (g/ha)		**1 × 150 g a.s./ha**				
Acute toxicity (mg/kg bw)		**1465**				
TER criterion		**10**				
Crop scenario	**Growth stage**	**Generic focal species**	**SV_{90th}**	**MAF**	**DDD (mg/kg bw/d)**	**TER_a**
Cereals	Early (shoots) autumn-winter BBCH 10-29	Large herbivorous bird "goose"	30.5	1	4.6	600.4
	BBCH 10-29	Small omnivorous bird “lark”	24.0	1	3.6	763.0
	BBCH 30-39	Small omnivorous bird “lark”	12.0	1	1.8	1526.0
Grassland	New sown grass seeds	Small granivorous bird "sparrow"	20.4	1	3.1	897.7
	Late season (seed heads)	Small granivorous bird "finch"	24.7	1	3.7	741.4
	Growing shoots	Large herbivorous bird "goose"	30.5	1	4.6	600.4
	Growing shoots	Small insectivorous bird “wagtail”	26.8	1	4.0	683.3
Reproductive toxicity (mg/kg bw)		**118**				
TER criterion		**5**				
Crop scenario	**Growth stage**	**Generic focal species**	**SV_{mean}**	**MAF x fTWA**	**DDD (mg/kg bw/d)**	**TER_{lt}**
Cereals	Early (shoots) autumn-winter BBCH 10-29	Large herbivorous bird "goose"	16.2	0.53	1.3	171.8
	BBCH 10-29	Small omnivorous bird “lark”	10.9	0.53	0.9	255.3
	BBCH 30-39	Small omnivorous bird “lark”	5.4	0.53	0.4	515.4
Grassland	New sown grass seeds	Small granivorous bird "sparrow"	9.4	0.53	0.7	296.1
	Late season (seed heads)	Small granivorous bird "finch"	11.4	0.53	0.9	244.1
	Growing shoots	Large herbivorous bird "goose"	16.2	0.53	1.3	171.8
	Growing shoots	Small insectivorous bird “wagtail”	11.3	0.53	0.9	246.3

~~**Table 9.2-6: First-tier assessment of the long-term/reproductive risk for birds due to Kinvara**~~

~~**Product**~~	~~Kinvara~~
~~**Application rate (g/ha)**~~	~~3 L formulation/ha~~
~~**TER criterion**~~	~~5~~

~~**Crop scenario**~~	~~**Growth stage**~~	~~**Generic focal species**~~	~~**DDD (mg/kg bw/d)**~~			~~**HI sum (sum < 1)**~~
			~~**MCPA**~~	~~**Fluroxypyr-acid**~~	~~**Clopyralid**~~	
~~Cereals~~	~~Early (shoots) autumn-winter BBCH 10-29~~	~~Large herbivorous bird "goose"~~	~~6.0~~	~~1.3~~	~~1.3~~	~~**8.6**~~
	~~BBCH 10-29~~	~~Small omnivorous bird “lark”~~	~~4.0~~	~~0.9~~	~~0.9~~	~~**5.8**~~
	~~BBCH 30-39~~	~~Small omnivorous bird “lark”~~	~~2.0~~	~~0.4~~	~~0.4~~	~~**2.8**~~
~~Grassland~~	~~New sown grass seeds~~	~~Small granivorous bird "sparrow"~~	~~3.5~~	~~0.7~~	~~0.7~~	~~**4.9**~~
	~~Late season (seed heads)~~	~~Small granivorous bird "finch"~~	~~4.2~~	~~0.9~~	~~0.9~~	~~**6.0**~~
	~~Growing shoots~~	~~Large herbivorous bird "goose"~~	~~6.0~~	~~1.3~~	~~1.3~~	~~**8.6**~~
	~~Growing shoots~~	~~Small insectivorous bird “wagtail”~~	~~4.2~~	~~0.9~~	~~0.9~~	~~**6.0**~~

First-tier assessment of the long-term/reproductive risk for birds due to Kinvara using the TER approach is reported in Table 9.2-11

9.2.2.1 Higher-tier risk assessment

The risk assessments performed so far have used the EFSA (2009) default assumption that the DT_{50} for the applied residues is 10 days, corresponding to a f_{twa} of 0.53. However, the residue decline studies in MCPA DAR (DAR Section B.6.5), demonstrate that MCPA declines significantly faster than the assumed default value when applied to cereals (Table 9.2-7). The geometric mean of the 8 new trials (Table 9.2-8) yields a F_{TWA} value of 0.16 which will be used to refine the risk assessment, refinement already accepted by CEU in previous registration.

Table 9.2-7: Residue decline data for MCPA for cereals and grassland (DAR section b.6.5)

Crop	Day	Residue (mg/kg)	% loss between initial application and 1st sample (number of days
Oats	0 18	22.1 0.15	99.3 (18 days)
Barley	0 13	17.5 0.09	99.5 (13 days)
Wheat	0 15	16.1 0.70	95.7 (15 days)
Grassland – Location 1	0 13	54.3 0.92	98.3 (13 days)
Grassland – Location 2	0 14	59.6 1.40	97.7 (14 days)
Grassland – Location 3	0 12	57.3 1.42	97.5 (12 days)
Grassland – Location 4	0 12	50.0 1.80	96.4 (12 days)

The data summarised indicate that >95% of the initial MCPA residues are lost within 12-18 days. This suggests that it is likely that >50% of residues would be lost within 10 days, and thus the default DT_{50} of 10 days is overly conservative for MCPA. An estimate of the DT_{50} value for MCPA can be calculated using the available residue data and assuming first-order kinetics.

Table 9.2-8: Calculation of DT50 based on available residue decline data for MCPA in cereals and grassland

C_0	C_t	*t*	DT *
22.1	0.15	18	2.50
17.5	0.09	13	1.71
16.1	0.7	15	3.32
54.3	0.92	13	2.21
59.6	1.4	14	2.59
57.3	1.42	12	2.25
50	1.8	12	2.50
		Geometric mean	**2.40**

C_0 = initial residue (mg/kg)
C_t = residue at time interval *t*
t = time interval between C_0 and C_t samples
*DT_{50} calculated using $t½ = t * \ln(2) / \ln(Co/Ct)$, which is converted from the standard first-order kinetic model [$C_t = C_0 \exp(-\ln(2)* t / t½)$]

Table 9.2-9: Higher-tier assessment of the long-term/reproductive risk for birds due to MCPA exposure following the use of Kinvara

Intended use		**Kinvara**				
Active substance/product		**MCPA**				
Application rate (g/ha)		**1 × 700 g a.s./ha**				
Reproductive toxicity (mg/kg bw)		**22**				
TER criterion		**5**				
Crop scenario	**Growth stage**	**Generic focal species**	**SV_{mean}**	**MAF x f_{TWA}***	**DDD (mg/kg bw/d)**	**TER_{lt}**
Cereals	Early (shoots) autumn-winter BBCH 10-29	Large herbivorous bird "goose"	16.2	0.16	1.81	12.13
	BBCH 10-29	Small omnivorous bird “lark”	10.9	0.16	1.22	18.02
	BBCH 30-39	Small omnivorous bird “lark”	5.4	0.16	0.60	36.38
Grassland	New sown grass seeds	Small granivorous bird "sparrow"	9.4	0.16	1.05	20.90
	Late season (seed heads)	Small granivorous bird "finch"	11.4	0.16	1.28	17.23
	Growing shoots	Large herbivorous bird "goose"	16.2	0.16	1.81	12.13
	Growing shoots	Small insectivorous bird “wagtail”	11.3	0.16	1.27	17.38

* refined parameters, Ftwa of 0.16 from geomean DT50 of 2.40 days.

Assessment of mixture toxicity

Kinvara contains 233 g/L MCPA, 50 g/L fluroxypyr and 28 g/L clopyralid.

To achieve a basis for a comparison of single active substance and mixture toxicity in terms of potential risk, a “tox per fraction” is calculated for each active substance and compared to the corresponding quotient for the mixture using the following equation, according to the EFSA guidance:

$$tox\ per\ fraction\ (a.s.) = \frac{LD_{50}(a.s._i)}{X(a.s._i)}$$

$$tox\ per\ fraction\ (a.s.) = \frac{LD_{50}(mix)}{\sum_i X(a.s._i)}$$

Table 9.2-10: Avian "Tox per fraction" quotient for active substances

Test substance	Fraction of active substance in the formulation mixture, X(a.s.) [a)]	Acute toxicity endpoint (mg/kg bw)	Tox per fraction (a.s)	Tox per fraction of the formulation mixture	Deviation (%) [b)]
MCPA	0.749	220	293.65	282	96.00 %
Fluroxypyr	0.161	2000	12440.00		2.27 %
Clopyralid	0.090	1465	16271.96		1.73 %

Test substance	Fraction of active substance in the formulation mixture, X(a.s.) [a)]	Reprod toxicity endpoint (mg/kg bw/d)	Tox per fraction (a.s)	Tox per fraction of the formulation mixture	Deviation (%) [b)]
MCPA	0.749	22	29.37	26	87.69 %
Fluroxypyr	0.161	40.1	249.07		10.34 %
Clopyralid	0.090	118	1311.11		1.96 %

a) Concentration of an active substance in the formulation, divided by, the total concentration of all active substances in the formulation.
b) Deviation % = Tox per fraction (mix) / Tox per fraction (a.s.) * 100

In accordance with the EFSA Birds and Mammals Guidance (2009), the ratio of 'tox per fraction (a.s) and tox per fraction (mix)' of MCPA within Kinvara deviates by <10 % indicating the acute toxicity risk assessment can be performed on the basis of this most toxic substance (Table 9.2-10).

A combined assessment is instead necessary to assess the reproductive toxicity of the formulation towards birds as neither substance contributes > 90 % to the mixture toxicity. The long-term combined risk assessment was revised to reflect the changes made in the higher-tier assessment of MCPA.

Table 9.2-11. Combined assessment of the avian long-term/reproductive risk for due to the use of Kinvara

Active substance/product	Kinvara
Application rate (g/ha)	3.0 L formulation/ha
TER criterion	5

Crop scenario	**Growth stage**	**Generic focal species**	**TER**			**RQ**			**RQ sum**
			MCPA*	**Fluroxypyr-acid**	**Clopyralid**	**MCPA**	**Fluroxypyr-acid**	**Clopyralid**	
Cereals	Early (shoots) autumn-winter BBCH 10-29	Large herbivorous bird "goose"	12.13	31.1	171.8	0.412	0.161	0.029	0.602
	BBCH 10-29	Small omnivorous bird “lark”	18.02	46.3	255.3	0.277	0.108	0.020	0.405
	BBCH 30-39	Small omnivorous bird “lark”	36.38	93.4	515.4	0.137	0.054	0.010	0.201
Grassland	New sown grass seeds	Small granivorous bird "Sparrow"	20.90	53.7	296.1	0.239	0.093	0.017	0.349
	Late season (seed heads)	Small granivorous bird "finch"	17.23	44.2	244.1	0.290	0.113	0.020	0.424
	Growing shoots	Large herbivorous bird "goose"	12.13	31.1	171.8	0.412	0.161	0.029	0.602
	Growing shoots	Small insectivorous bird “wagtail”	17.38	44.6	246.3	0.288	0.112	0.020	0.420

*The TER values for MCPA have been refined based on the experimental DT_{50} values.

9.2.2.2 Drinking water exposure

Leaf scenario
Since Kinvara is not a product for spray applications / not intended to be applied on leafy vegetables forming heads or crop plants with comparable water collecting structures at principal growth stage 4 or later, the leaf scenario does not have to be considered.

Puddle scenario

Given that the proposed use of Kinvara is on cereals and grassland, the relevant scenario for exposure via drinking water is the puddle scenario (see Section 5.5 of EFSA (2009)). As such, both acute and reproductive risks must be assessed for birds and mammals.

Due to the characteristics of the exposure scenario in connection with the standard assumptions for water uptake by animals, no specific calculations of exposure and TER are necessary when the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed 50 in the case of less sorptive substances (Koc < 500 L/kg) or 3000 in the case of more sorptive substances (Koc ≥ 500 L/kg). An initial screening step was conducted on this basis and the results are shown in Table 9.2-12. No further assessment is necessary for the drinking water risk to birds.

Table 9.2-12: Ratio of effective application rate to toxicity endpoints for birds

Substance	MCPA	Fluroxypyr-MHE	Fluroxypyr	Clopyralid
Mean KfOC (mL/g)	<500	>500	<500	<500
Effective application Rate (g a.s./ha)	700	216.1	150	80
Acute Toxicity (mg/kg bw/day)	220	2000	2000	1465
Reproductive Toxicity (mg/kg bw/d)	22	57.8	40.1	118
Acute Ratio	3.18	0.11	0.08	0.05
Chronic Ratio	31.81	3.74	3.74	0.68
Risk Assessment Required?	No	No	No	No

9.2.2.3 Effects of secondary poisoning

Since the Log P_{ow} of MCPA (pH dependent; -1.07 to 2.80) and clopyralid (-2.63) are < 3 the risks from secondary poisoning to birds and mammals do not require assessment. However, the Log P_{ow} for fluroxypyr (meptyl) is >5 and therefore requires assessment for secondary poisoning.

The EFSA final conclusion for fluroxypyr concludes although the log P_{OW} of fluroxypyr-meptyl is greater than 3, the risk assessment for secondary poisoning of birds and mammals was not conducted because fluroxypyr-meptyl rapidly hydrolyses to fluroxypyr in the environment and it does not bioaccumulate in fish (measured BCF = 26).

The log P_{OW} of metabolite fluroxypyr methoxypyridine is 3.09, which is higher than the EFSA (2009) trigger of 3. Therefore, foodchain transfer may occur and further assessment is required. All calculations are performed according to Section 5.6 of EFSA (2009). The endpoints used in the risk assessment are taken from the risk assessment performed in the Addendum to the DAR (December 2010).

Risk assessment for earthworm-eating birds via secondary poisoning

Table 9.2-13: Assessment of the risk for earthworm-eating birds due to exposure methoxypyridine via bioaccumulation in earthworms (secondary poisoning)

Parameter	Methoxypyridine	Comments
PEC_{soil} (accumulation) (mg/kg soil)	0.190	PEC soil acc max from all uses (from winter cereals, annual applications) (dRR B8, section 8.7.2.2)
log P_{ow} / P_{ow}	3.09	
Koc	311	Geometric Mean (n =4)
foc	0.02	Default
BCF_{worm}	0.195	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= ((0.84 + (0.12 \times P_{ow})) / foc \times Koc$
PEC_{worm}	0.037	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.039	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	4.01	
TER_{lt}	103.26	

TER values shown in bold fall below the relevant trigger.

Risk assessment for fish-eating birds via secondary poisoning

Table 9.2-14: Assessment of the risk for fish-eating birds due to exposure to methoxypyridine via bioaccumulation in fish (secondary poisoning)

Parameter	Methoxypyridine	Comments
PEC_{sw} (twa = 21 d) (mg/L)	0.00343	Step 2, 21 d TWA PECsw, max for all uses (from winter cereals, annual applications, see Appendix 3)
BCF_{fish}	1.41	
BMF	-	biomagnification factor (relevant for BCF ≥ 2000)
PEC_{fish}	0.00484	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.00077	$DDD = PEC_{fish} \times 0.159$
NOEL (mg/kg bw/d)	4.01	
TER_{lt}	5215	

TER values shown in bold fall below the relevant trigger.

9.2.2.4 Biomagnification in terrestrial food chains

The evaluation of the toxicokinetic studies at Annex I inclusion for all active substances concluded that the potential for bioaccumulation is low for MCPA, fluroxypyr and clopyralid. It is therefore considered that there is no biomagnification along the food chain.

9.2.3 Risk assessment for baits, pellets, granules, prills or treated seed

Not relevant.

9.2.4 Overall conclusions

A Tier 1 assessment demonstrates acceptable acute risks to birds exposed to MCPA, fluroxypyr and clopyralid via contaminated food items, based on extreme worst-case assumptions about residue intake. A higher-tier assessment demonstrates acceptable long-term risk to birds exposed under the same circumstances. A conservative screening assessment indicates acceptable acute and long-term risk to birds potentially exposed to MCPA, fluroxypyr or clopyralid residues by drinking water from contaminated puddles. It may therefore be concluded that the proposed use of Kinvara in cereal and grassland crops in accordance with Good Agricultural Practice poses no unacceptable acute risk to birds.

9.3 Effects on terrestrial vertebrates other than birds (KCP 10.1.2)

zRMS Comments:

The submitted screening step and first tier assessment of the acute and long-term risk for mammals, respectively, due to the use of Kinvara in proposed uses were accepted.
The used endpoints for all active substances were agreed at the EU level.

MCPA. The TER_A values are above the trigger value of 10 at first-tier assessment indicating an acceptable acute risk for mammals due to use of Kinvara in cereals and grasslands.

The TER_{LT} values for long-term risk are below the trigger value of 5 at first tier step for small herbivorous mammal, vole. The further refinement was required. The submitted refinement based on residue decline was accepted (geometric mean of DT_{50} of 2.4 d and the Ftwa of 0.16 (calculated) were accepted). The refined RUD value based on new EFSA 2023 Birds and Mammal guidance was not accepted as the guidance mixing is not allowed.
In this case it is proposed to use the risk refinement based on PD (proportions of feed item in the diet) in accordance with Rinke, 1991.
A PD of 25% non-grass herbs and 75% grass and cereals is considered relevant for the risk refinement to vole in grasslands.

Taking into account the refined voles diet, the FIR/bw was calculated in line with Appendix G of EFSA (2009).

BW vole (g)	DEE (kj)	RUD unit	PD	FE (kJ/g dry)	Moisture Fraction	Assimilation efficiency fraction	$FE_{total\ fresh}$ (kJ/g fresh weight)	$FIR_{total\ fresh}$ (g fresh weight/d)	FIR/BW
25	65.09	Grass + cereals	0.75	17.6	0.764	0.47	1.867	34.87	1.395
		Non-grass weeds	0.25	17.8	0.881	0.76			

The risk assessment based on the refined diet is presented in table below.

Scenario	Generic focal species	FIR/BW	RUD unit	Fraction in diet	mean RUD [1)]	App. Rate (kg a.s./ha)	MAF × TWA	DDD (mg a.s./kg bw)	Endpoint (mg a.s./kg bw)	TER	Trigger value
Grass	Small herbivorous mammal "vole"	1.395	Grass + cereals	0.75	54.2	0.700	0.16	6.35	37.8	5.06	5
			Non-grass weeds	0.25	28.7			1.12			
								Σ 7.47			

[1)] According to Appendix F of EFA (2009)

	In higher tier assessment the TER_{LT} values for long-term risk for vole is equal the trigger value of 5 are indicating an acceptable long-term risk for mammals. No further refinement is required. **Fluroxypyr (acid)** as representing a worse case**.** The TER_A values for mammals are above the trigger value of 10 at first-tier assessment indicating an acceptable acute risk for mammals. The TER_{LT} values for long-term risk are above the trigger value of 5 at first tier assessment indicating an acceptable long-term risk for mammals. No further refinement is required. **Clopyralid**. The TER_A values for mammals are above the trigger value of 10 at first-tier assessment indicating an acceptable acute risk for mammals. The TER_{LT} values for long-term risk are above the trigger value of 5 at first tier assessment indicating an acceptable long-term risk for mammals. No further refinement is required. **Combined risk assessment**. The MCPA was identified as a driver in the mixture toxicity assessment. The submitted risk assessment was corrected in accordance refined TER_{LT} value for MCPA of 5.06. The combined assessment of the long-term/reproductive risk for mammals due to the use of Kinvara was added (Table 9.3-9a). **The risk is still unacceptable for voles in grasslands. No safe use could be concluded for grasslands.** **For lower application rate of 2.5 L/ha the safe use was confirmed** (please refer to p. 35). **The safe use in grasslands was confirmed.** The German approach considering reducing the TER criteria for the vole to 5 for the acute assessment and 2 for the chronic assessment is not accepted. It could be considered at cMS level. **Drinking water exposure**. The submitted exposure assessment via drinking water, the puddle scenario, was accepted. The justification concerning the leaf scenario was accepted. **Secondary poisoning**. Risk assessment for earthworm-eating mammals and for fish-eating mammals via secondary poisoning was accepted. The risk to mammals following application of Kinvara in accordance with the proposed pattern use only in cereals is acceptable.

9.3.1 Toxicity data

Mammalian toxicity studies for all the active substances are available within their respective EFSA peer reviews. Full details of these studies are provided in the respective EU DAR and related documents and a summary of the endpoints has been reproduced in Table 9.3-1.

Effects on mammals of the formulation were not evaluated as part of the EU assessment and no new studies have been provided here. The provision of further data on the formulation is not considered essential, because the toxicity of the formulation can be conservatively estimated based on the existing active substance data. A combined risk assessment has been performed in accordance with EFSA (2009) for both

the acute and long-term risk and the predicted acute mixture toxicity calculations are provided in Table 9.3-9.

Table 9.3-1: Endpoints and effect values relevant for the risk assessment for mammals

Species	Test substance	Endpoint	Value	Reference
Rat	Clopyralid	Acute oral toxicity	LD_{50}>5000mg as/kg bw	EFSA (2018)
Rat	Fluroxypyr-meptyl	Acute oral toxicity	LD_{50}>2000	EFSA (2011)
Rat	Fluroxypyr-acid	Acute oral toxicity	LD_{50}>1390	Endpoint based on fluroxypyr-MHE value adjusted for molecular weight
Rat	MCPA	Acute oral toxicity	LD_{50}=962 mg/kg bw	EC (2008)
Rat	Clopyralid	Long-term toxicity (2 year study)	NOAEL= 50 mg as/kg bw/day	MCPA - Review Report
Rabbit	Fluroxypyr-meptyl	Long-term toxicity (developmental)	NOAEL= 144	No reproductive endpoint for fluroxypyr-meptyl in EFSA conclusion (2011); therefore endpoint (144 mg/kg bw/d) obtained by converting reproductive endpoint for fluroxypyr-acid (100 mg/kg bw/d)
Rabbit	Fluroxypyr-acid	Long-term toxicity (developmental)	NOAEL= 100	EFSA (2011)- Peer review notes its unclear if MHE or acid was used in the study
Rat	MCPA	Long-term toxicity and reproduction	NOAEL=37.8 mg as/kg bw/day	MCPA - Review Report

9.3.1.1 Justification for new endpoints.

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9.3.2 Risk assessment for spray applications

The risk assessment is based on the methods presented in the Guidance Document on Risk Assessment for Mammals and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438; hereafter referred to as EFSA (2009). All representative uses were assessed to achieve a comprehensive assessment.

9.3.2.1 First-tier assessment (screening/generic focal species)

The results of the acute and reproductive first-tier risk assessments are summarised for the active substances in

Table 9.3-2, Table 9.3-3 and Table 9.3-5. A risk assessment for the combined risk of the active substances is provided in Table 9.3-9.

Table 9.3-2: First-tier assessment of the acute and long-term/reproductive risk for mammals due to MCPA following the use of Kinvara

Intended use		**Kinvara**				
Active substance/product		MCPA				
Application rate (g/ha)		1 × 700 g a.s./ha				
Acute toxicity (mg/kg bw)		962				
Crop scenario	**Growth stage**	**Generic focal species**	**SV_{90th}**	**MAF**	**DDD (mg/kg bw/d)**	**TER_a**
Cereals	BBCH >20	Small insectivorous mammal "shrew"	5.4	1	3.8	254.5
	BBCH 10-29	Small omnivorous mammal "mouse"	17.2	1	12	79.9
	BBCH >20	Small insectivorous mammal "shrew"	5.4	1	3.8	254.5
	BBCH 30-39	Small omnivorous mammal "mouse"	8.6	1	6	159.8
Grassland	All season	Large herbivorous mammal "lagomorph"	32.6	1	22.8	42.2
	Late season	Small insectivorous mammal "shrew"	5.4	1	3.8	254.5
	All season	Small herbivorous mammal "vole"	136.4	1	95.5	10.1
	Late season (seed heads)	Small omnivorous mammal "mouse"	14.4	1	10.1	95.4
	Newly sown grass seeds	Small omnivorous mammal "mouse"	14.4	1	10.1	95.4
Reproductive toxicity (mg/kg bw)		**37.8**				
TER criterion		**5**				
Crop scenario	**Growth stage**	**Generic focal species**	**SV_{mean}**	**MAF x fTWA**	**DDD (mg/kg bw/d)**	**TER_{lt}**
Cereals	BBCH >20	Small insectivorous mammal "shrew"	1.9	0.53	0.705	53.62
	BBCH 10-29	Small omnivorous mammal "mouse"	7.8	0.53	2.894	13.06
	BBCH >20	Small insectivorous mammal "shrew"	1.9	0.53	0.705	53.62
	BBCH 30-39	Small omnivorous mammal "mouse"	3.9	0.53	1.447	26.12
Grassland	All season	Large herbivorous mammal "lagomorph"	17.3	0.53	6.418	5.89
	Late season	Small insectivorous mammal "shrew"	1.9	0.53	0.705	53.62
	All season	Small herbivorous mammal "vole"	72.3	0.53	26.823	**1.41**
	Late season (seed heads)	Small omnivorous mammal "mouse"	6.6	0.53	2.449	15.43
	New sown grass seeds	Small omnivorous mammal "mouse"	6.6	0.53	2.449	15.43

Table 9.3-3: First-tier assessment of the acute and long-term/reproductive risk for mammals due to fluroxypyr-meptyl following the use of Kinvara

Intended use		**Kinvara**				
Active substance/product		Fluroxypyr-meptyl				
Application rate (g/ha)		1 × 216.1 g a.s./ha				
Acute toxicity (mg/kg bw)		2000				
Crop scenario	**Growth stage**	**Generic focal species**	**SV_{90th}**	**MAF**	**DDD (mg/kg bw/d)**	**TER_a**
Cereals	BBCH >20	Small insectivorous mammal "shrew"	5.4	1	1.17	1713.9
	BBCH 10-29	Small omnivorous mammal "mouse"	17.2	1	3.72	538.1
	BBCH >20	Small insectivorous mammal "shrew"	5.4	1	1.17	1713.9
	BBCH 30-39	Small omnivorous mammal "mouse"	8.6	1	1.86	1076.2
Grassland	All season	Large herbivorous mammal "lagomorph"	32.6	1	7.04	283.9
	Late season	Small insectivorous mammal "shrew"	5.4	1	1.17	1713.9
	All season	Small herbivorous mammal "vole"	136.4	1	29.48	67.9
	Late season (seed heads)	Small omnivorous mammal "mouse"	14.4	1	3.11	642.7
	New sown grass seeds	Small omnivorous mammal "mouse"	14.4	1	3.11	642.7
Reproductive toxicity (mg/kg bw)		144				
TER criterion		5				
Crop scenario	**Growth stage**	**Generic focal species**	**SV_{mean}**	**MAF x fTWA**	**DDD (mg/kg bw/d)**	**TER_{lt}**
Cereals	BBCH >20	Small insectivorous mammal "shrew"	1.9	0.53	0.218	661.7
	BBCH 10-29	Small omnivorous mammal "mouse"	7.8	0.53	0.893	161.2
	BBCH >20	Small insectivorous mammal "shrew"	1.9	0.53	0.218	661.7
	BBCH 30-39	Small omnivorous mammal "mouse"	3.9	0.53	0.447	322.4
Grassland	All season	Large herbivorous mammal "lagomorph"	17.3	0.53	1.981	72.7
	Late season	Small insectivorous mammal "shrew"	1.9	0.53	0.218	661.7
	All season	Small herbivorous mammal "vole"	72.3	0.53	8.281	17.4
	Late season (seed heads)	Small omnivorous mammal "mouse"	6.6	0.53	0.756	190.5
	New sown grass seeds	Small omnivorous mammal "mouse"	6.6	0.53	0.756	190.5

Table 9.3-4: First-tier assessment of the acute and long-term/reproductive risk for mammals due to fluroxypyr (acid) following the use of Kinvara

Intended use		**Kinvara**				
Active substance/product		Fluroxypyr (acid)				
Application rate (g/ha)		1 × 150 g a.s./ha				
Acute toxicity (mg/kg bw)		1390				
Crop scenario	**Growth stage**	**Generic focal species**	**SV_{90th}**	**MAF**	**DDD (mg/kg bw/d)**	**TER_a**
Cereals	BBCH >20	Small insectivorous mammal "shrew"	5.4	1	0.81	1716.0
	BBCH 10-29	Small omnivorous mammal "mouse"	17.2	1	2.58	538.8
	BBCH >20	Small insectivorous mammal "shrew"	5.4	1	0.81	1716.0
	BBCH 30-39	Small omnivorous mammal "mouse"	8.6	1	1.29	1077.5
Grassland	All season	Large herbivorous mammal "lagomorph"	32.6	1	4.89	284.3
	Late season	Small insectivorous mammal "shrew"	5.4	1	0.81	1716.0
	All season	Small herbivorous mammal "vole"	136.4	1	20.46	67.9
	Late season (seed heads)	Small omnivorous mammal "mouse"	14.4	1	2.16	643.5
	New sown grass seeds	Small omnivorous mammal "mouse"	14.4	1	2.16	643.5
Reproductive toxicity		100				

(mg/kg bw)						
TER criterion		5				
Crop scenario	**Growth stage**	**Generic focal species**	**SV_{mean}**	**MAF x fTWA**	**DDD (mg/kg bw/d)**	**TER_{lt}**
Cereals	BBCH >20	Small insectivorous mammal "shrew"	1.9	0.53	0.151	662.0
	BBCH 10-29	Small omnivorous mammal "mouse"	7.8	0.53	0.620	161.3
	BBCH >20	Small insectivorous mammal "shrew"	1.9	0.53	0.151	662.0
	BBCH 30-39	Small omnivorous mammal "mouse"	3.9	0.53	0.310	322.5
Grassland	All season	Large herbivorous mammal "lagomorph"	17.3	0.53	1.375	72.7
	Late season	Small insectivorous mammal "shrew"	1.9	0.53	0.151	662.0
	All season	Small herbivorous mammal "vole"	72.3	0.53	5.748	17.4
	Late season (seed heads)	Small omnivorous mammal "mouse"	6.6	0.53	0.525	190.6
	New sown grass seeds	Small omnivorous mammal "mouse"	6.6	0.53	0.525	190.6

Table 9.3-5: First-tier assessment of the acute and long-term/reproductive risk for mammals due to clopyralid following the use of Kinvara

Intended use		**Kinvara**				
Active substance/product		Clopyralid				
Application rate (g/ha)		1 × 80 g a.s./ha				
Acute toxicity (mg/kg bw)		5000				
Crop scenario	**Growth stage**	**Generic focal species**	**SV_{90th}**	**MAF**	**DDD (mg/kg bw/d)**	**TER_a**
Cereals	BBCH >20	Small insectivorous mammal "shrew"	5.4	1	0.43	11574.1
	BBCH 10-29	Small omnivorous mammal "mouse"	17.2	1	1.38	3633.7
	BBCH >20	Small insectivorous mammal "shrew"	5.4	1	0.43	11574.1
	BBCH 30-39	Small omnivorous mammal "mouse"	8.6	1	0.69	7267.4
Grassland	All season	Large herbivorous mammal "lagomorph"	32.6	1	2.61	1917.2
	Late season	Small insectivorous mammal "shrew"	5.4	1	0.43	11574.1
	All season	Small herbivorous mammal "vole"	136.4	1	10.91	458.2
	Late season (seed heads)	Small omnivorous mammal "mouse"	14.4	1	1.15	4340.3
	New sown grass seeds	Small omnivorous mammal "mouse"	14.4	1	1.15	4340.3
Reproductive toxicity (mg/kg bw)		50				
TER criterion		5				
Crop scenario	**Growth stage**	**Generic focal species**	**SV_{mean}**	**MAF x fTWA**	**DDD (mg/kg bw/d)**	**TER_{lt}**
Cereals	BBCH >20	Small insectivorous mammal "shrew"	1.9	0.53	0.081	620.7
	BBCH 10-29	Small omnivorous mammal "mouse"	7.8	0.53	0.331	151.2
	BBCH >20	Small insectivorous mammal "shrew"	1.9	0.53	0.081	620.7
	BBCH 30-39	Small omnivorous mammal "mouse"	3.9	0.53	0.165	302.4
Grassland	All season	Large herbivorous mammal "lagomorph"	17.3	0.53	0.734	68.2
	Late season	Small insectivorous mammal "shrew"	1.9	0.53	0.081	620.7
	All season	Small herbivorous mammal "vole"	72.3	0.53	3.066	16.3
	Late season (seed heads)	Small omnivorous mammal "mouse"	6.6	0.53	0.280	178.7
	New sown grass seeds	Small omnivorous mammal "mouse"	6.6	0.53	0.280	178.7

9.3.2.2 Higher-tier risk assessment

Residue decline data

The risk assessment of the Kinvara formulation was refined by accounting for the residue decline data for MPCA from the DAR. Based on the field trials the geomean DT50 value of 2.47 days is estimated, corresponding to a ftwa of 0.16 (see Table 9.2-7 and Table 9.2-8). This refinement already accepted by CEU in previous registration and the ftwa for MCPA have then been used to resolve the chronic mammals risk assessment of Kinvara as shown in Table 9.3-6.

Table 9.3-6: Refined risk assessment of MCPA using a residue DT_{50} values of 2.47days

Reproductive toxicity (mg/kg bw)		**37.8**				
TER criterion		**5**				
Crop scenario	**Growth stage**	**Generic focal species**	**SV_{mean}**	**MAF x fTWA**	**DDD (mg/kg bw/d)**	**TER_{lt}**
Cereals	BBCH >20	Small insectivorous mammal "shrew"	1.9	0.16	0.213	177.63
	BBCH 10-29	Small omnivorous mammal "mouse"	7.8	0.16	0.874	43.27
	BBCH >20	Small insectivorous mammal "shrew"	1.9	0.16	0.213	177.63
	BBCH 30-39	Small omnivorous mammal "mouse"	3.9	0.16	0.437	86.54
Grassland	All season	Large herbivorous mammal "lagomorph"	17.3	0.16	1.938	19.51
	Late season	Small insectivorous mammal "shrew"	1.9	0.16	0.213	177.63
	All season	Small herbivorous mammal "vole"	72.3	0.16	8.098	**4.67**
	Late season (seed heads)	Small omnivorous mammal "mouse"	6.6	0.16	0.739	51.14
	New sown grass seeds	Small omnivorous mammal "mouse"	6.6	0.16	0.739	51.14

~~**RUD refinement**~~

~~Risk assessment for voles is further refined by using the updated default RUD value for cereal+grassland of 47.2 mg/kg in the EFSA 2023 Birds and Mammal guidance document.~~

~~**Table 9.3-7: Refined risk assessment of MCPA using a residue DT_{50} values of 2.47days and RUD value for grassland of 47.2 mg/kg**~~

~~Generic focal species~~	~~FIR/bw~~	~~RUDm~~	~~MAFm x TWA~~	~~SV~~	~~Dose (kg as /ha)~~	~~DDD~~	~~Tox mg/kg/day~~	~~TER~~
~~vole~~	~~1.33~~	~~47.2~~	~~0.16~~	~~17.452~~	~~0.524~~	~~5.263~~	~~37.8~~	~~7.18~~

~~The TER values for all generic focal species are above the trigger of 5. Therefore a safe use for the MCPA for the proposed uses in the GAP is concluded.~~

Assessment of mixture toxicity

Kinvara contains 233 g/L MCPA, 50 g/L fluroxypyr and 28 g/L clopyralid.

To achieve a basis for a comparison of single active substance and mixture toxicity in terms of potential risk, a "tox per fraction" is calculated for each active substance and compared to the corresponding quotient for the mixture using the following equation, according to the EFSA guidance:

$$tox\ per\ fraction\ (a.s.) = \frac{LD_{50}(a.s._i)}{X(a.s._i)}$$

$$tox\ per\ fraction\ (a.s.) = \frac{LD_{50}(mix)}{\sum_i X(a.s._i)}$$

Table 9.3-8: Mammal "Tox per fraction" quotient for active substances.

Test substance	Fraction of active substance in the formulation mixture, X(a.s.) [a)]	Acute toxicity endpoint (mg/kg bw)	Tox per fraction (a.s)	Tox per fraction of the formulation mixture	Deviation (%) [b)]
MCPA	0.749	962	1284.04	1140	88.78%
Fluroxypyr	0.161	2000	~~8645.80~~ 12422.4		9.16%
Clopyralid	0.090	5000	~~55535.71~~ 55555.56		2.05%

Test substance	Fraction of active substance in the formulation mixture, X(a.s.) [a)]	Reprod toxicity endpoint (mg/kg bw/d)	Tox per fraction (a.s)	Tox per fraction of the formulation mixture	Deviation (%) [b)]
MCPA	0.749	37.8	50.45	44	87.21%
Fluroxypyr	0.161	144	895.68		4.91%
Clopyralid	0.090	50	555.36		7.92%

a) Concentration of an active substance in the formulation, divided by, the total concentration of all active substances in the formulation.
b) Deviation % = Tox per fraction (mix) / Tox per fraction (a.s.) * 100

As neither substance contributes > 90 % to the mixture toxicity, a combined assessment is necessary.

Table 9.3-9: Combination combined assessment of the acute risk for mammals due to the use of Kinvara

Intended use		Kinvara				
Application rate (L/ha)		3.0				
Acute toxicity (mg/kg bw)		1140				
Crop scenario	Growth stage	Generic focal species	SV_{90th}	MAF	DDD (mg/kg bw/d)	TER_a
Cereals	BBCH >20	Small insectivorous mammal "shrew"	5.4	1	5.022	227.00
	BBCH 10-29	Small omnivorous mammal "mouse"	17.2	1	15.996	71.27
	BBCH >20	Small insectivorous mammal "shrew"	5.4	1	5.022	227.00
	BBCH 30-39	Small omnivorous mammal "mouse"	8.6	1	7.998	142.54

Grassland	All season	Large herbivorous mammal "lagomorph"	32.6	1	30.318	37.60
	Late season	Small insectivorous mammal "shrew"	5.4	1	5.022	227.00
	All season	Small herbivorous mammal "vole"	136.4	1	126.852	**8.99**
	Late season (seed heads)	Small omnivorous mammal "mouse"	14.4	1	13.392	85.13
	Newly sown grass seeds	Small omnivorous mammal "mouse"	14.4	1	13.392	85.13

Table 9.3-10: Combination combined assessment of the long-term/reproductive risk for mammals due to the use of Kinvara

Active substance/product		Kinvara						
Application rate (g/ha)		3.0 L formulation/ha						
TER criterion		10						
Crop scenario	**Growth stage**	**Generic focal species**	**TER**			**RQ**			**RQ sum**
			MCPA*	**Fluroxypyr-acid**	**Clopyralid**	**MCPA**	**Fluroxypyr-acid**	**Clopyralid**	
Cereals	BBCH >20	Small insectivorous mammal "shrew"	177.63	1713.9	11574.1	0.056	0.006	0.001	0.063
	BBCH 10-29	Small omnivorous mammal "mouse"	43.27	538.1	3633.7	0.231	0.019	0.003	0.252
	BBCH >20	Small insectivorous mammal "shrew"	177.63	1713.9	11574.1	0.056	0.006	0.001	0.063
	BBCH 30-39	Small omnivorous mammal "mouse"	86.54	1076.2	7267.4	0.116	0.009	0.001	0.126
Grassland	All season	Large herbivorous mammal "lagomorph"	19.51	283.9	1917.2	0.513	0.035	0.005	0.553
	Late	Small insectivorous mammal "shrew"	177.63	1713.9	11574.1	0.056	0.006	0.001	0.063
	All season	Small herbivorous mammal "vole"	7.18[1] 5.06	67.9	458.2	1.393 1.976	0.147	0.022	**1.562** **2.145**
	Late season (seed heads)	Small omnivorous mammal "mouse"	51.14	642.7	4340.3	0.196	0.016	0.002	0.213
	New sown grass seeds	Small omnivorous mammal "mouse"	51.14	642.7	4340.3	0.196	0.016	0.002	0.213

* HT TER for MCPA by using refined DT50 value of 2.47 days

~~1 Refined TER value for voles using RUD for grass+cereals of 47.2 mg/kg in the EFSA 2023 Birds and Mammal GD~~

Table 9.3-11a: Combination combined assessment of the long-term/reproductive risk for mammals due to the use of Kinvara

Active substance/product		Kinvara							
Application rate (g/ha)		3.0 L formulation/ha							
TER criterion		5							
Crop scenario	Growth stage	Generic focal species	TER			RQ			RQ sum
			MCPA*	Fluroxypyr-acid	Clopyralid	MCPA	Fluroxypyr-acid	Clopyralid	
Cereals	BBCH >20	Small insectivorous mammal "shrew"	177.63	662	620.7	0.056	0.008	0.008	0.044
	BBCH 10-29	Small omnivorous mammal "mouse"	43.27	161.3	151.2	0.231	0.031	0.033	0.180
	BBCH >20	Small insectivorous mammal "shrew"	177.63	662	620.7	0.056	0.008	0.008	0.044
	BBCH 30-39	Small omnivorous mammal "mouse"	86.54	322.5	302.4	0.116	0.016	0.017	0.090
Grassland	All season	Large herbivorous mammal "lagomorph"	19.51	72.7	68.2	0.513	0.069	0.073	0.398
	Late	Small insectivorous mammal "shrew"	177.63	662	620.7	0.056	0.008	0.008	0.044
	All season	Small herbivorous mammal "vole"	~~7.18~~[1] 5.06	17.4	16.3	~~1.393~~ 1.976	0.287	0.307	**1.582**
	Late season (seed heads)	Small omnivorous mammal "mouse"	51.14	190.6	178.7	0.196	0.026	0.028	0.152
	New sown grass seeds	Small omnivorous mammal "mouse"	51.14	190.6	178.7	0.196	0.026	0.028	0.152

On a combination basis the risk to voles in grassland cannot be resolved using the standard EU TER thresholds. This is no different from the previous registration of Kinvara in Central Zone, as neither the endpoints nor the EU guidance has changed meaning the calculations are identical. Previous registrations were granted based on either dropping the application rate to 2.5 L/ha, which is the maximum rate that passes the acute assessment, or accepting the full rate and resolving the assessment following German standard guidance. The EU definition of the vole is vastly over-conservative for application in the Central Zone. This has long been recognized in Germany and resolved by reducing the TER criteria for the vole to 5 for the acute assessment and 2 for the chronic assessment. This has long been accepted as a more realistic representation of the risk to small mammals and allows the assessment to be fully resolved.

The risk assessment for lower application rate of 2.5 L/ha is presented below.

Application rate of 2.5 L/ha

Intended use			**Kinvara MCPA**			
Application rate (g/ha)			1 x 583.3			
Acute toxicity (mg/kg bw)			962			
TER			10			
Crop scenario	**Growth stage**	**Generic focal species**	**SV_{90th}**	**MAF**	**DDD (mg/kg bw/d)**	**TER_a**
Grassland	All season	Small herbivorous mammal "vole"	136.4	1	79.6	12.1
Reproductive toxicity (mg/kg bw)		**37.8**				
TER criterion		**5**				
Grassland	All season	Small herbivorous mammal "vole"	72.3	0.53	22.3	**1.70**
Refined risk assessment of MCPA using a residue DT50 values of 2.47days						
Grassland	All season	Small herbivorous mammal "vole"	72.3	0.16	6.75	5.6

Intended use			**Kinvara Fluroxypyr meptyl**			
Application rate (g/ha)			217.6			
Acute toxicity (mg/kg bw)			2000			
TER			10			
Crop scenario	**Growth stage**	**Generic focal species**	**SV_{90th}**	**MAF**	**DDD (mg/kg bw/d)**	**TER_a**
Grassland	All season	Small herbivorous mammal "vole"	136.4	1	29.68	67.4
Reproductive toxicity (mg/kg bw)		**144**				
TER criterion		**5**				
Grassland	All season	Small herbivorous mammal "vole"	72.3	0.53	8.34	17.3

Intended use		Kinvara Fluroxypyr acid				
Application rate (g/ha)		125				
Acute toxicity (mg/kg bw)		1390				
TER		10				
Crop scenario	**Growth stage**	**Generic focal species**	**SV_{90th}**	**MAF**	**DDD (mg/kg bw/d)**	**TER_a**
Grassland	All season	Small herbivorous mammal "vole"	136.4	1	17.05	81.5
Reproductive toxicity (mg/kg bw)		**100**				
TER criterion		**5**				
Grassland	All season	Small herbivorous mammal "vole"	72.3	0.53	4.79	20.9

Intended use		Kinvara Clopyralid				
Application rate (g/ha)		66.7				
Acute toxicity (mg/kg bw)		5000				
TER		10				
Crop scenario	**Growth stage**	**Generic focal species**	**SV_{90th}**	**MAF**	**DDD (mg/kg bw/d)**	**TER_a**
Grassland	All season	Small herbivorous mammal "vole"	136.4	1	9.10	549
Reproductive toxicity (mg/kg bw)		**50**				
TER criterion		**5**				
Grassland	All season	Small herbivorous mammal "vole"	72.3	0.53	2.56	19.5

Combination combined assessment of the acute risk for mammals due to the use of Kinvara

Intended use		Kinvara				
Application rate (L/ha)		2.5 (777.g g S scz)				
Acute toxicity (mg/kg bw)		1140				
Crop scenario	**Growth stage**	**Generic focal species**	**SV_{90th}**	**MAF**	**DDD (mg/kg bw/d)**	**TER_a**
Grassland	All season	Small herbivorous mammal "vole"	136.4	1	106	10.75

Combination combined assessment of the reproductive long-term risk for mammals due to the use of Kinvara

Active substance/product		Kinvara							
Application rate (g/ha)		2.5 L formulation/ha							
TER criterion		5							
Crop scenario	**Growth stage**	**Generic focal species**	**TER**			**RQ**			**RQ sum**
			MCPA*	**Fluroxypyr-acid**	**Clopyralid**	**MCPA**	**Fluroxypyr-acid**	**Clopyralid**	
	All season	Small herbivorous mammal "vole"	5.6	17.3	19.5	0.178	0.058	0.051	0.287

9.3.2.3 Drinking water exposure

Given that the proposed use of Kinvara is on cereals and grassland, the relevant scenario for exposure via drinking water is the puddle scenario (see Section 5.5 of EFSA (2009)). As such, both acute and reproductive risks must be assessed for birds and mammals.

Puddle scenario

According to the EFSA (2009) guidance document, '*no specific calculation of exposure and TER are necessary when the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed 50 in the case of less sorptive substances (Koc <500 L/kg) or 3000 in the case of more sorptive substances (Koc >500 L/kg)*'. As a result no further calculations are necessary (see Table 9.3-12).

Table 9.3-12: Substance summary table for puddle scenario

Substance	MCPA	Fluroxypyr-MHE	Fluroxypyr	Clopyralid
Mean KfOC (mL/g)	<500	>500	<500	<500
Effective application Rate (g a.s./ha)	700	216.1	150	80
Acute Toxicity (mg/kg bw/day)	220	2000	2000	1465
Reproductive Toxicity (mg/kg bw/d)	22	57.8	40.1	118
Acute Ratio	3.18	0.11	0.08	0.05
Chronic Ratio	31.8	3.74	3.74	0.68
Risk Assessment Required?	No	No	No	No

9.3.2.4 Effects of secondary poisoning

Since the Log P_{OW} of MCPA (pH dependent; -1.07 to 2.80) and clopyralid (-2.63) are < 3 the risks from secondary poisoning to birds and mammals do not require assessment. However, the Log Pow for fluroxypyr (meptyl) is >5 and therefore requires assessment for secondary poisoning.

The EFSA final conclusions for fluroxypyr conclude although the log P_{OW} of fluroxypyr-meptyl is greater than 3, the risk assessment for secondary poisoning of birds and mammals was not conducted because fluroxypyr-meptyl rapidly hydrolyses to fluroxypyr in the environment and it does not bioaccumulate in fish (measured BCF = 26).

The log P_{OW} of the fluroxypyr metabolite methoxypyridine is 3.09, which is greater than the EFSA (2009) trigger of 3. Therefore foodchain transfer may occur and further assessment is required. All calculations are performed according to Section 5.6 of EFSA (2009). The endpoints used in the risk assessment are taken from the risk assessment performed in the Addendum to the DAR (December 2010).

Risk assessment for earthworm-eating mammals via secondary poisoning

Table 9.3-13: Assessment of the risk for earthworm-eating mammals due to exposure to methoxypyridine via bioaccumulation in earthworms (secondary poisoning)

Parameter	Methoxypyridine	Comments
PEC_{soil} (accumulation) (mg/kg soil)	0.190	PEC soil acc max from all uses (from winter cereals, annual applications) (dRR B8, section 8.7.2.2)
log P_{ow} / P_{ow}	3.09	
Koc	311	Mean (n = 4)
foc	0.02	Default

BCF_{worm}	0.195	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$
PEC_{worm}	0.037	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.039	$DDD = PEC_{worm} \times 1.28$
NOEL (mg/kg bw/d)	10	EFSA (2011)
TER_{lt}	257.5	

Risk assessment for fish-eating mammals via secondary poisoning

Table 9.3-14: Assessment of the risk for fish-eating mammals due to exposure to methoxypyridine via bioaccumulation in fish (secondary poisoning)

Parameter	Methoxypyridine	Comments
PEC_{sw} (twa = 21 d) (mg/L)	0.00343	Step 2, 21 d TWA PECsw, max for all uses (from winter cereals, annual applications, see Appendix 3)
BCF_{fish}	1.41	
BMF	-	biomagnification factor (relevant for BCF ≥ 2000)
PEC_{fish}	0.00484	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.0007	$DDD = PEC_{fish} \times 0.142$
NOEL (mg/kg bw/d)	10.0	EFSA (2011)
TER_{lt}	13004.4	

9.3.2.5 Biomagnification in terrestrial food chains

Not relevant.

9.3.3 Risk assessment for baits, pellets, granules, prills or treated seed

Not relevant.

9.3.4 Overall conclusions

The combined and individual risk of the active substances in Kinvara can be resolved at Tier-1 for the proposed applications to cereals. The assessment of applications to grassland needs to be refined using residue decline data for MCPA in order to demonstrate safe usage for voles.

A safe risk from the mixture toxicity assessment is concluded for all species except voles. The risk to voles in grassland cannot be resolved using the standard EU TER thresholds. This is no different from the previous registration of Kinvara in Central Zone, as neither the endpoints nor the EU guidance has changed meaning the calculations are identical. Previous registrations were granted accepting the full rate and resolving the assessment following German standard guidance. The EU definition of the vole is vastly over-conservative for application in the Central Zone. This has long been recognized in Germany and resolved by reducing the TER criteria for the vole to 5 for the acute assessment and 2 for the chronic assessment. This has long been accepted as a more realistic representation of the risk to small mammals and allows the assessment to be fully resolved.

9.4 Effects on other terrestrial vertebrate wildlife (reptiles and amphibians) (KCP 10.1.3)

Not relevant.

9.5 Effects on aquatic organisms (KCP 10.2)

zRMS Comments:

New studies were submitted and evaluated in Appendix 2.

The following application pattern was taken into consideration:

- winter and spring cereals,
- grasslands.

The scenarios D1 and D2 were not considered in the risk assessment as they are not relevant for Central Zone.

MCPA. The endpoints agreed at the EU level were used in risk assessment.
For risk assessment the PECsw and PECsed values evaluated in Section 8 were taken into consideration.

No mitigation measure is required for winter cereals.
The mitigation measure of 10 m VBS and 10 NSS or 10 VBS is required for spring cereals (R4 scenario).
No mitigation measure is required for grasslands.
The proper mitigation measures should be considered at MS level in accordance with the national requirements.

Metabolite of MCPA
The relevant metabolite, PCOC, was taken into consideration. The submitted risk assessment is based on PECsw i PECsed values reported in Section 8 (Step 2 assessment is sufficient).

Fluroxypyr. The fluroxypyr meptyl and floroxypyr acid were considered.
Most of the endpoints used in risk assessment for fluroxypyr meptyl were agreed at the EU level. In case of chronic risk assessment, the relevant endpoint of 60.5 µg/L should be used. The update risk assessment will change the final conclusion. For fluroxypyr acid the used endpoints were agreed at the EU level.
For risk assessment the PECsw and PECsed values evaluated in Section 8 were taken into consideration.
No mitigation measure is required for all crops.
Metabolite of Fluroxypyr. All relevant were considered. The submitted risk assessment is based on PECsw i PECsed values reported in Section 8 (for Step 1/2 assessment is sufficient).

Clopyralid. The endpoints agreed at the EU level were used in risk assessment.
For risk assessment the PECsw and PECsed values evaluated in Section 8 were taken into consideration. The submitted risk assessment is based on PECsw i PECsed values reported in Section 8 (for Step 1/2 assessment is sufficient).

Mixture toxicity assessment. The correct NOEC value of 0.0605 mg a.s./L for Fluroxypyr-MHE for aquatic invertebrates should be used. As the contribution to toxicity is above 99.17% for NOEC= 0.100 mg a.s./L the update to corrected NOEC value will not effect on final assessment. The correction is provided in Table 9.5-19.

Formulation Kinvara.
The submitted by the Applicant risk assessment for formulation was accepted.
The risk considering the drift exposure for aquatic plants as the worst case (RAC=22.1 µg f.p./L) was added by evaluator. Using the Drift Calculator of SWASH model the mitigation measures are proposed:

Crop/Application pattern	No spray buffer (m)	PECsw µg/L
Winter cereals Spring cereals	1	21.68

	Grass			
	To ensure safe use of Kinvara no mitigation measure is required.			

9.5.1 Toxicity data

Studies on the toxicity to aquatic organisms have been carried out with MCPA, fluroxypyr, clopyralid and all relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents, as well as in Appendix 2 of this document (new studies). The new studies which have been submitted fulfil data requirements but have not been used to change the agreed EU endpoints for any of the active substances. The agreed list of aquatic endpoints are provided in Table 9.5-1 and Table 9.5-2.

Effects on aquatic organisms from the Kinvara formulation have not been previously evaluated as part of active substance approvals. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2. In accordance with the EFSA (2013) edge-of-field surface water guidance testing was conducted with aquatic invertebrates, algae and macrophytes, but not with fish in order to avoid excessive vertebrate testing. The active substance data for MCPA, fluroxypyr and clopyralid indicates that a mixture with the composition of Kinvara will be more than 10 times more toxic to aquatic macrophytes than fish. This is consistent with the herbicidal mode of action of all the substances within Kinvara. It is noted that fluroxypyr-MHE is potentially as toxic to fish as it is to aquatic macrophytes, however it is a short-lived substance and the other active substances are far less toxic to fish. Also, in line with the EFSA (2013) guidance, the toxicity of Kinvara was tested against both *Lemna* and *Myriophyllum* due to its herbicidal mode of action.

Table 9.5-1: Agreed effects values for fish, aquatic invertebrates and sediment dwellers endpoints

Substance	Value (mg a.s/L)	Endpoint	Species	Reference
Fish-Acute (AF 100)				
MCPA	50	96 h, static LC_{50}	*Oncorhynchus mykiss*	EC (2008)
PCOC	2.12	24hr, static, LC_{50}	*Salmo trutta*	UNEP (1998)
Fluroxypyr-MHE	>0.225	96 h, static LC_{50}	*Oncorhynchus mykiss*	EFSA (2011)
Fluroxypyr (acid)	14.3	96 h, static LC_{50}	*Lepomis macrochirus*	EFSA (2011)
Pyridinol	39	96 h, static LC_{50}	*Oncorhynchus mykiss*	EFSA (2011)
3-CP	95.1	96 h, static LC_{50}	*Oncorhynchus mykiss*	EFSA (2011)
Clopyralid	>99.9	96 h, static LC_{50}	*Oncorhynchus mykiss*	EFSA (2018)
Fish-Chronic (AF 10)				
MCPA	15	28 d, NOEC	*Pimephales promelas*	EC (2008)
PCOC	0.5	28d, NOEC	*Salmo trutta*	UNEP (1998)
Fluroxypyr-MHE	0.2	21 d, NOEC	*Pimephales promelas*	EFSA (2011)
Fluroxypyr (acid)	100	21 d, NOEC	*Oncorhynchus mykiss*	EFSA (2011)
Clopyralid	10.8	28 d, NOEC	*Pimephales promelas*	EFSA (2018)
Aquatic Invertebrates-Acute (AF 100)				
MCPA	>190	48 h, static, EC_{50}	*Daphnia magna*	EC (2008)
PCOC	0.29	48 h, static, EC_{50}	*Daphnia magna*	UNEP (1998)
Fluroxypyr-MHE	>0.183	48 h, LD_{50}	*Daphnia magna*	EFSA (2011)
Fluroxypyr (acid)	>100	48 h	*Daphnia magna*	EFSA (2011)
Pyridinol	>49	48 h, NOEC	*Daphnia magna*	EFSA (2011)
3-CP	7.56	48 h, NOEC	*Daphnia magna*	EFSA (2011)
Clopyralid	>99	48 h, static, EC50	*Daphnia magna*	EFSA (2018)
Kinvara	9.8	48 h, NOEC	*Daphnia magna*	Seeland-Fremer & Wydra (2014)
Kinvara	>2.8	Predicted Formulation Toxicity		
Aquatic Invertebrates-Chronic (AF 10)				

MCPA	50	21 d, NOEC	*Daphnia magna*	EC (2008)
PCOC	0.55	21 day, NOEC	*Daphnia magna*	UNEP (1998)
Fluroxypyr-MHE	~~0.1~~ 0.0605	21 d, NOEC	*Daphnia magna*	EFSA (2011)
Fluroxypyr (acid)	56	21 d, NOEC	*Daphnia magna*	EFSA (2011)
Clopyralid	17	21 d, NOEC	*Daphnia magna*	EFSA (2018)
Sediment Dwellers				
Fluroxypyr-MHE	0.13	28 d water-spiked study	*Chironomus riparius*	EFSA (2011)
Clopyralid	50	28 d static. NOEC	*Chironomus riparius*	EFSA (2018)

Table 9.5-2: Agreed Effects Values for Algae and Aquatic Macrophytes

Substance	Value (mg a.s/L)	Endpoint	Species	Reference
Algae (AF=10)				
MCPA	32.9	72 h, EC_{50}	*Pseudokirchneriella subcapitata*	EC (2008)
PCOC	8.2	96h, E_bC_{50}	*Scenedesmus subspicatus*	
Fluroxypyr-MHE	>0.208	72 h, E_rC_{50}	*Navicula pelliculosa*	EFSA (2011)
Fluroxypyr (acid)	26	72 hr E_rC_{50}	*Navicula pelliculosa*	EFSA (2011)
Pyridinol	0.640	120 h, EC_{50} (cell density)	*Navicula pelliculosa*	EFSA (2011)
Methoxypyridine	1.12	72 h EC_{50} (cell density)	*Anabaena flos-aquae*	EFSA (2011)
3-CP	35	72 h, EC_{50} (cell density)	*Selanastrum capricornutum*	EFSA (2011)
Clopyralid	30	72 h, E_rC_{50}	*Selanastrum capricornutum*	EFSA (2018)
Kinvara formulation	3.79	7 d, E_rC_{50}	*Anabaena flos-aquae*	Seeland-Fremer. A & Wydra, V. (2014)
Kinvara formulation	>1.44	Predicted Formulation Toxicity		
Aquatic Macrophytes (AF=10)				
MCPA	0.152	7 d, I_rC_{50}	*Lemna gibba*	EC (2008)
PCOC	93	48h (static), EC_{50}	*Lemna minor*	UNEP (1998)
Fluroxypyr-MHE	>2.31	14 d (static), Fronds, EC_{50}	*Lemna gibba*	EFSA (2011)
Fluroxypyr (acid)	12.3	14 d (static), Fronds, EC_{50}	*Lemna gibba*	EFSA (2011)
Pyridinol	>3.2	14 d (static), Fronds, EC_{50}	*Lemna gibba*	EFSA (2011)
Methoxypyridine	10.6	14 d (static), Fronds, EC_{50}	*Lemna gibba*	EFSA (2011)
Clopyralid	>3	14 d, E_rC_{50}	*Myriophyllum* sp.	EFSA (2018)
Kinvara formulation	38.3	7 d, E_rC_{50} (frond number)	*Lemna gibba*	Seeland-Fremer & Wydra (2014)
Kinvara formulation	0.221	14d E_rC_{50} (mean measured, fresh weight)	*Myriophyllum* sp.	Wenzel (2016)

9.5.1.1 Justification for new endpoints

No changes have been made to available agreed EFSA endpoints in this assessment. Toxicity data on the Kinvara formulation has been added as it was not previously evaluated by EFSA.

9.5.2 Risk assessment

The evaluation of the risk for aquatic and sediment-dwelling organisms was performed in accordance with the recommendations of the "Guidance document on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters in the context of Regulation (EC) No 1107/2009", as provided by the Commission Services (SANTE-2015-00080, 15 January 2015).

The relevant global maximum FOCUS Step 1, 2 and 3 PEC_{SW} for risk assessments covering the proposed use pattern and the resulting PEC/RAC ratios are presented in the tables below. For Step 1 and 2 calculations the values for spring and winter cereals are identical and therefore only one set of the results has been presented which covers both proposed uses.

In Table 9.5-3 to

Table 9.5-18, the ratios between predicted environmental concentrations in surface water bodies (PEC_{SW}) and regulatory acceptable concentrations (RAC) for aquatic organisms are given for each active substance and metabolite for each FOCUS scenario at Step 1, 2 and 3 and each organism group A formulation risk assessment has been provided in Table 9.5-20.

MCPA

Table 9.5-3: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for MCPA for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of Kinvara in winter cereals

Group			**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Macrophytes**
Test species			*O.mykiss*	*P. promelas*	*D.magna*	*D. magna*	*P. subcapitata*	*L. gibba*
Endpoint			LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}	IrC50
(µg/L)			50000	15000	>190000	50000	32900	152
AF			100	10	100	10	10	10
RAC (µg/L)			500	1500	1900	5000	3290	15.2
FOCUS Scenario PEC gl-max (µg/L)			**PEC/RAC**					
Step 1		223.694	0.447	0.149	0.118	0.045	0.068	**14.717**
Step 2	Northern Europe	36.703	0.073	0.024	0.019	0.007	0.011	**2.415**
	Southern Europe	68.035	0.136	0.045	0.036	0.014	0.021	**4.476**
D1	ditch	5.656	0.011	0.004	0.003	0.001	0.002	0.372
D1	stream	4.457	0.009	0.003	0.002	0.001	0.001	0.293
D2	ditch	73.61	0.147	0.049	0.039	0.015	0.022	**4.843**
D2	stream	47.02	0.094	0.031	0.025	0.009	0.014	**3.093**
D3	ditch	4.431	0.009	0.003	0.002	0.001	0.001	0.292
D4	pond	0.164	0.000	0.000	0.000	0.000	0.000	0.011
D4	stream	3.279	0.007	0.002	0.002	0.001	0.001	0.216
D5	pond	0.207	0.000	0.000	0.000	0.000	0.000	0.014
D5	stream	3.578	0.007	0.002	0.002	0.001	0.001	0.235
D6	ditch	4.438	0.009	0.003	0.002	0.001	0.001	0.292
R1	pond	0.227	0.000	0.000	0.000	0.000	0.000	0.015
R1	stream	5.759	0.012	0.004	0.003	0.001	0.002	0.379
R3	stream	6.131	0.012	0.004	0.003	0.001	0.002	0.403
R4	stream	2.894	0.006	0.002	0.002	0.001	0.001	0.190

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Table 9.5-4: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for MCPA for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of Kinvara in spring cereals

Group			Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Macrophytes
Test species			*O.mykiss*	*P. promelas*	*D.magna*	*D. magna*	*P. subcapitata*	*L. gibba*
Endpoint			LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}	IrC50
(µg/L)			50000	15000	>190000	50000	32900	152
AF			100	10	100	10	10	10
RAC (µg/L)			500	1500	1900	5000	3290	15.2
FOCUS Scenario PEC gl-max (µg/L)			PEC/RAC					
Step 1		223.694	0.447	0.149	0.118	0.045	0.068	**14.717**
Step 2	Northern Europe	36.703	0.073	0.024	0.019	0.007	0.011	**2.415**
	Southern Europe	68.035	0.136	0.045	0.036	0.014	0.021	**4.476**
D1	ditch	5.804	0.012	0.004	0.003	0.001	0.002	0.382
D1	stream	4.961	0.010	0.003	0.003	0.001	0.002	0.326
D3	ditch	4.435	0.009	0.003	0.002	0.001	0.001	0.292
D4	pond	0.248	0.000	0.000	0.000	0.000	0.000	0.016
D4	stream	3.633	0.007	0.002	0.002	0.001	0.001	0.239
D5	pond	0.191	0.000	0.000	0.000	0.000	0.000	0.013
D5	stream	3.549	0.007	0.002	0.002	0.001	0.001	0.233
R4	stream	16.49	0.033	0.011	0.009	0.003	0.005	**1.085**
Step 4 (volatilization/deposition and 10m drift/runoff VBS)								
D1	ditch	5.802	0.012	0.004	0.003	0.001	0.002	0.382
D1	stream	4.961	0.010	0.003	0.003	0.001	0.002	0.326
D3	ditch	0.638	0.001	0.000	0.000	0.000	0.000	0.042
D4	pond	0.248	0.000	0.000	0.000	0.000	0.000	0.016
D4	stream	0.728	0.001	0.000	0.000	0.000	0.000	0.048
D5	pond	0.15	0.000	0.000	0.000	0.000	0.000	0.010
D5	stream	0.72	0.001	0.000	0.000	0.000	0.000	0.047
R4	stream	7.443	0.015	0.005	0.004	0.001	0.002	0.490

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Table 9.5-5: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for MCPA for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of Kinvara in grassland

Group	Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Macrophytes

Test species			*O.mykiss*	*P. promelas*	*D.magna*	*D. magna*	*P. subcapitata*	*L. gibba*
Endpoint			LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}	IrC50
(µg/L)			50000	15000	>190000	50000	32900	152
AF			100	10	100	10	10	10
RAC (µg/L)			500	1500	1900	5000	3290	15.2
FOCUS Scenario PEC gl-max (µg/L)			**PEC/RAC**					
Step 1		233.694	0.467	0.156	0.123	0.047	0.071	**15.375**
Step 2	Northern Europe	28.869	0.058	0.019	0.015	0.006	0.009	**1.899**
	Southern Europe	52.369	0.105	0.035	0.028	0.010	0.016	**3.445**
Step 3- Spring Applications								
D1	Ditch	58.86	0.118	0.039	0.031	0.012	0.018	**3.872**
D1	Stream	36.88	0.074	0.025	0.019	0.007	0.011	**2.426**
D2	Ditch	95.66	0.191	0.064	0.050	0.019	0.029	**6.293**
D2	Stream	62.49	0.125	0.042	0.033	0.012	0.019	**4.111**
D3	Ditch	4.437	0.009	0.003	0.002	0.001	0.001	0.292
D4	Pond	0.153	0.000	0.000	0.000	0.000	0.000	0.010
D4	Stream	3.393	0.007	0.002	0.002	0.001	0.001	0.223
D5	Pond	0.178	0.000	0.000	0.000	0.000	0.000	0.012
D5	Stream	3.676	0.007	0.002	0.002	0.001	0.001	0.242
R2	Stream	2.318	0.005	0.002	0.001	0.000	0.001	0.153
R3	Stream	2.965	0.006	0.002	0.002	0.001	0.001	0.195
Step 3- Summer Applications								
D1	Ditch	4.755	0.010	0.003	0.003	0.001	0.001	0.313
D1	Stream	3.928	0.008	0.003	0.002	0.001	0.001	0.258
D2	Ditch	4.722	0.009	0.003	0.002	0.001	0.001	0.311
D2	Stream	7.782	0.016	0.005	0.004	0.002	0.002	0.512
D3	Ditch	4.459	0.009	0.003	0.002	0.001	0.001	0.293
D4	Pond	0.153	0.000	0.000	0.000	0.000	0.000	0.010
D4	Stream	3.837	0.008	0.003	0.002	0.001	0.001	0.252
D5	Pond	0.242	0.000	0.000	0.000	0.000	0.000	0.016
D5	Stream	4.755	0.010	0.003	0.003	0.001	0.001	0.313
R2	Stream	4.782	0.010	0.003	0.003	0.001	0.001	0.315
R3	Stream	2.482	0.005	0.002	0.001	0.000	0.001	0.163

Step 3- Fall Applications								
D1	Ditch	13.5	0.027	0.009	0.007	0.003	0.004	0.888
D1	Stream	9.435	0.019	0.006	0.005	0.002	0.003	0.621
D2	Ditch	22.57	0.045	0.015	0.012	0.005	0.007	**1.485**
D2	Stream	27.04	0.054	0.018	0.014	0.005	0.008	**1.779**
D3	Ditch	4.453	0.009	0.003	0.002	0.001	0.001	0.293
D4	Pond	0.153	0.000	0.000	0.000	0.000	0.000	0.010
D4	Stream	3.837	0.008	0.003	0.002	0.001	0.001	0.252
D5	Pond	0.795	0.002	0.001	0.000	0.000	0.000	0.052
D5	Stream	4.139	0.008	0.003	0.002	0.001	0.001	0.272
R2	Stream	2.661	0.005	0.002	0.001	0.001	0.001	0.175
R3	Stream	2.478	0.005	0.002	0.001	0.000	0.001	0.163

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Table 9.5-6: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for PCOC for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of Kinvara in winter cereals

Group			**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Macrophytes**
Test species			*S. trutta*	*S. trutta*	*D. magna*	*D. magna*	*S. subspicatus*	*L. minor*
Endpoint			LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}	EC_{50}
(µg/L)			2120	500	290	550	8200	93000
AF			100	10	100	10	10	10
RAC (µg/L)			21.2	50	2.9	55	820	9300
FOCUS Scenario PEC gl-max (µg/L)			**PEC/RAC**					
Step 1		72.576	**3.423**	**1.452**	**25.026**	**1.320**	0.089	0.008
Step 2	Northern Europe	10.503	0.495	0.210	**3.622**	0.191	0.013	0.001
	Southern Europe	20.655	0.974	0.413	**7.122**	0.376	0.025	0.002
D1	ditch	2.535	0.120	0.051	0.874	0.046	0.003	0.000
D1	stream	1.625	0.077	0.033	0.560	0.030	0.002	0.000
D2	ditch	2.458	0.116	0.049	0.848	0.045	0.003	0.000
D2	stream	1.561	0.074	0.031	0.538	0.028	0.002	0.000
D3	ditch	0.001	0.000	0.000	0.000	0.000	0.000	0.000
D4	pond	0.232	0.011	0.005	0.080	0.004	0.000	0.000
D4	stream	0.278	0.013	0.006	0.096	0.005	0.000	0.000
D5	pond	0.096	0.005	0.002	0.033	0.002	0.000	0.000
D5	stream	0.085	0.004	0.002	0.029	0.002	0.000	0.000
D6	ditch	0.042	0.002	0.001	0.014	0.001	0.000	0.000
R1	pond	0.018	0.001	0.000	0.006	0.000	0.000	0.000
R1	stream	0.26	0.012	0.005	0.090	0.005	0.000	0.000
R3	stream	1.261	0.059	0.025	0.435	0.023	0.002	0.000
R4	stream	0.122	0.006	0.002	0.042	0.002	0.000	0.000

Table 9.5-7: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for PCOC for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of Kinvara in spring cereals

Group			**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Macrophytes**
Test species			*S. Trutta*	*S. Trutta*	*D.magna*	*D. magna*	*S. subspicatus*	*L. minor*
Endpoint			LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}	EC_{50}
(µg/L)			2120	500	290	550	8200	93000
AF			100	10	100	10	10	10
RAC (µg/L)			21.2	50	2.9	55	820	9300
FOCUS Scenario PEC gl-max (µg/L)			**PEC/RAC**					
Step 1		72.575	**3.423**	**1.452**	**25.026**	**1.320**	0.089	0.008
Step 2	Northern Europe	10.503	0.495	0.210	**3.622**	0.191	0.013	0.001
	Southern Europe	20.655	0.974	0.413	**7.122**	0.376	0.025	0.002
D1	ditch	2.529	0.119	0.051	0.872	0.046	0.003	0.000
D1	stream	1.606	0.076	0.032	0.554	0.029	0.002	0.000
D3	ditch	0.001	0.000	0.000	0.000	0.000	0.000	0.000
D4	pond	0.327	0.015	0.007	0.113	0.006	0.000	0.000
D4	stream	0.382	0.018	0.008	0.132	0.007	0.000	0.000
D5	pond	0.123	0.006	0.002	0.042	0.002	0.000	0.000
D5	stream	0.116	0.005	0.002	0.040	0.002	0.000	0.000
R4	stream	0.706	0.033	0.014	0.243	0.013	0.001	0.000

Table 9.5-8: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for PCOC for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of Kinvara in grassland

Group			Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Macrophytes
Test species			*S. Trutta*	*S. Trutta*	*D.magna*	*D. magna*	*S. subspicatus*	*L. minor*
Endpoint			LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}	EC_{50}
(µg/L)			2120	500	290	550	8200	9300
AF			100	10	100	10	10	10
RAC (µg/L)			21.2	50	2.9	55	820	930
FOCUS Scenario PEC gl-max (µg/L)			PEC/RAC					
Step 1		72.576	**3.423**	**1.452**	**25.026**	**1.320**	0.089	0.008
Step 2	Northern Europe	7.965	0.376	0.159	**2.747**	0.145	0.010	0.001
	Southern Europe	15.579	0.735	0.312	**5.372**	0.283	0.019	0.002
Step 3- Spring Applications								
D1	Ditch	2.121	0.100	0.042	0.731	0.039	0.003	0.000
D1	Stream	1.389	0.066	0.028	0.479	0.025	0.002	0.000
D2	Ditch	2.215	0.104	0.044	0.764	0.040	0.003	0.000
D2	Stream	1.418	0.067	0.028	0.489	0.026	0.002	0.000
D3	Ditch	0.001	0.000	0.000	0.000	0.000	0.000	0.000
D4	Pond	0.001	0.000	0.000	0.000	0.000	0.000	0.000
D4	Stream	0.001	0.000	0.000	0.000	0.000	0.000	0.000
D5	Pond	0.108	0.005	0.002	0.037	0.002	0.000	0.000
D5	Stream	0.141	0.007	0.003	0.049	0.003	0.000	0.000
R2	Stream	0.167	0.008	0.003	0.058	0.003	0.000	0.000
R3	Stream	0.493	0.023	0.010	0.170	0.009	0.001	0.000
Step 3- Summer Applications								
D1	Ditch	3.084	0.145	0.062	**1.063**	0.056	0.004	0.000
D1	Stream	2.173	0.103	0.043	0.749	0.040	0.003	0.000
D2	Ditch	2.854	0.135	0.057	0.984	0.052	0.003	0.000
D2	Stream	1.798	0.085	0.036	0.620	0.033	0.002	0.000
D3	Ditch	0.001	0.000	0.000	0.000	0.000	0.000	0.000
D4	Pond	0.001	0.000	0.000	0.000	0.000	0.000	0.000
D4	Stream	0.001	0.000	0.000	0.000	0.000	0.000	0.000
D5	Pond	0.226	0.011	0.005	0.078	0.004	0.000	0.000
D5	Stream	0.341	0.016	0.007	0.118	0.006	0.000	0.000
R2	Stream	0.099	0.005	0.002	0.034	0.002	0.000	0.000
R3	Stream	0.179	0.008	0.004	0.062	0.003	0.000	0.000

Step 3- Fall Applications								
D1	Ditch	3.657	0.173	0.073	**1.261**	0.066	0.004	0.000
D1	Stream	2.28	0.108	0.046	0.786	0.041	0.003	0.000
D2	Ditch	4.482	0.211	0.090	**1.546**	0.081	0.005	0.000
D2	Stream	2.819	0.133	0.056	0.972	0.051	0.003	0.000
D3	Ditch	0.001	0.000	0.000	0.000	0.000	0.000	0.000
D4	Pond	0.001	0.000	0.000	0.000	0.000	0.000	0.000
D4	Stream	0.002	0.000	0.000	0.001	0.000	0.000	0.000
D5	Pond	0.481	0.023	0.010	0.166	0.009	0.001	0.000
D5	Stream	0.68	0.032	0.014	0.234	0.012	0.001	0.000
R2	Stream	0.495	0.023	0.010	0.171	0.009	0.001	0.000
R3	Stream	0.514	0.024	0.010	0.177	0.009	0.001	0.000

Fluroxypyr

Table 9.5-9: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for fluroxypyr-MHE for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of Kinvara in winter cereals

Group			**Fish acute**	**Fish pro-longed**	**Inver-teb. acute**	**Inverteb. prolonged**		**Algae**	**Macro-phytes**	**Sediment Dweller**
Test species			*O. mykiss*	*P. promelas*	*D. magna*	*D. magna*		*N. pellicu losa*	*L. gibba*	*C. riparius*
Endpoint			LC_{50}	NOEC	EC_{50}	NOEC		E_rC_{50}	ErC50	NOEC (spiked water)
(µg/L)			>225	200	183	~~100~~	60.5	208	2310	130
AF			100	10	100	10		10	10	10
RAC (µg/L)			2.25	20	1.83	~~10~~	6.05	20.8	231	13
FOCUS Scenario PEC gl-max (µg/L)			**PEC/RAC**							
Step 1		4.649	**2.066**	0.232	**2.540**	~~0.465~~	0.768	0.224	0.020	0.159
Step 2	Northern Europe	1.987	0.883	0.099	**1.086**	~~0.199~~	0.328	0.096	0.009	0.068
	Southern Europe	1.987	0.883	0.099	**1.086**	~~0.199~~	0.328	0.096	0.009	0.068
D1	ditch	1.363	0.606	0.068	0.745	~~0.136~~		0.066	0.006	0.047
D1	stream	1.163	0.517	0.058	0.636	~~0.116~~		0.056	0.005	0.040
D2	ditch	1.359	0.604	0.068	0.743	~~0.136~~		0.065	0.006	0.046
D2	stream	1.124	0.500	0.056	0.614	~~0.112~~		0.054	0.005	0.038
D3	ditch	1.348	0.599	0.067	0.737	~~0.135~~	0.223	0.065	0.006	0.046
D4	pond	0.047	0.021	0.002	0.026	~~0.005~~	0.008	0.002	0.000	0.002
D4	stream	0.996	0.443	0.050	0.544	~~0.100~~	0.165	0.048	0.004	0.034
D5	pond	0.047	0.021	0.002	0.026	~~0.005~~	0.008	0.002	0.000	0.002
D5	stream	1.077	0.479	0.054	0.589	~~0.108~~	0.178	0.052	0.005	0.037
D6	ditch	1.333	0.592	0.067	0.728	~~0.133~~	0.220	0.064	0.006	0.046
R1	pond	0.047	0.021	0.002	0.026	~~0.005~~	0.008	0.002	0.000	0.002

R1	stream	0.888	0.395	0.044	0.485	~~0.089~~	0.147	0.043	0.004	0.030
R3	stream	1.248	0.555	0.062	0.682	~~0.125~~	0.206	0.060	0.005	0.043
R4	stream	0.88	0.391	0.044	0.481	~~0.088~~	0.145	0.042	0.004	0.030

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Table 9.5-10: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for fluroxypyr-MHE for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of Kinvara in spring cereals

Group			**Fish acute**	**Fish pro-longed**	**Inver-teb. acute**	**Inverteb. prolonged**		**Algae**	**Macro-phytes**	**Sediment Dweller**
Test species			*O. mykiss*	*P. promelas*	*D. magna*	*D. magna*		*N. pellicul osa*	*L. gibba*	*C. riparius*
Endpoint			LC_{50}	NOEC	EC_{50}	NOEC		E_rC_{50}	ErC_{50}	NOEC (spiked water)
(µg/L)			>225	200	183	~~100~~	60.5	208	2310	130
AF			100	10	100	10		10	10	10
RAC (µg/L)			2.25	20	1.83	~~10~~	6.05	20.8	231	13
FOCUS Scenario PEC gl-max (µg/L)			**PEC/RAC**							
Step 1		4.645	**2.064**	0.232	**2.538**	~~0.465~~	0.768	0.223	0.020	0.159
Step 2	Northern Europe	1.987	0.883	0.099	**1.086**	~~0.199~~	0.328	0.096	0.009	0.068
	Southern Europe	1.987	0.883	0.099	**1.086**	~~0.199~~	0.328	0.096	0.009	0.068
D1	ditch	1.365	0.607	0.068	0.746	0.137		0.066	0.006	0.047
D1	stream	1.194	0.531	0.060	0.652	0.119		0.057	0.005	0.041
D3	ditch	1.349	0.600	0.067	0.737	~~0.135~~	0.223	0.065	0.006	0.046
D4	pond	0.047	0.021	0.002	0.026	~~0.005~~	0.008	0.002	0.000	0.002
D4	stream	1.104	0.491	0.055	0.603	0.110	0.182	0.053	0.005	0.038
D5	pond	0.047	0.021	0.002	0.026	~~0.005~~	0.008	0.002	0.000	0.002
D5	stream	1.072	0.476	0.054	0.586	~~0.107~~	0.177	0.052	0.005	0.037
R4	stream	0.891	0.396	0.045	0.487	0.089	0.147	0.043	0.004	0.030

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Table 9.5-11: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for fluroxypyr-MHE for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of Kinvara in grassland

Group			Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged		Algae	Macroph.	Sediment Dweller
Test species			*O. mykiss*	*P. promelas*	*D. magna*	*D. magna*		*N. pelliculosa*	*L. gibba*	*C. riparius*
Endpoint			LC_{50}	NOEC	EC_{50}	NOEC		E_rC_{50}	ErC_{50}	NOEC (spiked water)
(µg/L)			>225	200	183	~~100~~	60.5	208	2310	130
AF			100	10	100	10		10	10	10
RAC (µg/L)			2.25	20	1.83	~~10~~	6.05	20.8	231	13
FOCUS Scenario PEC gl-max (µg/L)			PEC/RAC							
Step 1		4.649	**2.066**	0.232	**2.540**	~~0.465~~	0.768	0.224	0.020	0.159
Step 2	Northern Europe	1.987	0.883	0.099	**1.086**	~~0.199~~	0.328	0.096	0.009	0.068
	Southern Europe	1.987	0.883	0.099	**1.086**	~~0.199~~	0.328	0.096	0.009	0.068
Step 3- Spring Applications										
D1	Ditch	1.358	0.604	0.068	0.742	~~0.136~~		0.065	0.006	0.046
D1	Stream	1.109	0.493	0.055	0.606	~~0.111~~		0.053	0.005	0.038
D2	Ditch	1.367	0.608	0.068	0.747	~~0.137~~		0.066	0.006	0.047
D2	Stream	1.216	0.540	0.061	0.664	~~0.122~~		0.058	0.005	0.042
D3	Ditch	1.349	0.600	0.067	0.737	~~0.135~~	0.223	0.065	0.006	0.046
D4	Pond	0.047	0.021	0.002	0.026	~~0.005~~	0.008	0.002	0.000	0.002
D4	Stream	1.032	0.459	0.052	0.564	~~0.103~~	0.171	0.050	0.004	0.035
D5	Pond	0.047	0.021	0.002	0.026	~~0.005~~	0.008	0.002	0.000	0.002
D5	Stream	1.113	0.495	0.056	0.608	~~0.111~~	0.184	0.054	0.005	0.038
R2	Stream	0.705	0.313	0.035	0.385	~~0.071~~	0.117	0.034	0.003	0.024
R3	Stream	0.75	0.333	0.038	0.410	~~0.075~~	0.124	0.036	0.003	0.026
Step 3- Summer Applications										
D1	Ditch	1.365	0.607	0.068	0.746	~~0.137~~		0.066	0.006	0.047
D1	Stream	1.194	0.531	0.060	0.652	~~0.119~~		0.057	0.005	0.041
D2	Ditch	1.367	0.608	0.068	0.747	~~0.137~~		0.066	0.006	0.047
D2	Stream	1.216	0.540	0.061	0.664	~~0.122~~		0.058	0.005	0.042
D3	Ditch	1.356	0.603	0.068	0.741	~~0.136~~	0.224	0.065	0.006	0.046
D4	Pond	0.047	0.021	0.002	0.026	~~0.005~~	0.008	0.002	0.000	0.002
D4	Stream	1.167	0.519	0.058	0.638	~~0.117~~	0.193	0.056	0.005	0.040
D5	Pond	0.047	0.021	0.002	0.026	~~0.005~~	0.008	0.002	0.000	0.002
D5	Stream	1.259	0.560	0.063	0.688	~~0.126~~	0.208	0.061	0.005	0.043
R2	Stream	0.718	0.319	0.036	0.392	~~0.072~~	0.119	0.035	0.003	0.025
R3	Stream	0.755	0.336	0.038	0.413	~~0.076~~	0.125	0.036	0.003	0.026
Step 3- Fall Applications										
D1	Ditch	1.365	0.607	0.068	0.746	0.137		0.066	0.006	0.047
D1	Stream	1.194	0.531	0.060	0.652	0.119		0.057	0.005	0.041

D2	Ditch	1.367	0.608	0.068	0.747	0.137		0.066	0.006	0.047
D2	Stream	1.216	0.540	0.061	0.664	0.122		0.058	0.005	0.042
D3	Ditch	1.354	0.602	0.068	0.740	~~0.135~~	0.224	0.065	0.006	0.046
D4	Pond	0.047	0.021	0.002	0.026	~~0.005~~	0.008	0.002	0.000	0.002
D4	Stream	1.167	0.519	0.058	0.638	~~0.117~~	0.193	0.056	0.005	0.040
D5	Pond	0.047	0.021	0.002	0.026	~~0.005~~	0.008	0.002	0.000	0.002
D5	Stream	1.259	0.560	0.063	0.688	~~0.126~~	0.208	0.061	0.005	0.043
R2	Stream	0.718	0.319	0.036	0.392	~~0.072~~	0.119	0.035	0.003	0.025
R3	Stream	0.754	0.335	0.038	0.412	~~0.075~~	0.125	0.036	0.003	0.026

Table 9.5-12: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for fluroxypyr (acid) for each organism group based on FOCUS Steps 1 and 2 calculations for the use of Kinvara in cereals(winter and spring) and grassland

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Macrophytes**
Test species		*L. macrochirus*	*O. mykiss*	*D. magna*	*D. magna*	*N. pelliculosa*	*L. gibba*
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}	E_rC_{50}
(µg/L)		14300	100000	>100000	56000	26000	12300
AF		100	10	100	10	10	10
RAC (µg/L)		143	10000	1000	5600	2600	1230
FOCUS Scenario PEC gl-max (µg/L)		**PEC/RAC**					
Winter and spring cereal							
Step 1		93.197	0.652	0.009	0.093	0.017	0.036	0.076
Step 2	Northern Europe	7.691	0.054	0.001	0.008	0.001	0.003	0.006
	Southern Europe	14.166	0.099	0.001	0.014	0.003	0.005	0.012
Grassland							
Step 1		93.197	0.652	0.009	0.093	0.017	0.036	0.076
Step 2	Northern Europe	6.072	0.042	0.001	0.006	0.001	0.002	0.005
	Southern Europe	10.929	0.076	0.001	0.011	0.002	0.004	0.009

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Table 9.5-13: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for pyridinol for each organism group based on FOCUS Steps 1, 2 calculations for the use of Kinvara in cereals (spring and winter) and grassland

Group		Fish acute	Inverteb. acute	Algae	Macrophytes
Test species		*O. mykiss*	*D. magna*	*N. pelliculosa*	*L. gibba*
Endpoint		LC_{50}	EC_{50}	E_rC_{50}	ErC_{50}
(µg/L)		39000	>49000	640	>3200
AF		100	100	10	10
RAC (µg/L)		390	490	64	320
FOCUS Scenario PEC gl-max (µg/L)		**PEC/RAC**			
Spring and winter cereals					
Step 1		28.695	0.074	0.059	0.448	0.090
Step 2	Northern Europe	4.248	0.011	0.009	0.066	0.013
	Southern Europe	7.979	0.020	0.016	0.125	0.025
Grassland					
Step 1		28.695	0.074	0.059	0.448	0.090
Step 2	3.32	3.316	0.009	0.007	0.052	0.010
	6.12	6.114	0.016	0.012	0.096	0.019

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Table 9.5-14: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for methoxypyridine for each organism group based on FOCUS Steps 1and 2 calculations for the use of Kinvara in cereals (spring and winter) and grassland

Group		Algae	Macrophytes
Test species		*N. pelliculosa*	*L. gibba*
Endpoint		E_rC_{50}	E_rC_{50}
(µg/L)		1120	10600
AF		10	10
RAC (µg/L)		112	1060
FOCUS Scenario PEC gl-max (µg/L)		**PEC/RAC**	
Winter and spring cereals			
Step 1		11.085	0.099	0.010
Step 2	1.73	0.015	0.002	0.002
	3.46	0.031	0.003	0.004
Grassland				
Step 1		11.085	0.099	0.010
Step 2	1.297	0.012	0.001	0.001
	2.595	0.023	0.002	0.004

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Table 9.5-15: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for 3-CP for each organism group based on FOCUS Steps 1 and 2 calculations for the use of Kinvara in cereals (spring and winter) and grassland

Group		Fish acute	Inverteb. acute	Algae	Macrophytes
Test species		*O. mykiss*	*D. magna*	*P. subcapitata*	*L. gibba*
Endpoint		LC_{50}	EC_{50}	E_rC_{50}	ErC_{50}
(µg/L)		95100	7560	35000	>3200
AF		100	100	10	10
RAC (µg/L)		95.1	75.6	3500	320
FOCUS Scenario PEC gl-max (µg/L)		**PEC/RAC**			
Winter and spring cereals					
Step 1		7.965	0.084	0.105	0.002	0.025
Step 2	Northern Europe	1.207	0.013	0.016	0.000	0.004
	Southern Europe	2.223	0.023	0.029	0.001	0.007
Grassland					
Step 1		7.965	0.084	0.105	0.002	0.025
Step 2	Northern Europe	0.953	0.010	0.013	0.000	0.003
	Southern Europe	1.715	0.018	0.023	0.000	0.005

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Table 9.5-16: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for clopyralid for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of Kinvara in winter cereals

Group			Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Macrophytes
Test species			*O. mykiss*	*P. promelas*	*D. magna*	*D. magna*	*S. capricornutum*	*M. spicatum*
Endpoint			LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}	ErC_{50}
(µg/L)			>99900	10800	>99000	17000	30000	>3000
AF			100	10	100	10	10	10
RAC (µg/L)			999	1080	990	1700	3000	300
FOCUS Scenario PEC gl-max (µg/L)			**PEC/RAC**					
Step 1		28.72	0.029	0.027	0.029	0.017	0.010	0.096
Step 2	Northern Europe	3.787	0.004	0.004	0.004	0.002	0.001	0.013
	Southern Europe	6.805	0.007	0.006	0.007	0.004	0.002	0.023
D1	ditch	4.225	0.004	0.004	0.004	0.002	0.001	0.014
D1	stream	2.892	0.003	0.003	0.003	0.002	0.001	0.010
D2	ditch	11.24	0.011	0.010	0.011	0.007	0.004	0.037
D2	stream	7.476	0.007	0.007	0.008	0.004	0.002	0.025

D3	ditch	0.534	0.001	0.000	0.001	0.000	0.000	0.002
D4	pond	0.019	0.000	0.000	0.000	0.000	0.000	0.000
D4	stream	0.394	0.000	0.000	0.000	0.000	0.000	0.001
D5	pond	0.019	0.000	0.000	0.000	0.000	0.000	0.000
D5	stream	0.497	0.000	0.000	0.001	0.000	0.000	0.002
D6	ditch	0.535	0.001	0.000	0.001	0.000	0.000	0.002
R1	pond	0.023	0.000	0.000	0.000	0.000	0.000	0.000
R1	stream	0.796	0.001	0.001	0.001	0.000	0.000	0.003
R3	stream	0.493	0.000	0.000	0.000	0.000	0.000	0.002
R4	stream	0.347	0.000	0.000	0.000	0.000	0.000	0.001

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Table 9.5-17: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for clopyralid for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of Kinvara in spring cereals

Group			**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Macrophytes**
Test species			*O. mykiss*	*P. promelas*	*D. magna*	*D. magna*	*S. capricornutum*	*M. spicatum*
Endpoint			LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}	ErC_{50}
(µg/L)			>99900	10800	>99000	17000	30000	>3000
AF			100	10	100	10	10	10
RAC (µg/L)			999	1080	990	1700	3000	300
FOCUS Scenario PEC gl-max (µg/L)			**PEC/RAC**					
Step 1		28.72	0.029	0.027	0.029	0.017	0.010	0.096
Step 2	Northern Europe	3.787	0.004	0.004	0.004	0.002	0.001	0.013
	Southern Europe	6.805	0.007	0.006	0.007	0.004	0.002	0.023
D1	ditch	7.572	0.008	0.007	0.008	0.004	0.003	0.025
D1	stream	5.611	0.006	0.005	0.006	0.003	0.002	0.019
D3	ditch	6.385	0.006	0.006	0.006	0.004	0.002	0.021
D4	pond	0.261	0.000	0.000	0.000	0.000	0.000	0.001
D4	stream	5.206	0.005	0.005	0.005	0.003	0.002	0.017
D5	pond	0.221	0.000	0.000	0.000	0.000	0.000	0.001
D5	stream	5.036	0.005	0.005	0.005	0.003	0.002	0.017
R4	stream	15.47	0.015	0.014	0.016	0.009	0.005	0.052

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Table 9.5-18: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for clopyralid for each organism group based on FOCUS Steps 1 and 2 calculations for the use of Kinvara in grassland

Group			Fish acute	Inverteb. acute	Algae	Macrophytes
Test species			*O. mykiss*	*D. magna*	*P. subcapitata*	*L. gibba*
Endpoint			LC_{50}	EC_{50}	E_rC_{50}	ErC_{50}
(µg/L)			95100	7560	35000	>3200
AF			100	100	10	10
RAC (µg/L)			95.1	75.6	3500	320
FOCUS Scenario PEC gl-max (µg/L)			PEC/RAC			
Step 1		28.72	0.029	0.027	0.029	0.017
Step 2	Northern Europe	3.033	0.003	0.003	0.003	0.002
	Southern Europe	5.26	0.005	0.005	0.005	0.003

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Mixture toxicity assessment

The mixture toxicity assessment is conducted using data on MCPA, clopyralid and fluroxypyr-meptyl (fluroxypyr-MHE) toxicity and PEC values. This is considered worst-case as fluroxypyr-meptyl hydrolyses to fluroxypyr-acid, which is less toxic to aquatic organisms.

The toxicity driver assessment is conducted using the proportion of active substances in the formulated product. Based on the toxicity driver assessment, fluroxypyr-MHE drives the toxicity for fish, Daphnids and algae, whereas MCPA drives the toxicity for aquatic macrophytes (*Lemna sp*. and/or *Myriophyllum spicatum*) (see Table 9.5-19).

With regard to the mixture risk assessment EFSA states that if the toxicity of the mixture is largely explained by the toxicity of a single active substance, a sufficient protection level might be achieved by simply basing the RA on the toxicity data for that single 'driver'. Hence the risk assessments for the formulation product based on single-substance toxicity data for fluroxypyr-MHE and MCPA are sufficient given that they are identified as the drivers of the mixture toxicity.

Table 9.5-19. Toxicity driver assessment for Kinvara formulation

Acute fish			
Test Substance	96h LC_{50} (mg a.i/L)	"toxic unit"	"contribution to toxicity" %
MCPA	50	0.014	1.43
Fluroxypyr-MHE	0.225	0.978	98.50
Clopyralid	99.9	0.001	0.07
Total a.s.'s		0.993	100.00
Chronic fish			
Test Substance	NOEC (mg a.i/L)	"toxic unit"	"contribution to toxicity" %
MCPA	15	0.047	4.10
Fluroxypyr-MHE	0.2	1.100	95.34
Clopyralid	10.8	0.006	0.56
Total a.s.'s		1.154	100.00
Acute Daphnia			
	EC_{50} (mg/l)	"toxic unit"	"contribution to toxicity" %
MCPA	190	0.004	0.31
Fluroxypyr-MHE	0.183	1.202	99.63

Clopyralid	99	0.001	0.06
Total a.s.'s		1.207	100.00
Chronic Daphnia			
Test Substance	NOEC (mg a.i/L)	"toxic unit"	"contribution to toxicity" %
MCPA	50	0.014	~~0.64~~ 1.04
Fluroxypyr-MHE	~~0.1~~ 0.0605	~~2.20~~ 1.33	~~99.17~~ 98.66
Clopyralid	17	0.004	~~0.19~~ 0.30
total a.s.'s		~~2.218~~ 1.348	100.00
Algae			
	EC_{50} (mg/l)		
MCPA	32.9	0.022	2.00
Fluroxypyr-MHE	0.208	1.058	97.79
Clopyralid	30	0.003	0.22
Total a.s.'s		1.082	100.00
Aquatic macrophytes			
	EC_{50} (mg/l)	"toxic unit"	"contribution to toxicity" %
MCPA	0.152	4.671	97.52
Fluroxypyr-MHE	2.31	0.095	1.99
Clopyralid	3	0.023	0.49
Total a.s.'s		4.790	100.00

Toxicity studies on the effects of the formulated product on *Daphnia magna*, *Lemna gibba* and *Pseudokirchneriella subcapitata* and *Anabaena flos-aquae* are available. Acceptable risk is demonstrated in Table 9.5-20.

Table 9.5-20. Risk assessment based on toxicity studies on the formulated product and formulation PEC_{sw} values

Species	**EC_{50} µg f.p./L**	**RAC µg f.p./L**	**PEC_{sw} (µg f.p/L)**	**PEC_{sw} > RAC ?**
Daphnia magna	9800	980	6.505	No
Lemna gibba	3830	383	6.505	No
Myriophyllum sp.	221	22.1	6.505	No
Anabaena flos-aquae	3790	379	6.505	No

9.5.3 Overall conclusions

Active substances and formulation risk assessments result in PEC/RAC values above the trigger value of 1 for all scenario using either step 1, Step 2 or Step 3 FOCUS PECsw. MCPA and the metabolite PCOC result in PEC/RAC >1 for D1 and D2 scenarios that are however not relevant for the Central Zone. Therefore it can be concluded that applications of Kinvara to cereals and grassland do not pose a risk to aquatic organisms.

9.6 Effects on bees (KCP 10.3.1)

zRMS Comments:	The submitted risk assessment based on SANCO guidance, 2002, was accepted. The studies for acute and chronic toxicity to honeybees were submitted and accepted. The toxicity studies for bumblebees were submitted and accepted.

	The EU agreed endpoints for active substances and accepted endpoints from submitted studies for formulation were used in acute risk assessment. The hazard quotients are below the trigger values considering SANCO guidance indicating that the active substance and formulation pose an acceptable risk to bees. Therefore, an acceptable risk to bees is expected from the application of Kinvara. It is recommended to submit the risk assessment for bees using the new (not agreed up to now), EFSA, 2013 guidance. Some cMS require risk assessment provided in accordance with the mentioned approach. The submitted risk assessment according to EFSA, 2013 was accepted and relevant mitigation measures (precautionary statements) will be considered at cMS level. According to Commission regulation (EU) No 284/2013, point 10.3.1. (Effects on bees): The Applicant should provide chronic test on bees and evaluation of effects on honey bee development with formulated product. The chronic studies were not performed, therefore, the deficiencies need to be fulfilled by the entry into force of the revised EFSA bee guideline. Concerned Member States must decide on the consideration of data requirements on national level.

9.6.1 Toxicity data

Toxicity data on all the active substances have been reviewed at the EU level and agreed endpoints are available in the respective peer reviews. Full details of these studies are provided in the respective EU DAR and related documents. Toxicity data on the Kinvara formulation has not been previously assessed and new studies have been provided to cover the current data requirements. The details of these studies have been provided in Appendix 2. The chronic and non-lethal toxicity of Kinvara to bees was determined using a higher-tier field scale test.

Table 9.6-1: Endpoints and effect values relevant for the risk assessment for bees

Species	Substance	Exposure system	Results	Reference
Apis mellifera	MCPA	Acute oral	LD_{50}> 200 µg/bee	EC (2008)
Apis mellifera	MCPA	Acute contact	LD_{50}> 200 µg/bee	EC (2008)
Apis mellifera	Fluroxypyr-meptyl	Acute oral	LD_{50}> 100 µg/bee	EFSA (2011)
Apis mellifera	Fluroxypyr-meptyl	Acute contact	LD_{50}> 100 µg/bee	EFSA (2011)
Apis mellifera	Fluroxypyr-acid	Acute oral	LD_{50}=37.1 µg/bee	EFSA (2011)
Apis mellifera	Fluroxypyr-acid	Acute contact	LD_{50}> 180 µg/bee	EFSA (2011)
Apis mellifera	Clopyralid	Acute oral	LD_{50}> 100 µg/bee	EFSA (2018)
Apis mellifera	Clopyralid	Acute contact	LD_{50}>98.1 µg/bee	EFSA (2018)
Apis mellifera	Clopyralid	Chronic oral	LDD_{50}> 71.2 µg/bee/d	EFSA (2018)
Apis mellifera (larvae)	Clopyralid	Larval feeding chronic oral	LD_{10} = 12.5 µg/larva	EFSA (2018)
Apis mellifera	Kinvara	Acute oral	LD_{50}>210 µg/bee	Ehmke (2014a)
Apis mellifera	Kinvara	Acute contact	LD_{50}>200 µg/bee	Ehmke (2014a)
Apis mellifera	Kinvara	Chronic oral	LDD_{50}> 124 µg/bee/d	Wilkins (2019) FR/001855-10
Bombus terrestris	Kinvara	Acute Oral	LD_{50}>512.8 µg/bee	Wright (2019)
Bombus terrestris	Kinvara	Acute Contact	LD_{50}>1281 µg/bee	Wright (2019)
Higher-tier studies				

A higher-tier brood feeding test at the field level was conducted with Kinvara by Ehmke (2014b) according to Oomen and Steen (1992) testing guidelines. Kinvara was applied to fields at application rates of 2 and 3 L formulation/ha and ontogenesis of the bee brood was observed for 21 days. No statistical differences were found in the rate of mean egg termination rate between the control and the treatments indicating application rates of 3 L Kinvara/ha do not adversely affect honey bee colonies.

9.6.1.1 Justification for new endpoints

9.6.2 Risk assessment

9.6.2.1 First tier risk assessment for honeybees

SANCO Approach

The evaluation of the risk for bees was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002).

Table 9.6-2: First-tier assessment of the risk for bees due to the use of Kinvara in cereals and grassland

Intended use		Kinvara	
Active substance		MCPA	
Application rate (g/ha)		1 × 700	
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	200	700	3.5
Contact toxicity	200		3.5
Active substance		Fluroxypyr-meptyl	
Application rate (g/ha)		1 × 216.1	
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	100	216.1	2.2
Contact toxicity	100		2.2
Active substance		Fluroxypyr (acid)	
Application rate (g/ha)		1 × 150	
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	37.1	150	4.0
Contact toxicity	180		0.8
Active substance		Clopyralid	
Application rate (g/ha)		1 × 84	
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	100	84	0.84
Contact toxicity	98.1		0.86
Product		Kinvara (formulation test data)	
Application rate (L/ha)		1 × 3 L/ha	
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	210	3375*	16.1
Contact toxicity	200		16.9
Product		Kinvara (predicted toxicity from a.s. data)	

Application rate (g/ha)		1 × 3 L/ha		
Test design	**Q_H**			**$\sum Q_H$**
	MCPA	Fluroxypyr	Clopyralid	
Oral toxicity	3.5	4.0	0.84	8.34
Contact toxicity	3.5	0.8	0.86	5.16

Q_{HO}, Q_{HC}: Hazard quotients for oral and contact exposure. Q_H values shown in bold breach the relevant trigger.
* Based on a product density of 1.125 g/mL and a maximum application rate of 3.0 L/ha

Hazard quotients for oral and contact exposure for Kinvara and its active substance are all below the trigger value indicating a safe use can be concluded.

EFSA 2013 Approach

In addition to the risk assessments for bees using the SANCO approach, a risk assessment for the formulated product Kinvara was performed following the EFSA GD 2013 approach using the EFSA calculator tool (Bee-Tool v.3) and is presented in the tables below.

Table 9.6-3: EFSA 2013 honeybees risk assessment scheme for the risk of Kinvara

Product		**Kinvara**		
Application rate (g/ha)		**3375**		
Acute contact LD_{50} (µg/bee)		**200**		
Acute contact LD_{50} (µg/bee)		**210**		
Adult chronic LDD_{50} (µg/bee)		**124**		
Screening assessment				
Contact route of exposure				
	"calculation factor"	**HQ**	**Trigger**	**Risk indicator**
HB	1	16.9	42	OK
Oral route of exposure				
	"calculation factor" (Ef x SV)	**ETR**	**Trigger**	**Risk indicator**
HB - acute	7.6	0.12	0.2	OK
HB - chronic	7.6	0.07	0.207	!
1st tier Oral Assessment – HB chronic				
category	**scenario**	**BBCH**	**Honeybees**	
			ETR	**trigger**
acute	treated crop	10 - 29	0.02	0.03
acute	treated crop	30 - 39	0.02	0.03
acute	weeds	10 - 29	**0.06**	0.03
acute	weeds	30 - 39	0.03	0.03
acute	field margin	10 - 29	<0.001	0.03
acute	field margin	30 - 39	<0.001	0.03
acute	adjacent crop	10 - 29	<0.001	0.03
acute	adjacent crop	30 - 39	<0.001	0.03
acute	next crop	10 - 29	0.01	0.03
acute	next crop	30 - 39	0.01	0.03

* Based on a product density of 1.125 g/mL and a maximum application rate of 3.0 L/ha

A safe use for the product Kinvara for the acute risk to honeybees is concluded at the screening tier, while for the chronic risk a safe use is concluded for most of the scenarios assessed in the first tier oral assessment. Only for weeds at crop growth stage 10-29 the ETR value of 0.06 exceeds the trigger value of 0.03. Therefore the following SPe8 is requested: *Do not apply when flowering weeds are present./Remove weeds*

before flowering.

9.6.2.2 Higher-tier risk assessment for bees (tunnel test, field studies)

A higher-tier brood feeding test at the field level was conducted with Kinvara by Ehmke (2014b) according the Oomen and Steen (1992) testing guidelines. Kinvara was applied to fields at application rates of 2 and 3 L formulation/ha and ontogenesis of the bee brood was observed for 21 days. No statistical differences were found in the rate of mean egg termination between the control and the treatments indicating application rates of 3 L Kinvara/ha do not adversely affect honey bee colonies.

9.6.3 Effects on bumble bees

The evaluation of the acute oral and contact toxicity risk for bees was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002) and is presented in Table 9.6-4.

Table 9.6-4: First-tier assessment of the acute oral and contact toxicity risk for bumblebees due to the use of Kinvara in cereals

Intended use	Applications to cereals		
Product	Kinvara (formulation test data)		
Application rate (L/ha)	1 × 3 L/ha		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	512.8	3375*	6.58
Contact toxicity	1281		2.63

* Based on a product density of 1.125 g/mL and a maximum application rate of 3.0 L/ha

Hazard quotients for bumblebees oral and contact exposure for Kinvara are below the trigger value indicating a safe use can be concluded.

EFSA 2013 Approach

In addition to the risk assessments for bumblebees using the SANCO approach, a risk assessment for the formulated product Kinvara was performed following the EFSA GD 2013 approach using the EFSA calculator tool (Bee-Tool v.3) and is presented in the tables below.

Table 9.6-5: EFSA 2013 bumblebees risk assessment scheme for the acute toxicity risk of Kinvara

Product		**Kinvara**		
Application rate (g/ha)		**3375**		
Acute contact LD_{50} (µg/bee)		**1281**		
Acute contact LD_{50} (µg/bee)		**512.8**		
Screening assessment				
Contact route of exposure				
	"calculation factor"	**HQ**	**Trigger**	**Risk indicator**
BB	1	2.6	7	OK
Oral route of exposure				
	"calculation factor" (Ef x SV)	**ETR**	**Trigger**	**Risk indicator**
BB - acute	11.2	0.07	0.036	!
1st tier Oral Assessment – BB acute				

category	scenario	BBCH	Bumblebee	
			ETR	trigger
acute	treated crop	10 - 29	0.02	0.036
acute	treated crop	30 - 39	0.02	0.036
acute	weeds	10 - 29	**0.04**	0.036
acute	weeds	30 - 39	0.02	0.036
acute	field margin	10 - 29	<0.001	0.036
acute	field margin	30 - 39	<0.001	0.036
acute	adjacent crop	10 - 29	<0.001	0.036
acute	adjacent crop	30 - 39	<0.001	0.036
acute	next crop	10 - 29	0.01	0.036
acute	next crop	30 - 39	0.01	0.036

A safe use for the product Kinvara for the chronic risk for adult bumblebees is concluded for most of the scenarios assessed in the first tier oral assessment. Only for weeds at crop growth stage 10-29 the ETR value of 0.04 exceeds the trigger value of 0.03. Therefore the following SPe8 is requested: *Do not apply when flowering weeds are present./Remove weeds before flowering.*

9.6.4 Effects on solitary bees

No required

9.6.5 Overall conclusions

Neither the Kinvara formulation, nor any of the relevant active substance pose an acute risk to honeybees and bumblebees according to the standard acute risk assessment. A higher-tier field study on Kinvara indicates the formulation also does not pose a risk to honeybee larvae.

Following the EFSA 2013 approach, a safe use for the product Kinvara can be concluded for most scenarios, only for weeds at crop growth stage 10-29 the ETR value exceeds the trigger value thus the following SPe8 is requested: *Do not apply when flowering weeds are present./Remove weeds before flowering.*

9.7 Effects on arthropods other than bees (KCP 10.3.2)

zRMS Comments:	The submitted risk assessment based on the "Guidance Document on Terrestrial Ecotoxicology", 2002, was accepted. New studies for formulation at Tier 1 and Tier 2 were submitted and accepted. The risk assessment for each active substance is based on endpoints accepted at the EU level The hazard quotients for each active substance are below the trigger value (HQ ≤ 2) at tier 1, the refinement at higher tier is not required. **Formulation**. The following endpoints at Tier 1 were used in risk assessment: • *Typhlodromus pyri:* LR_{50} = 1567 mL/ha • *Aphidius rhopalosiphi: :* LR_{50} = 769 mL/ha As the hazard quotients are higher than trigger value (HQ ≤ 2) at tier 1, the refinement at higher tier was provided. indicating that the formulation poses an acceptable risk to arthropods other than bees.

	Therefore, an acceptable risk to arthropods other than bees is expected if the application of Kinvara is in accordance with intended uses.

9.7.1 Toxicity data

Laboratory studies on the toxicity of MCPA, fluroxypyr and clopyralid to non-target arthropods are available within their respective EU reviews. A summary of the agreed endpoint values are provided in Table 9.7-1. Studies on the toxicity of Kinvara were not considered during the EU active substance reviews and new studies have been supplied with this application. The new studies have been summarized in Appendix 2 and the endpoints have been provided in Table 9.7-1.

Table 9.7-1: Endpoints and effect values relevant for the risk assessment for non-target arthropods

Species	Exposure Sytem	Substance	LR_{50} (g a.s./ha)	Reference
T. pyri	Mortality (glass plate)	EF-1136 (clopyralid)	>200	EFSA (2018)
T. pyri	Mortality (extended study)	GF-1374 (clopyralid)	2000	EFSA (2018)
A. rhopalosiphi	Mortality (glass plate)	EF-1136 (clopyralid)	>200	EFSA (2018)
A. rhopalosiphi	Mortality (extended study)	GF-1374 (clopyralid)	>2 (L/ha)	EFSA (2018)
C. carnea	Mortality (glass plate)	EF-1136 (clopyralid)	>200	EFSA (2018)
C. carnea	Mortality (extended study)	GF-1374 (clopyralid)	> 2 (L/ha)	EFSA (2018)
P. cupreus	Mortality (spray application)	EF-1136 (clopyralid)	>120	EFSA (2018)
A. bilinetata	Mortality (spray application)	Lontreal 100 (clopyralid)	>120	EFSA (2018)
Pardosa sp.	Mortality (spray application)	Lontreal 100 (clopyralid)	>120	EFSA (2018)
T. pyri	Mortality (leaf discs)	EF 1502 (fluroxypyr)	>2000	EFSA (2011)
A. rhopalosiphi	Mortality (residues on dried barley)	EF 1502 (fluroxypyr)	>2000	EFSA (2011)
C. carnea	Mortality (glass plate)	EF 1502 (fluroxypyr)	>2000	EFSA (2011)
T. pyri	Mortality	MCPA (formulation not known)	>2 (L/ha)	EC (2008)
A. rhopalosiphi	Mortality (residues on dried barley)	MCPA (formulation not known)	>25	EC (2008)
C. carnea	Mortality (spray application)	MCPA (formulation not known)	>4 (L/ha)	EC (2008)
T. pyri	Mortality (glass plate)	Kinvara	1567 (ml/ha)	Moll (2014)
A. rhopalosiphi	Mortality (glass plate)	Kinvara	769 (ml/ha)	Moll (2014)
A. rhopalosiphi	Mortality (extended study)	Kinvara	>3000 (mL/ha)	Moll (2017)
C. septempunctata	Mortality (extended study)	Kinvara	>3000 (mL/ha)	Vaughan (2017)

9.7.1.1 Justification for new endpoints

No deviations have been made from the EU agreed endpoints for the active substances. New endpoints have been proposed for the formulation based on new studies provided with this application.

9.7.2 Risk assessment

The evaluation of the risk for non-target arthropods was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002), and in consideration of the recommendations of the guidance document ESCORT 2.

9.7.2.1 Risk assessment for in-field exposure

A risk assessment is provided below for in-field exposure to NTAs from the individual active substances, the theoretical combined risk and the actual formulation.

Table 9.7-2: First- and higher-tier assessment of the in-field risk for non-target arthropods due to the use of Kinvara

Intended use	**Kinvara**		
Active substance	**MCPA**		
Application rate (g/ha)	1 × 700		
Test species	LR_{50} (lab.) (g/ha)	$PER_{in\text{-}field\ (g/ha)}$	$HQ_{in\text{-}field}$, (criterion: HQ ≤ 2)
Tier I			
Typhlodromus pyri	>2000	700	0.38
Pardosa sp	>2000		0.38
Chrysoperla carnea	>4000		0.20
Higher-tier			$HQ_{in\text{-}field,}$ (criterion: HQ ≤ 1)
Aphidius rhopalosiphi	>2100	750	0.36
Active substance	**Fluroxypyr-MHE**		
Application rate (g/ha)	1 × 216.1		
Test species	LR_{50} (lab.) (g/ha)	$PER_{in\text{-}field\ (g/ha)}$	$HQ_{in\text{-}field}$, (criterion: HQ ≤ 2)
Tier I			
Typhlodromus pyri	>2000	216.1	0.11
Pardosa spp	>2000		0.11
Chrysoperla carnea	>2000		0.11
Active substance	**Clopyralid**		
Application rate (g/ha)	1 × 84		
Test species	LR_{50} (lab.) (g/ha)	$PER_{in\text{-}field\ (g/ha)}$	$HQ_{in\text{-}field,}$ (criterion: HQ ≤ 2)
Tier I			
Typhlodromus pyri	>200	84	0.42
Aphidius rhopalosiphi	>200		0.42
Chrysoperla carnea	>200		0.42
Combined Risk Quotients			
Tier I			
Typhlodromus pyri			0.89
Aphidius rhopalosiphi			0.87
Chrysoperla carnea			0.71
Formulation	**Kinvara**		
Application rate (L/ha)	1 × 3		
Test species	LR_{50} (lab.) (mL/ha)	$PER_{in\text{-}field\ (mL/ha)}$	$HQ_{in\text{-}field}$, (criterion: HQ ≤ 2)
Tier I			
Typhlodromus pyri	1567	3000	**1.9**
Aphidius rhopalosiphi	769		**3.9**
Higher-tier			HQin-field, (criterion: HQ ≤ 1)
Aphidius rhopalosiphi	>3000	3000	1.0
Coccinella. septempunctata	>3000		1.0

9.7.2.2 Risk assessment for off-field exposure

To achieve a concise risk assessment, the risk envelope approach is applied. As demonstrated in the previous section, the highest risk to NTAs is associated with the Tier 1 data for the Kinvara formulation. Therefore the off-field assessment at Tier 1 of the formulation covers the risk of the individual actives and their combined toxicity.

Table 9.7-3: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of Kinvara

Intended use	Kinvara				
Active substance/product	Kinvara				
Application rate (L/ha)	1 x 3 L/ha ≡ 3375 g/ha				
MAF	1				
vdf	10 (Tier 1)				
Test species	**LR_{50} (lab.)**	**Drift**	**$PER_{off\text{-}field}$**	**CF**	**$HQ_{off\text{-}field}$**
Tier I	**(g/ha)**	**rate**	**(g/ha)**		**criterion: HQ ≤ 2**
Typhlodromus pyri	1567	2.77	9.35	10	0.06
Aphidius rhopalosiphi	769				0.12

MAF: Multiple application factor; vdf: Vegetation distribution factor; (corr.) PER: (corrected) Predicted environmental rate; CF: Correction factor; HQ: Hazard quotient. Criteria values shown in bold breach the relevant trigger.

* If an LR_{50} or ER_{50} from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50 % effect.

9.7.2.3 Additional higher-tier risk assessment

Not relevant.

9.7.2.4 Risk mitigation measures

No risk mitigation needed.

9.7.3 Overall conclusions

Applications of Kinvara to winter and spring cereals and grassland do not pose a risk to non-target arthropods either in-field or off-field.

9.8 Effects on non-target soil meso- and macrofauna (KCP 10.4)

zRMS Comments:	The submitted risk assessment was accepted. The risk assessment for formulation is based on accepted endpoints. The max PECs values for active substances, their metabolites and formulation (see Section 8. Fate and behavior) were used for acute and long-term risk assessment Since risk assessment for non-target soil meso- and macrofauna (earthworm and other organisms) is not acceptable at Tier 1, then further, higher-tier risk assessment was required and submitted. Based on confirmatory data, *H. aculeifer* reproduction study considering exposure to methoxypyridine (metabolite of fluroxypyr), the justification was accepted. An acceptable risk to non-target soil organisms meso- and macrofauna is expected if the Kinvara is used in accordance with proposed uses.

9.8.1 Toxicity data

Studies on the toxicity to earthworms and other non-target soil organisms (meso- and macrofauna) have been carried out with MCPA, fluroxypyr and clopyralid. During the EFSA (2011) peer review of fluroxypyr it was concluded additional testing was required for the chronic risk of methoxypyridine. The requested tests were subsequently submitted as confirmatory data including a higher tier exposure study. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on earthworms and other non-target soil organisms (meso- and macrofauna) of Kinvara were not evaluated as part of the EU active substance renewal process and have been provided below.

Table 9.8-1: Endpoints and effect values relevant for the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna)

Species	Substance	Exposure System	Results	Reference
Eisenia fetida	MCPA	14d	LC_{50} =325 mg/kg dw	EC (2008)
Eisenia fetida	Fluroxypyr-meptyl	14 d, 10 % peat content	LC50>1000 mg/kg dw $LC50_{corr}$ >500 mg/kg dw*	EFSA (2011) including studies accepted in the confirmatory data
Eisenia fetida	Fluroxypyr-meptyl	56 d, 10 % peat content	NOEC=3.92 mg/kg dw $NOEC_{corr}$ =**1.96 mg/kg dw***	
Eisenia fetida	Fluroxypyr (acid)	14d	LC_{50} =64.8 mg/kg dw	
Eisenia fetida	Fluroxypyr (acid)	56 d	NOEC =**3.05 mg/kg dw**	
Eisenia fetida	Pyridinol	14d	LC_{50} =79 mg/kg dw	
Eisenia fetida	Pyridinol	56 d	NOEC =**0.720 mg/kg dw**	
Eisenia fetida	Methoxypyridine	14d	LC50>313 mg/kg dw $LC50_{corr}$ >156.5 mg/kg dw*	
Eisenia fetida	Methoxypyridine	56 d	NOEC =1.17 mg/kg dw **$NOEC_{corr}$ = 0.585 mg/kg dw**	
Folsomia candida	Methoxypyridine	28 d	NOEC =1.0 mg/kg dw	
Hypoaspsi aculeifer	Methoxypyridine	14 d	NOEC =0.25mg/kg dw **$NOEC_{corr}$ =0.125mg/kg dw**	
Eisenia fetida	Clopyralid	28 d, 10% peat content	**NOEC =1.97 mg/kg dw**	EFSA (2018)
Eisenia fetida	Kinvara	56d, 10% peat content	NOEC >184 mg/kg dw **$NOEC_{corr}$ =92 mg/kg dw***	Luhrs (2014)
Hypoaspsi aculeifer	Kinvara	14 d, 5% peat content	NOEC >1000 mg/kg dw	Straube (2017a)
Folsomia candida	Kinvara	28 d. 5% peat content	NOEC = 250 mg/kg dw	Straube (2018b)

			$NOEC_{corr}$ = 125 mg/kg dw*	
Higher Tier- A higher tier *H. aculeifer* reproduction study considering exposure to methoxypyridine was sumitted and accepted as confirmatory data to EFSA (2011). The study, Luhrs, (2012) utilised the natural LUFA 2.4 soils for the exposure study rather than the artificial soil media used in the standard study guidelines. On study derived an effects endpoint of NOEC=2.5 mg/ kg dws which was agreed as an acceptable means to refine the soil risk assessment of methoxypyridine.				

* Corrected value derived by dividing the endpoint by a factor of 2 in accordance with the EPPO earthworm scheme 2002.

9.8.1.1 Justification for new endpoints

New studies have been submitted on the toxicity of the formulation, but all active substance data are taken from the agreed list of endpoints.

9.8.2 Risk assessment

The evaluation of the risk for earthworms and other non-target soil organisms (meso- and macrofauna) was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev 2 (final), October 17, 2002).

9.8.2.1 First-tier risk assessment

The relevant PEC_{soil} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate). According to the assessment of environmental-fate data, multi-annual accumulation in soil does not need to be considered for any of the active substances but does need to be considered for pyridinol and methoxypyridine. The risk assessment can be resolved at Tier 1 for all substances excluding methoxypyridine.

Table 9.8-2: First-tier assessment of the acute and chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of Kinvara

Intended use	Kinvara		
~~Acute effects on earthworms~~			
~~Product/active substance~~	**~~LC_{50}~~ ~~(mg/kg dw)~~**	**~~PEC_{soil}~~ ~~(maximum considering accumulation)~~ ~~(mg/kg dw)~~**	**~~TER_a~~ ~~(criterion TER ≥ 10)~~**
~~MCPA~~	~~325~~	~~0.747~~	~~435~~
~~Fluroxypyr-meptyl~~	~~>500~~	~~0.230~~	~~2174~~
~~Fluroxypyr (acid)~~	~~64.8~~	~~0.160~~	~~405~~
~~Pyridinol~~	~~79~~	~~0.032~~	~~2469~~
~~Methoxypyridine (corr)~~	~~156.5~~	~~0.190~~	~~824~~
Chronic effects on earthworms			
Product/active substance	**NOEC (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
Fluroxypyr-meptyl	1.96	0.230	8.5
Fluroxypyr (acid)	3.05	0.160	19.1
Pyridinol	0.72	0.032	22.5
Methoxypyridine	~~1.17~~ 0.585	0.190	~~6.2~~ **3.07**
Clopyralid	1.97	0.087	22.6
Formulation	>~~184~~ 92	3.6	~~51.1~~ 25.56
Chronic effects on other soil macro- and mesofauna			
Product/active substance	**NOEC**	**PEC_{soil}**	**TER_{lt}**

	(mg/kg dw)	(mg/kg dw)	(criterion TER ≥ 5)
Methoxypyridine (*H. aculeifer*)	~~0.25~~ 0.125	0.190	**~~1.3~~ 0.66**
Methoxypyridine (*H. aculeifer*)	~~0.25~~ 0.125	0.128 (refined, ESCAPE v2.0)	**~~2.0~~ 0.98**
Kinvara	~~250~~ 125	3.6	~~64.9~~ 34.72

TER values shown in bold fall below the relevant trigger.

9.8.2.2 Higher-tier risk assessment

The chronic exposure assessment of methoxypyridine to soil organisms can be refined based on a higher tier study submitted and accepted as confirmatory information to EFSA (2011). The Tier-I critical endpoint was set by a reproductive endpoint for *H. aculeifer*. This Tier-I study was conducted using an artificial soil medium as is recommended in the guidelines. A higher-tier study by Lührs (2012) refined the standard approach through the implementation of a real soil as the test medium and found the reproductive endpoint for *H. aculeifer* is NOEC>2.5 mg/kg dws. This refined endpoint is greater than the earthworm reproductive endpoint (i.e. 1.17 mg/kg dw) indicating the risk of methoxypyridine to soil organism can be covered by the Tier 1 chronic earthworm assessment.

9.8.3 Overall conclusions

The risk MCPA, fluroxypyr-MHE, fluroxypyr (acid), pyridinol, clopyralid and the Kinvara formulation to soil organisms can all be resolved at Tier-1. A previously accepted higher-tier study on methoxypyridine allows the assessment of that substance to be resolved without the need for further testing.

9.9 Effects on soil microbial activity (KCP 10.5)

zRMS Comments:	The submitted information and data were accepted. A new study conducted on formulation was submitted and accepted. The formulation Kinvara poses no adverse effect on nitrate formation in soil. An acceptable risk to soil microorganisms is expected if the application of the Kinvara is in accordance with proposed pattern use.

9.9.1 Toxicity data

Effects soil microorganisms studies have been carried out with MCPA, fluroxypyr, clopyralid and all relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents. A new study has been conducted on the formulation and details are provided below.

Table 9.9-1: Endpoints and effect values relevant for the risk assessment for soil microorganisms

Active substance	Test design	EU agreed endpoints	Reference
MCPA	C	No effect (28 d) at 2.67 mg as/kg dry wt soil corresponding to the highest recommended rate of application (2kg/ha) and at 26.7 mg as/kg dry wt soil corresponding to 10 times the highest recommended rate of application (20 kg as /ha). (Respiration). <25% of deviation at 1.224 kg as/ha (28 d).	EC (2008)
	N	No effect (28 d) at 2.67 mg as/kg dry wt. soil corresponding to the highest recommended rate of application (2kg/ha) and at 26.7 mg as/kg dry wt soil corresponding to 10 times the highest recommended rate of application (20 kg as /ha). (Ammonification and nitrification) <25% of deviation at 1.224 kg as/ha (28 d).	EC (2008)
EF-1502 (representative Fluroxypyr formulation)	C	<25% effect at 12.6 mg f.p. /kg dry soil <25% effect at 3.25* mg a.s /kg dry soil	EFSA (2011)
	N	<25% effect at 7.36 mg f.p. /kg dry soil <25% effect at 1.9* mg a.s. /kg dry soil	Addendum: Confirmatory Information (Volume 3 - B.5, B.7, B.8, B.9) December 2014 [Feil 2009, Study No. 090432 Fluroxypyr]
Pyridinol	C	<25% effect at 0.441 mg/kg dry soil	EFSA (2011)
	N	<25% effect at 0.240 mg/kg dry soil	Addendum: Confirmatory Information (Volume 3 - B.5, B.7, B.8, B.9) December 2014 [Feil 2009, Study No. 090430 Fluroxypyr]
Methoxypyridine	C	<25% effect at 0.132 mg/kg dry soil <25% effect at 0.66 mg/kg dry soil	EFSA (2011)
	N	<25% effect at 0.132 mg/kg dry soil <25% effect at 0.66 mg/kg dry soil	EFSA (2011)
Clopyralid	C	<25% effect at 13.9 mg/kg dry soil	EFSA (2018)
	N	<25% effect at 13.9 mg/kg dry soil	
Kinvara	C	<25% effect at 22.4 mg/kg dry soil	Hammesfahr, 2017. Project No.: 122621080
	N	<25% effect at 22.4 mg/kg dry soil	

* Active substance endpoint derived from the 25.8% fluroxypyr-meptyl content in the formulation

9.9.1.1 Justification for new endpoints

Not Required.

9.9.2 Risk assessment

The evaluation of the risk for soil microorganisms was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev 2 (final), October 17, 2002).

The relevant PEC_{soil} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.2, Table 8.7-3 and were already used in the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) (see 9.8).

Table 9.9-2: Assessment of the risk for effects on soil micro-organisms due to the use of Kinvara

Intended use	Kinvara		
N-mineralisation			
Product/active substance	**Max. conc. with effects ≤ 25 % (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**Risk acceptable?**
MCPA	1.224	0.747	yes
Fluroxypyr-meptyl	1.9	0.230	yes
Clopyralid	13.9	0.087	yes
Pyridinol	0.24	0.032	yes
Methoxypyridine	0.66	0.190	yes
Kinvara	22.4	3.6	yes
C-mineralisation			
Product/active substance	**Max. conc. with effects ≤ 25 % (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**Risk acceptable?**
MCPA	1.224	0.747	yes
Fluroxypyr-meptyl	3.25	0.230	yes
Clopyralid	13.9	0.087	yes
Pyridinol	0.441	0.032	yes
Methoxypyridine	0.66	0.190	yes
Kinvara	22.4	3.6	yes

9.9.3 Overall conclusions

The proposed applications of Kinvara results in an acceptable risk assessment for soil microorganisms.

9.10 Effects on non-target terrestrial plants (KCP 10.6)

zRMS Comments:	Toxicity effects of herbicide Kinvara on the vegetative vigor and seedling emergence were submitted. The following endpoints for formulation were accepted at zonal level: ER_{50} = 25.7 mL/ha (seedling emergence, cabbage); ER_{50} = 32.9 mL/ha (vegetative vigour, lettuce); and were used in risk assessment. An acceptable risk to non-target terrestrial plants is expected if the following mitigation measures are applied: • 5 m with 75% drift reducing nozzle or • 10 m with 50% drift reducing nozzle, or • 20 m.

9.10.1 Toxicity data

Studies on the toxicity to non-target terrestrial plants were not carried out as part of the EU approval of MCPA. As MCPA is the major component within Kinvara and has a herbicidal mode of action which will be toxic to non-target plants it was determined the risk assessment could only be based on direct formulation testing. That is individual assessment of fluroxypyr and/or clopyralid will not be informative about the overall risk of the Kinvara formulation. This new formulation based assessment has been provided below.

Table 9.10-1: Endpoints and effect values relevant for the risk assessment for non-target terrestrial plants

Species	Clade	Substance	Exposure System	Results (ER_{50} mL/ha)	Reference
Lolium perenne	m	Kinvara	21 d Seedling emergence	n.d.*	Bützler &Münz (2014a)
Avena sativa	m			2685	
Allium cepa	m			61.2	
Brassica oleracea	d			**25.7**	
Vicia faba	d			612	
Lactuca sativa	d			69.3	
Lycopersicon esculentum	d			234	
Cucumis sativus	d			300	
Daucus carota	d			214	
Gossypium herbaceum	d			1416	
Lolium perenne	m	Kinvara	21 d Vegetative vigour	n.d.	Bützler &Münz (2014b)
Avena sativa	m			n.d	
Allium cepa	m			1748	
Brassica oleracea	d			68.9	
Vicia faba	d			149	
Lactuca sativa	d			**32.9**	
Lycopersicon esculentum	d			78.2	
Cucumis sativus	d			1394	
Daucus carota	d			107	
Gossypium herbaceum	d			185	

m: monocotyledonous; d: dicotyledonous
* n.d. not determined due to mathematical reasons or inappropriate data

9.10.1.1 Justification for new endpoints

Not relevant.

9.10.2 Risk assessment

9.10.2.1 Tier-1 risk assessment (based screening data)

Not relevant.

9.10.2.2 Tier-2 risk assessment (based on dose-response data)

The risk assessment is based on the "Guidance Document on Terrestrial Ecotoxicology", (SANCO/10329/2002 rev.2 final, 2002). It is restricted to off-field situations, as non-target plants are non-crop plants located outside the treated area.

Table 9.10-2: Assessment of the risk for non-target plants due to the use of Kinvara

Intended use	Applications to cereals			
Active substance/product	Kinvara			
Application rate (L/ha)	1 × 3			
MAF	1			
Test species	**ER_{50} (~~g~~ mL/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (g/ha)**	**TER criterion: TER ≥ 5**
~~HC_5 from Vegetative vigour~~ *Brassica oleracea*	25.7	2.77	83.1	**0.31**

MAF: Multiple application factor; PER: Predicted environmental rate; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

9.10.2.3 Higher-tier risk assessment

Not relevant.

9.10.2.4 Risk mitigation measures

In order to reduce the off-field exposure, risk mitigation measures can be implemented. These correspond to unsprayed in-field buffer strips of a given width and/or the usage of drift reducing nozzles. The results of the risk assessment using typical mitigation measures (no-spray buffer zones of 5 or 10 m; drift-reducing nozzles with reduction by 50 %, 75 %, or 90 %) are summarised in the following table. Only mitigation options which results in less than a 95% reduction in the total dirft value have been considered.

Table 9.10-3: Risk assessment for non-target terrestrial plants due to the use of Kinvara in cereals considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles)

Intended use		Kinvara to cereals			
Active substance/product		Kinvara			
Application rate (L/ha)		1 × 3			
MAF		1			
Buffer strip	**Drift rate**	**$PER_{off\text{-}field}$**	**$PER_{off\text{-}field}$**	**$PER_{off\text{-}field}$**	**$PER_{off\text{-}field}$**
(m)	**(%)**	**(g/ha)**	**50 % drift red.**	**75 % drift red.**	**90 % drift red.**
			(g/ha)	**(g/ha)**	**(g/ha)**
1	2.77	83.1	41.55	20.78	8.31
5	0.57	17.1	8.55	4.28	
10	0.29	8.7	4.35		
15	0.20	6			
20	0.15	4			
Toxicity value		**TER**			
ER_{50} = 25.7 ~~g/ha~~ mL/ha		**criterion: TER ≥ 5**			

1	**0.31**	**0.62**	**1.24**	**3.09**
5	**1.50**	**3.01**	6.00	
10	**2.95**	5.91		
15	**4.28**			
20	5.71			

MAF: Multiple application factor; PER: Predicted environmental rates; TER: toxicity to exposure ratio. Criteria values shown in bold breach the relevant trigger.

9.10.3 Overall conclusions

Spray drift mitigation measures are required to protect non-target plants from the risk of Kinvara. Either a 20 m no spray buffer, 10 m no spray buffer and 50 % spray reducing nozzle or 5 m no spray buffer and 75 % spray reducing nozzle will be required.

9.11 Effects on other terrestrial organisms (flora and fauna) (KCP 10.7)

Not required.

9.12 Monitoring data (KCP 10.8)

Not required.

9.13 Classification and Labelling

zRMS Comment	Applicant has submitted the formulation composition update with actual classification (according to CLP Regulation 1272/2008 with amendments). The submitted information was accepted.

Please see dRR Part C for full composition details. The Environmental Hazard classification of the product is calculated as follows:

Constituent	R Phrases or H Statements	% Content
MCPA (*present as 40 % w/w MCPA potassium aq. salt solution*)	Xn; R22, Xi; R36 Warning, H302, H319	≥ 10 – < 30
Fluroxypyr methylheptyl ester	N; R50/53 Warning, H410	≥ 5 – < 10
Clopyralid (*present as 55 % clopyralid ethanolamine aq. salt solution*)	Xi, R41 Danger, H318	≥ 1 – < 5
Alcohols, C12-15, ethoxylated	Xn; R22, R41, R52/53 Danger, H302, H318, H412	≥ 10 – < 30
Hydrocarbons, C10 aromatics, <1 % naphthalene	Xn, N; R10, R20, R22, R36/37/38, R40, R50/53 Danger, H304, H336, H411	≥ 5 – < 10

The environmental risk phrase R50/53 applies to the individual components fluroxypyr methylheptyl ester and hydrocarbons with a total composition of 15.3 % w/w present in the formulation. This level is within the range 2.5 % ≤ C_n ≤ 25 % for classification as R51/53. This translates to the CLP hazard statement H411.

The risk phrase R52/53 also applies to the alcohol component of the formulation at a level of 16 % w/w. This level is below the classification trigger of C_n ≥ 25 %, therefore no risk phrase applies based on this.

H Statements		Basis	Obligatory or recommended	Pictogram
Signal Word	No signal word	Based on the % w/w content of fluroxypyr methylheptyl ester and hydrocarbons	Obligatory	
H411	Toxic to aquatic life with long lasting effects			

Also, in line with Article 10, 1.2 of Directive (1999/45/EC) the phrase ‘To avoid risks to man and the environment, comply with instructions for use’ should be included on the label.

Appendix 1 Lists of data considered in support of the evaluation

Tables considered not relevant can be deleted as appropriate.

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

List of data submitted by the applicant and relied on

Annex Point	Author	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or Unpublished	Data Protection claimed Y/N	Owner	Relied on (Y/N)
KIIIA 10.2.1/01	A. Seeland-Fremer & V. Wydra	2015	Acute Toxicity of Kinvara to *Daphnia magna* in a Static 48-hour Immobilisation Test Report No. 89201220 GLP Unpublished	Y	XXXX	Y
KIIIA 10.2.1/02	A. Seeland-Fremer & V. Wydra	2014	Toxicity of Kinvara to *Pseudokirchneriella subcapitata* in an Algal Growth Inhibition Test Report No. 89201210 GLP Unpublished	Y	XXXX	Y
KIIIA 10.2.1/03	A. Seeland-Fremer & V. Wydra	2014	Toxicity of Kinvara to *Anabaena* in an Algal Growth Inhibition Test Report No. ~~89201210~~ 89202210 GLP Unpublished	Y	XXXX	Y
KIIIA 10.2.1/04	Podd G	2012b	Fluroxypyr Acid Algal Growth Inhibition Test with *Navicula pelliculosa* Report No. DWI0012 GLP Unpublished	Y	XXXX	N

KIIIA 10.2.1/05	Podd G	2012c	Fluroxypyr MHE Techincal: Algal Growth Inhibition Test with *Navicula pelliculosa* Report No.DWI0014 GLP Unpublished	Y	XXXX	N
KIIIA 10.2.1/06	Podd G	2012d	Monochloropyridinol Algal Growth Inhibition Assay Report No. DWI0017 GLP Unpublished	Y	XXXX	N
KIIIA 10.2.1/07	Podd G	2012e	Methoxypyridine: Algal Growth Inhibition Test with *Navicula pelliculosa* Report No. DWI0020 GLP Unpublished	Y	XXXX	N
KIIIA 10.2.1/08	Seeland-Fremer., A & Wydra, V.	2014	Toxicity of Kinvara to the Aquatic Plant *Lemna gibba* in a Static Growth Inhibition Test Report No. 89201240 GLP Unpublished	Y	XXXX	Y
KIIIA 10.2.1/09	Wenzel	2016	Macrophyte, water-sediment toxicity test (OECD 239), Kinvara Effects on the growth of *Myriophyllum spicatum* with exposure via the water column Document Number BCH-002/4-12/K GLP Unpublished	Y	XXXX	Y
KIIIA 10.3.1.1.1/01	A. Ehmke	2014	Effects of Kinvara (acute contact and oral) on Honey Bees (*Apis mellifera* L.) in the laboratory. Project Code: 89201035 GLP Unpublished	Y	XXXX	Y
KIIIA 10.3.1.1/02	E. Wright	2019	Kinvara: Acute contact and oral toxicity to bumblebees (*Bombus terrestris*) Study Number: FR/001855-09 GLP Unpublished	Y	XXXX	Y
KIIIA 10.3.1.2/01	S. Wilkins	2019	Kinvara: 10 Day chronic oral toxicity test for adult honey bees (*Apis mellifera* L.)	Y	XXXX	Y

			Study Number: FR/001855-10 GLP Unpublished			
KIIIA 10.3.1.3/01	A. Ehmke	2014	Study on the Effects of Kinvara on Honey Bee Brood (*Apis mellifera L.*) – Brood Feeding Test – Project Code: 89201031 GLP Unpublished	Y	XXXX	Y
KIIIA 10.3.2/01	Moll., M.	2014	Effects of Kinvara on the Predatory Mite *Typhlodromus pyri* in the Laboratory - Dose Response Test Report No. 89201063 GLP Unpublished	Y	XXXX	Y
KIIIA 10.3.2/02	Moll., M.	2014	The effects of Kinvara on the Parasitoid *Aphidius rhopalosiphi* in the Laboratory – Dose Response Test Report No. 81663001 GLP Unpublished	Y	XXXX	Y
KIIIA 10.3.2/03	Moll., M.	2017	Kinvara: Effects on the Parasitoid *Aphidius rhopalosiphi*, Extended Laboratory Study – Dose Response Test – Report No. 122621001 GLP Unpublished	Y	XXXX	Y
KIIIA 10.3.2/04	Vaughan, R.	2017	XXXX Kinvara- A rate-response extended laboratory test to evaluate the effects of fresh residues on the ladybird beetle *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) Report No. BAR-17-1 GLP Unpublished	Y	XXXX	Y
KIIIA 10.4.1.1/01	Luhrs., U	2014	Effects of Kinvara on Reproduction and Growth of Earthworms *Eisenia fetida* in Artificial Soil Report No. 89201022 GLP Unpuiblished	Y	XXXX	Y
KIIIA 10.4.2/01	Staube, D	2017a	Kinvara: Effects on Reproduction of the Predatory Mite *Hypoaspis aculeifer* in Artificial Soil with 5% Peat Report No. 122621089	Y	XXXX	Y

			GLP Unpublished			
KIIIA 10.4.2/02	Staube, D	2017b	Kinvara: Effects on Reproduction of the Collembola *Folsomia candida* in artifial soil with 5% peat Report No. 122621016 GLP Unpublished	Y	XXXX	Y
KIIIA 10.5.1/01	Hammesfahr, U	2017	Kinvara: Effects on the Activity of the Soil Microflora in the Laboratory Report No. 122621080 GLP Unpublished	Y	XXXX	Y
KIIIA 10.6.2/01	Butzler, R. & Munz, J.,	2014	Effects of Kinvara on Terrestrial (Non-Target) plants: Vegetative Vigour Test Report No. 89201087 GLP Unpublished	Y	XXXX	Y
KIIIA 10.6.2/02	Butzler, R. & Munz, J	2014	Effects of Kinvara on Terrestrial (Non-Target) plants: Seedling Emergence and Seedling Growth Test Report No. 89201086 GLP Unpublished	Y	XXXX	Y

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**
KCP XX	Author	YYYY	Title Company Report N Source GLP/non GLP/GEP/non GEP Published/Unpublished	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

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 new studies

In the following, summaries of all studies that were not previously assessed on EU level should be provided. Studies should be sorted by data points and within one data point by names of authors.
A grey box like presented below is intended for documenting the results of the study evaluation by the zRMS and must therefore be attached to each study summary.

A 2.1 KCP 10.1 Effects on birds and other terrestrial vertebrates

A 2.1.1 KCP 10.1.1 Effects on birds

A 2.1.1.1 KCP 10.1.1.1 Acute oral toxicity

A 2.1.1.2 KCP 10.1.1.2 Higher tier data on birds

A 2.1.2 KCP 10.1.2 Effects on terrestrial vertebrates other than birds

A 2.1.2.1 KCP 10.1.2.1 Acute oral toxicity to mammals

A 2.1.2.2 KCP 10.1.2.2 Higher tier data on mammals

A 2.1.3 KCP 10.1.3 Effects on other terrestrial vertebrate wildlife (reptiles and amphibians)

A 2.2 KCP 10.2 Effects on aquatic organisms

A 2.2.1 KCP 10.2.1 Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes

KCP 10.2.1/01 Seeland-Fremer and Wydra (2015)

Comments of zRMS:	The study was evaluated and accepted. The study was performed in accordance with GLP requirements and OECD 202 guideline. The validity criteria were met: • no daphnid showed signs of disease or stress in control • dissolved oxygen concentration was ≥ 8.3 mg O2/L in the control and test vessels at the end of the test. Some deviations from the guidelines were noted. They do not affect final results and can be used in regulatory risk assessment of Kinvara. The following endpoints were derived: • 48-h NOEC = 3.1 mg Kinvara/L • 48 h EC_{50} was calculated to be 9.8 mg Kinvara/L

	• 48 h EC_{10} was calculated to be 7.3 mg Kinvara/L

The following aquatic invertebrate toxicity study performed on Kinvara is provided in support of the assessment and has not been previously evaluated.

Report:	KIIIA1 10.2.1/01, Seeland-Fremer. A & Wydra, V. (2015)
Title:	Acute Toxicity of Kinvara to *Daphnia magna* in a Static 48-hour Immobilisation Test
Document No:	Project Number 89201220
Guidelines:	OECD Guideline 202
GLP	Yes (certified laboratory)

Executive summary

In an acute immobilisation test with Kinvara, juvenile *Daphnia magna* (<24 hours old) were exposed for 48-hours in a static test system at nominal test concentrations of 100, 31.3, 9.8, 3.1, 1.0, 0.3 and 0.1 mg test item/ L. The mobility of the daphnids was determined by visual observation after 24 and 48 hours. Toxic reference item potassium dichromate was tested in a separate study to verify the sensitivity of the test system. The 48-hour NOEC for Kinvara was determined to be 3.1 mg test item/L.

A. MATERIALS:

1. Test material: Kinvara
Description: Yellow / Amber liquid
Lot/Batch #: 13-3601
Purity: MCPA 233.6 g/L
Fluroxypyr-meptyl 73.3 g/L
Clopyralid 27.3 mg/L
Stability of test compound: Stable under normal conditions.

2. Vehicle and/or positive control: Vehicle: Dilution water

3. Test animals
Species: *Daphnia Magna* STRAUS
Age: From 5 to 21 hours old
Source: Held and bred in the test facility under standardised laboratory conditions. The daphnids introduced in the test were taken from IBACON's in-house laboratory culture

Environmental conditions-
Temperature: 20 ºC
Photoperiod: 16 hours light and 8 hours dark daily

B. STUDY DESIGN AND METHODS

1. In-life dates: 4th June 2014 to 6th June 2014

2. Experimental treatments:
Young (< 24 hours old) *Daphnia magna* were exposed for 48 hours in a static system to Kinvara added to test water at test concentrations of 100, 31.3, 9.8, 3.1, 1.0, 0.3 and 0.1 mg test item/ L.

The test units were 100 ml glass beakers containing approximately 60 ml test medium. At each test concentration 4 replicates were performed each containing 5 *Daphnia.* The pH of the test water was between 7.7 and 7.8.

A stock solution of 40 mg/L was prepared by dissolving 50.36 mg test item into 503.6 mL test water by intense stirring for 10 minutes. Adequate volumes of this stock solution were diluted with test water to obtain the test media of the desired test concentrations. Test media were prepared just prior to the introduction of the daphnids.

3. Observations:

The immobility and mortality of the *Daphnia* was determined by visual assessment after 24 and 48 hours. Animals not able to swim after 15 seconds gentle agitation of the test beaker were considered to be immobile.

The pH-values, oxygen concentrations and water temperature were measured at the test start, on day 1 in the old and new media and at the end of the test. The behaviour of Kinvara in water was also determined at the start of the test and after 24 and 48 hours test duration.

4. Statistical analysis:

The 24-hour and 48-hour EC_{50}, EC_{20} and EC_{10} and the 95% confidence limits were calculated by Probit analysis. The NOEC and LOEC after 24 and 48 hours were determined directly from the raw data. The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, ToxRat Solutions GmbH.

II. RESULTS AND DISCUSSION

The study is valid as the mortality of *Daphnia magna* in the control was 0.0% and no *Daphnia* were trapped at the water surface. At the end of the test, the dissolved oxygen concentration in the test media was ≥ 8.3 mg O_2 in the control and test vessels.

The *Daphnia* were observed for mortality or immobilisation after 24 and 48 hours. After 24 hours, the EC_{50}, EC_{20} and EC_{10} values were 9.80, 8.09 and 7.3mg Kinvara /L respectively. The NOEC and LOEC values were 3.1 mg Kinvara /L and 9.8 mg Kinvara /L respectively.

Table 10.2.2.2-1: Influence of Kinvara on the Mobility of *Daphnia magna*

Nominal Concentration [mg test item/L]	No. of *Daphnia* tested	No. of immobilised *Daphnia* after		% of immobilised *Daphnia* after	
		24 h	48 h	24 h	48 h
Control	20	0	0	0	0
0.1	20	0	0	0	0
0.3	20	0	0	0	0
1.0	20	0	0	0	0
3.1	20	0	0	0	0
9.8	20	10	10	50	50
31.3	20	20	20	100	100
100	20	20	20	100	100

III. CONCLUSION

The 48-hour NOEC was determined to be 3.1 mg Kinvara/L. The 48 hour LOEC was determined to be 9.8 mg Kinvara/L and the 48 hour EC_{50} was calculated to be 9.8 mg Kinvara/L.

(Seeland-Fremer. A & Wydra, V., 2015)

KCP 10.2.1/02 Seeland-Fremer and Wydra (2014)

Comments of zRMS:	The study was evaluated and accepted. The study was performed in accordance with GLP requirements and OECD 201 guideline. The validity criteria were met. • cell density: 186-fold increase within 72 hours; • coefficient of variation of daily growth rates in control cultures: 18.2 %; • coefficient of variation of average growth between control replicates: 1.3%. Some deviations from the guidelines were noted. They do not affect results and can be used in regulatory risk assessment of Kinvara. The following endpoints were derived: • 72 h E_rC_{50} (growth rate) = 7.67 mg product/L; 72 h NOEC = 0.98 mg product/L; 72 h E_rC_{10} = 3.27 mg product/L • 72 h E_yC_{50}(yield inhibition) = 3.70 mg product/L; 72 h NOEC = 0.98 mg product/L; 72 h E_rC_{10} = 1.87 mg product/L The relevant endpoints will be used in the risk assessment.

The following algal acute toxicity studies performed on Kinvara are provided in support of the assessment and have not been previously evaluated.

Report:	KIIIA1 10.2.1/02, A. Seeland-Fremer & V. Wydra. (2014)
Title:	Toxicity of Kinvara to *Pseudokirchneriella subcapitata* in an Algal Growth Inhibition Test
Document No:	Project Number 89201210
Guidelines:	OECD 201
GLP	Yes (certified laboratory)

Executive summary:
The inhibitory effect of Kinvara on the growth of the freshwater green algal species *Pseudokirchneriella subcapitata* was determined over a test period of 72 hours. The cultures were exposed to Kinvara at concentrations of 10.0, 3.13, 0.98, 0.31 and 0.1 mg Kinvara/L. The 72-hour EC_{50} for yield was 3.70 mg Kinvara/L. The 72-hour EC_{50} for growth rate was 7.67 mg Kinvara/L. The 72-hour NOEC for both yield and growth rate was 0.98 mg Kinvara/L. The 72-hour LOEC for both yield and growth rate was 3.13 mg Kinvara/L.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Kinvara
Description: Amber/Yellow liquid
Lot/Batch no: 13-3601
Active Content: 233.6 g/L MCPA
73.3 g/L Fluroxypyr-meptyl
27.3 g/L Clopyralid

Stability of test compound: Stable under normal conditions.

2. Vehicle and/or positive control: Control: Reconstituted water

3. Test animals –
Species: *Pseudokirchneriella subcapitata,* Strain No. 61.81 SAG
Source: SammLung Von Algenkulturen (SAG)
Initial cell count: 5000 cells/ml test medium

Acclimation period: Not applicable
Environmental conditions –
Temperature: 23 °C
Photoperiod: 4980 – 7200 lux continuous illumination

B. STUDY DESIGN AND METHODS:
1. In-life dates: 12th May 2014 to 19th May 2014

2. Experimental treatments:
The inhibitory effect of Kinvara on the green algal species *Pseudokirchneriella subcapitata* was determined in a static, non-renewal exposure system. Algae were cultivated in the laboratories of IBACON under standardised conditions. The test was started by inoculation of a biomass of 5000 algal cells per mL test medium. These cells were taken from an exponentially growing pre culture set up 3 days prior to the start of the test under the same conditions of the test.

The test was performed with three replicates per test concentration and six replicates of the control. Each replicate contained 50 mL algal suspension in test medium in a 50 mL Erlenmeyer flask. Each replicate was continuously stirred with a magnetic stirrer. An additional replicate per test concentration was prepared without algae as a "blank" for the spectrophotometrical measurements.

3. Observations/Sampling:
Samples were taken from all replicates after 24, 48 and 72 hours exposure. Cell densities were determined by spectrophotometrical measurement. The shape of all the treated cells compared to the control was microscopically examined.

4. Statistical analysis:
Based on the calculated cell densities the 72 hour E_rC_{50} and the E_yC_{50} values (50 % inhibition of algal growth rate and yield, respectively), together with their 95 % confidence values were calculated by Probit Analysis. For the determination of LOEC and NOEC values for algal growth, the calculated growth rates and mean biomass at the test concentrations were tested for significant differences compared to the control values using the Williams t-test.

5. Deviations from Study Plan
Deviation
In the case of clopyralid: The relative standard deviation of repeated injection of one standard solution was not determined.
In the case of MCPA: The relative standard deviation of repeated injection of one standard solution was 3.4%.
Reason for Deviation
In the case of clopyralid: Human error
In the case of MCPA: Reason unknown

Effect on Study
None. The clopyralid measurement series was done at the same day of analysis as the other active ingredients, where the repeatability was verified. The obtained value of 3.4 % in case of MCPA is regarded to be still acceptable

II. RESULTS AND DISCUSSION

The experiment is considered valid as cell density in the control cultures increased by a factor of 186 within 72 hours; the coefficient of variation for the sectional (daily) growth rates of the control cultures during the course of the test was 18.2 %; the coefficient of variation of average growth in the replicate control cultures was 1.3 %.

Growth inhibition:
The mean algal cell densities during the test period of 72 hours are given in Table 10.2.2.3-1

Table 10.2.2.3-1: Mean algal cell densities during the test period of 72 hours

Nominal Concentration (mg Kinvara/L)	24 h	48 h	72 h
Control	2.938	22.535	92.956
0.1	2.553	22.278	92.316
0.31	2.861	22.586	89.754
0.98	3.219	23.559	95.133
3.13	1.682	11.673	58.245
10	1.170	0.878	3.066

The 72-hour EC_{50} for yield was 3.70 mg Kinvara/L. The 72-hour EC_{50} for growth rate was 7.67 mg Kinvara/L. The 72-hour NOEC for both yield and growth rate was 0.98 mg Kinvara/L. The 72-hour LOEC for both yield and growth rate was 3.13 mg Kinvara/L. The influence of Kinvara on the growth of *Pseudokirchneriella subcapitata* is summarised in Table 10.2.2.3-3.

Table 10.2.2.3-2: Results of the influence of Kinvara on the growth of *Pseudokirchneriella subcapitata*

Parameter	Yield [mg test item/L]	Growth rate [mg test item/L]
72-hour EC_{50}	3.70	7.67
95 % conf. interval	3.47 - 4.05	7.43 - 7.91
72-hour EC_{20}	2.36	4.38
95 % conf. interval	2.04 - 2.59	4.13 - 4.61
72-hour EC_{10}	1.87	3.27
95 % conf. interval	1.48 - 2.14	3.02 - 3.50
72-hour NOEC	0.98	0.98
72-hour LOEC	3.13	3.13

n.d. = not determinable
Values refer to nominal test concentrations

The microscopic examination of the shape of the algal cells after 72 hours of test duration did not show any difference between between the algae that had been growing up to a nominal test concentration of 0.005 mg test item/L and the algal cells in the control. Beginning with 0.016 mg test item/L, the algal cells were deformed and spherical.

III. CONCLUSIONS

The 72-hour EC_{50} for yield was 3.70 mg Kinvara/L. The 72-hour EC_{50} for growth rate was 7.67 mg Kinvara/L. The 72-hour NOEC for both yield and growth rate was 0.98 mg Kinvara/L. The 72-hour LOEC for both yield and growth rate was 3.13 mg Kinvara/L.

(A. Seeland-Fremer & V. Wydra., 2014)

KCP 10.2.1/03 Seeland-Fremer and Wydra (2015)

Comments of zRMS:	The study was evaluated and accepted. The study was performed in accordance with GLP requirements and OECD 201 guideline. The validity criteria were met. • cell density: 23-fold increase within 72 hours; • coefficient of variation of daily growth rates in control cultures: 23.1 %; • coefficient of variation of average growth between control replicates: 1.81%. Some deviations from the guidelines were noted. They do not affect results and can be used in regulatory risk assessment of Kinvara. The following endpoints, based on nominal concentration, were derived: • 72 h E_rC_{50} (growth rate) = 3.79 mg product/L; 72 h NOEC = 1.00 mg product/L; 72 h E_rC_{10} = 2.67 mg product/L • 72 h E_yC_{50}(yield inhibition) = 2.87 mg product/L; 72 h NOEC < 1.00 mg product/L; 72 h E_rC_{10} = 1.26 mg product/L The relevant endpoints will be used in the risk assessment.

Report:	KIIIA1 10.2.1/03, A. Seeland-Fremer & V. Wydra. (2015)
Title:	Toxicity of Kinvara to *Anabaena flos aquae* in an Algal Growth Inhibition Test
Document No:	Project Number 89202210
Guidelines:	OECD 201
GLP	Yes (certified laboratory)

Executive summary:
The inhibitory effect of Kinvara on the growth of the freshwater green algal species *Anabaena flos aquae* was determined over a test period of 72 hours. The cultures were exposed to Kinvara at concentrations of 100, 31.3, 9.8, 3.1 and 1.0 mg Kinvara/L. The 72-hour EC_{50} for yield was 2.87 mg Kinvara/L. The 72-hour EC_{50} for growth rate was 3.79 mg Kinvara/L. The 72-hour NOEC for both yield and growth rate was 1.0 mg Kinvara/L. The 72-hour LOEC for yield and growth rate were 1.0 mg Kinvara/L and 3.1 mg Kinvara/L respectively.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Kinvara
Description: Amber/Yellow liquid
Lot/Batch no: 13-3601
Active Content: 233.6 g/L MCPA
73.3 g/L Fluroxypyr-meptyl
27.3 g/L Clopyralid

Stability of test compound: Stable under normal conditions.

2. Vehicle and/or positive control: Control: Reconstituted water

3. Test animals –
Species: *Anabaena flos-aquae,* UTEX B1444
Source: University of Texas
Initial cell count: 1500 cells/ml test medium
Acclimation period: Not applicable

Environmental conditions –
Temperature: 22-23 ºC
Light Instensity: 3280 – 4230 lux continuous illumination

B. STUDY DESIGN AND METHODS:

1. In-life dates: 21st July 2014

2. Experimental treatments:

The inhibitory effect of Kinvara on the green algal species *Anabaena flos-aquae* was determined in a growth inhibition test. Algae were cultivated in the laboratories of University of Texas. The test was started with a nominal algal cell density of 15000 algal cells per mL test medium. These cells were taken from an exponentially growing pre culture set up 4 days prior to the start of the test under the same conditions of the test.

The test was performed with three replicates per test concentration and six replicates of the control. Each replicate contained 50 mL algal suspension in test medium in a 50 mL Erlenmeyer flask. Each replicate was continuously stirred with a magnetic stirrer. An additional replicate per test concentration was prepared without algae as a "blank" for the spectrophotometrical measurements.

6. Observations/Sampling:

Samples were taken from all replicates after 24, 48 and 72 hours exposure. Cell densities were determined by spectrophotometrical measurement. The shape of all the treated cells compared to the control was microscopically examined.

7. Statistical analysis:

Based on the calculated cell densities the 72 hour E_rC_{50} and the E_yC_{50} values (50 % inhibition of algal growth rate and yield, respectively), together with their 95 % confidence values were calculated by Probit Analysis. For the determination of LOEC and NOEC values for algal growth, the calculated growth rates and mean biomass at the test concentrations were tested for significant differences compared to the control values using the Williams t-test.

8. Deviations from Study Plan

Deviation

For clopyralid:

1- the relative standard deviation of repeated injection of one standard solution was not determined.
2- The mean recovery was outside the limits of 70-110 %; 162 % for the fortification level of nominal 3mg test item/L.

For fluroxypyr:

1- The mean recovery was outside the limits of 70-110 %; 52 % for the fortification level of nominal 3 mg test item/L.

Reason for Deviation

For clopyralid:

1- Human error.
2- Measured value

For fluroxypyr:

1- Measured value

Effect on Study

For clopyralid:

1- None since the accuracy and precision of the analysis of clopyralid show good results.
2- None, the increased recovery is explained by the low measured concentrations. There was enough valid data for the analysis of the active ingredients that these minor deviations re regarded to have no negative impact on the study outcome.

For fluroxypyr:

1- None, as the two other fortification levels for fluroxypyr-1-meptylheptyl ester show good results. There was enough valid data for the analysis of the active ingredients that these minor deviations re regarded to have no negative impact on the study outcome.

II. RESULTS AND DISCUSSION

The experiment is considered valid as cell density in the control cultures increased by 23 fold within 72 hours; the coefficient of variation for the sectional (daily) growth rates of the control cultures during the course of the test was 23.1 %; the coefficient of variation of average growth in the replicate control cultures was 1.81 %.

Table 10.2.2.3-3: Results of the influence of Kinvara on the growth of *Anabaena flos-aquae*

Parameter (0-72 h)	Yield [mg test item/L]	Growth rate [mg test item/L]
72-hour EC_{50}	2.87	3.79
95 % conf. interval	2.71 - 3.04	n.d.
72-hour EC_{20}	1.67	3.01
95 % conf. interval	1.46 - 1.85	n.d.
72-hour EC_{10}	1.26	2.67
95 % conf. interval	1.04 - 1.44	n.d.
72-hour NOEC	< 1.0	1.0
72-hour LOEC	1.0	3.1

n.d. = not determinable
Values refer to nominal test concentrations

The 72-hour EC_{50} for yield was 2.87 mg Kinvara/L. The 72-hour EC_{50} for growth rate was 3.79 mg Kinvara/L. The 72-hour NOEC for both yield and growth rate was 1.0 mg Kinvara/L. The 72-hour LOEC for yield and growth rate was 1.0 mg Kinvara/L and 3.1 mg Kinvara/L respectively.

The microscopic examination of the shape of the algal cells after 72 hours of test duration did not show any difference between the algae that had been growing up to a nominal test concentration of 100 mg test item/L and the algal cells in the control.

IV. CONCLUSIONS

The 72-hour EC_{50} for yield was 2.87 mg Kinvara/L. The 72-hour EC_{50} for growth rate was 3.79 mg Kinvara/L. The 72-hour NOEC for both yield and growth rate was 1.0 mg Kinvara/L. The 72-hour LOEC for yield and growth rate was 1.0 mg Kinvara/L and 3.1 mg Kinvara/L respectively.

(A. Seeland-Fremer & V. Wydra., 2015)

KCP 10.2.1/04 Podd (2012b)
Fluroxypyr and its metabolites

The following algal acute toxicity studies performed on fluroxypyr technical and its metabolites were originally submitted by XXXX for re-registration of Hurler in the UK under COP 2013/02588 and have been accepted. The results of these studies are again summarised below for completeness.

Report:	KIIIA 10.2.1/04, Podd, G. (2012b)
Title:	Fluroxypyr Acid Algal Growth Inhibition Test with *Navicula pelliculosa*
Document No.:	Report No.: DWI0012
Guidelines:	OECD 201
GLP:	Yes (certified laboratory)

Executive summary:

The inhibitory effect of fluroxypyr acid on the growth of the freshwater green algal species *Navicula pelliculosa* was determined over a test period of 72 hours. Triplicate algal cultures, with an initial cell density of 1 x 10^4/mL were exposed to fluroxypyr acid dissolved in algal nutrient medium at nominal concentrations of 6.25, 12.5, 25.0, 50.0, and 100 mg/L. After 72 hours, the measured levels had been

maintained, ranging between 84 and 95 % of their nominal values (between 86 and 92 % of their starting values. The overall mean measured test levels of fluroxypyr acid were 5.98, 12.4, 25.0, 48.6 and 90.3 mg/L.

The 72-hour EC_{50} value for both yield and growth rate was >90.3 mg/L. The 72-hour NOEC was >90.3 mg/L.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Fluroxypyr Acid
Description: Off-White solid
Lot/Batch no: AG-MET3
Purity: 96.4%
Stability of test compound: Stable under normal conditions.

2. Vehicle and/or positive control: Vehicle: Treated mains water

3. Test animals –
Species: *Navicula pelliculosa* (Strain 1050-3)
Source: SammLung Von Algenkulturen (SAG)
Initial cell count: 1 x 10^4 cells/ml
Acclimation period: Not applicable

Environmental conditions –
Temperature: 21.0 – 24 ºC
Photoperiod: 4440-8880 lux continuous white light

B. STUDY DESIGN AND METHODS:

1. In-life dates: 28th October 2011 – 3rd January 2012

2. Experimental treatments:
The study comprised two range finding tests, a limit main test and a definitive test with five concentrations, plus an algal nutrient medium control group.

Algae were cultivated in the laboratory under standardised conditions. The test was started by inoculation of a biomass of 1 x 10^4 algal cells per mL test medium. Six control vessels and three test vessels at each treatment level were prepared, plus an additional flask at nominal concentrations of 6.25 and 100 mg/L, which contained test medium but no algal cells. Each replicate contained 100 mL algal suspension in test medium in a 250 mL conical flask. Six of the control and three test vessels per treatment level were inoculated with 100ml of inoculated test medium. The remaining non-inoculated control and treatment vessel were incubated with the inoculated test vessels, and used to establish background counts on each sampling occasion. Background counts were subtracted from the cell counting results for each of the inoculated test vessels. The resulting cell counts were then used to calculate the area under the growth curves and the corresponding specific growth rates. The cultures were incubated, without renewal of medium for 72 hours in an illuminated orbital incubator according to a random number sequence.

3. Observations/Sampling:
At approximately 24-hour intervals after the start of the incubation period, cultures were treated with ultrasound for ca 5 seconds and samples were taken from each incubated test vessel. Cell densities were measured using a haemacytometer (improved Neubauer). The estimate of cell numbers in each sample was based on the mean of four or eight consecutive counts depending on the cell density of the cultures. The presence of any abnormal cells was also noted during counting.

The test concentrations of fluroxypyr Acid were measured using a mass spectrrometric method of analysis. At the start of the test, the measured levels of fluroxypyr Acid in samples of the test cultures ranged between 97 and 106 % of their nominal values. After 72 hours, the measured levels had been maintained, ranging

between 84 and 95 % of their nominal values; between 86 and 92 % of their starting values. The overall mean measured levels of fluroxypyr Acid were 5.98, 12.4, 25.0, 48.6 and 90.3 mg/L.

4. Statistical analysis:
The data were compiled in an Excel spreadsheet and analysed using SAS 9.1 (SAS Institute 2002) using mean measured concentrations.

II. RESULTS AND DISCUSSION

During the initial 24 hours of exposure, algal growth appeared to have been affected at 25, 48.6 and 90.3 mg/L, with reductions in cell densities of 27, 36 and 39 % respectively. Thereafter cell densities grew at similar or higher rates to that of the control cultures; this excludes the latter 24 hours of the test at the two lowest levels where cell numbers were slightly lower. Since no inhibition was observed over the duration of the definitive test at the three highest levels employed and no inhibition was observed in the two range finding tests, fluroxyyr Acid was not considered to be inhibitory to *Navicula pelliculosa.* Stimulation of algal growth, which was pronounced at 48.6 and 90.3 mg/L, was not considered to be an adverse effect or of biological importance.
The experiment is considered valid as cell density in the control cultures increased by at least a factor of 16, the mean coefficient of variation for section-by-section specific growth rates (0-24, 24-48 and 48-72 hours) in the the control culture did not exceed 35 %, and the coefficient of variation for average specific growth rates in the replicate control cultures was less than 10 % for the whole time period. Therefore the validity criteria were met.
Growth inhibition:
The mean algal cell concentrations during the test period of 72 hours are given in Table 10.2.2.3-4.

Table 10.2.2.3-4: Mean cell concentrations of *Navicula pelliculos* cultures exposed to fluroxypyr Acid during the 72 hours test period

Nominal Concentration (mg /L)	Mean cell concentration (cells/mL)		
	24 h	48 h	72 h
Control	70417	161250	254375
6.25	67500	315833	204167
12.5	66667	359583	247500
25	51250	198750	263750
50	45417	183750	312917
100	42917	172500	292917

The influence of fluroxyyr Acid on the growth of *Navicula pelliculosa* is summarised in Table 10.2.2.3-5.

Table 10.2.2.3-5: Results of the influence of Fluroxyyr Acid on the growth of *Navicula pelliculosa*

Nominal Concentration (mg /L)	Area under curve to 72 h	Growth rate to 72 h	Yield to 72 h	0-24 h	24-48 h	48-72 h
Control	0.0	0.0	0.0	0.0	0.0	0.0
6.25	-37.9	7.0	20.5	0.4	-80.5	197.0
12.5	-57.3	0.8	2.8	1.7	-98.0	180.4
25	-6.9	-1.2	-3.8	15.6	-60.4	38.5
50	-8.0	-6.4	-24.0	22.1	-65.2	-16.2
100	-0.9	-4.5	-15.8	26.2	-66.7	-16.7

Note: negative values indicate stimulation of algal growth.

III. CONCLUSIONS

After 72 hours of exposure, fluroxyyr acid was not found to be inhibitory to *Navicula pelliculosa* at concentrations up to and including 90.3 mg/L (measured). Consequently, the E_bC_{50}, E_rC_{50} and E_yC_{50} could not be calculated but all were greater than 90.3 mg/L (measured) snd ‘’no observed effect concentration (NOEC) was equal to or greater that 90.3 mg/L.

(Podd, 2012b)

KCP 10.2.1/05 Podd (2012c)

Report:	KIIIA 10.2.1/05, Podd, G. (2012c)
Title:	Fluroxypyr MHE Technical: Algal Growth Inhibition Test with *Navicula pelliculosa*
Document No.:	Report No.: DWI0014
Guidelines:	OECD 201
GLP:	Yes (certified laboratory)

Executive summary:
The inhibitory effect of fluroxypyr MHE Technical on the growth of the freshwater diatom *Navicula pelliculosa* was determined over a test period of 72 hours.

Six replicate algal cultures, with an initial cell density of 1 x 10^4 were exposed to fluroxypyr MHE technical for 72 hours. As the test substance was known to be poorly soluble in water the test medium was prepared as a saturated solution from an aqueous mixture with an initial nominal concentration of 0.09 mg/L (limit of water solubility). To aid dissolution, the test substance was dissolved in dimethyl formamide (DMF) before its addition to the algal nutrient medium and the resultant medium was filtered (0.2 µm pore size) before use in the test. As fluroxypyr MHE technical (ester) was known to break down to fluroxypyr Acid on addition to water, prolonged stirring of the test medium was not undertaken.

The exposure level was monitored by measuring the concentrations of fluroxypyr MHE technical and fluroxypyr acid in samples taken at the start and end of the test. Test results were expressed in terms of fluroxypyr MHE technical.

The range finding test employed nominal concentrations of 0.01, 0.1 and 1.0 mg/L. After 72 hours, no significant inhibition was observed at any of the test levels compared to the control group. In the absence of any significant inhibition, the definitive test employed a single concentration of 0.09 mg/L. At the start of the test, the measured levels of fluroxypyr MHE technical in samples of the test cultures ranged between 104 and 123 % of the nominal value. After 72 hours, the measured levels had been maintained (122 % of nominal, 117 % of the mean starting value), with the majority of the test substance present as fluroxypyr Acid, the overall mean measured level was 0.105 mg/L.

The 72-hour EC_{50} value for growth rate was >0.105 mg/L. The 72-hour EC_{50} value for yield >0.105 mg/L. The 72-hour NOEC was >0.105 mg/L

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Fluroxypyr MHE Technical
Description: Off-White powder
Lot/Batch no: 20091207
Purity: 98.1%
Stability of test compound: Unstable

2. Vehicle and/or positive control: Vehicle: DMF solvent

3. Test animals –
Species: *Navicula pelliculosa* (Strain 1050-3)
Source: SammLung Von Algenkulturen (SAG)
Initial cell count: 1 x 10^4 cells/ml
Acclimation period: Not applicable

Environmental conditions –
Temperature: 21.0 – 25 ºC

Photoperiod: 4440-8880 lux continuous white light

B. STUDY DESIGN AND METHODS:

1. In-life dates: 14th October 2011 – 12th April 2012

2. Experimental treatments:

The study comprised a number of formulation trials, a range finding test and a definitive (limit) test, which employed one test concentration, plus an algal nutrient medium control group and a solvent control (100 µL DMF per litre).

Six flasks were established and incubated for the control group, the solvent control group and for the test group. The range finding test employed nominal concentrations of 0.01, 0.1 and 1.0 mg/L. After 72 hours, no significant inhibition was observed at any of the test levels compared to the control group. The results of the analysis confirmed that fluroxypyr MHE technical was poorly soluble and unstable in the test dilution system. A number of formulation trials were performed to identify a suitable method for formulating the test substance. In the absence of any significant inhibition, the definitive test employed a single concentration of 0.09 mg/L.

Each replicate contained 100 mL algal suspension in test medium in a 250 mL conical flask. All flasks were loosely capped and incubated in an illuminated orbital incubator.

3. Observations/Sampling:

At approximately 24-hour intervals after the start of the incubation period, samples were taken from each incubated test vessel. Cell densities were measured using a haemacytometer (improved Neubauer). The estimate of cell numbers in each sample was based on the mean of four or eight consecutive counts depending on the cell density of the cultures. The presence of any abnormal cells was also noted during counting.

The test concentrations of fluroxypyr MHE technical and fluroxypyr acid were measured using a mass spectrometric method of analysis. At the start of the definitive test, eight samples (10mL) were taken from the freshly prepared control, solvent control and test media. After 72 hours, the contents of the replicate flask for each group were pooled and a further eight samples taken for analysis. Additional samples were also taken from a flask containing fluroxypyr MHE technical at 0.09 mg/L but with no algal cells, in order to obtain information on the extent of adsorption/absorption of the test substance by the algal cells.

At the start of the test, the measured levels of fluroxypyr MHE technical in samples of the test cultures ranged between 104 and 123 % of the nominal vslue. After 72 hours, the measured levels had been maintained (122 % of nominal, 117 % of the mean starting value), with the majority of the test substance present as fluroxypyr Acid, the overall mean measured level was 0.105 mg/L.

4. Statistical analysis:

The data were compiled in an Excel spreadsheet and alnalysed using SAS 9.1 (SAS Institute 2002) using mean measured concentrations.

II. RESULTS AND DISCUSSION

For the test to be valid, cell density in the control cultures must increase by at least a factor of 16, the mean coefficient of variation for daily growth rates (0-24, 24-48 and 48-72 hours) in control culture must not exceed 35 %, and the coefficient of variation for average specific growth rates in the replicate control cultures must not exceed 10 % for the whole time period.

The mean coefficient of variation for daily average specific growth rates in replicate control cultures (49.6 and 42.9 % respectively for the control and solvent control groups) exceeded the criteria (35 %) recommended for algal studies. Due to the physical characteristic of the species of algae and the difficulties associated with determining cell density of an algal species that forms in clumps of cells as it grows, cell counts can vary greatly between samples, resulting in a higher coefficient of variation. Therefore, this

guideline criterion is not considered appropriate for this species of algae. This was not considered to have affected the determination of endpoints or to have had any impact on the integrity of the study.

The mean algal cell concentrations during the test period of 72 hours are given in Table 10.2.2.3-6.

Table 10.2.2.3-6: Mean cell concentrations of *Navicula pelliculos* cultures exposed to Fluroxypyr MHE Technical during the 72 hours test period

Nominal Concentration (mg /L)	Mean cell concentration (cells/mL)		
	24 h	48 h	72 h
Control	53542	431875	6954175
Solvent Control	50208	417292	724167
0.105	49583	368542	709167

The influence of fluroxypyr acid on the growth of *Navicula pelliculosa* is summarised in Table 10.2.2.3-7.

Table 10.2.2.3-7: Results of the influence of Fluroxypyr MHE Technical on the growth of *Navicula pelliculosa*

Nominal Concentration (mg /L)	Area under curve to 72 h	Growth rate to 72 h	Yield	0-24 h	24-48 h	48-72 h
Control	-0.4	1.0	4.0	-5.6	2.2	14.5
Solvent Control	0.0	0.0	0.0	0.0	0.0	0.0
0.105	-57.3	0.5	2.1	0.3	5.0	-16.4

Note: negative values indicate stimulation of algal growth.

III. CONCLUSIONS

Under the conditions of the study, fluroxypyr MHE technical was not found to be acutely toxic to *Navicula pelliculosa* at a mean concentration of 0.105 mg/L (measured). Consequently, the 72 hour $E_rC_{50,}$ and E_yC_{50} could not be calculated but all must be > 0.105 mg/L and the 'no observed effect concentration' (NOEC) was > 0.105 mg/L.

(Podd, 2012c)

KCP 10.2.1/06 Podd (2012d)

Report:	KIIIA 10.2.1/06, Podd, G. (2012d)
Title:	Monochloropyridinol Algal Growth Inhibition Assay
Document No.:	Report No.: DWI0017
Guidelines:	OECD 201
GLP:	Yes (certified laboratory)

Executive summary:

The inhibitory effect of monochloropyridinol on the growth of the unicellular green algal species *Pseudokirchneriella subcapitata* was determined over a test period of 72 hours. Triplicate algal cultures, with an intial cell density of 1 x 10^4 /mL, were exposed to monochloropyridinol dissolved in algal nutrient medium at nominal concentrations of 6.25, 12.5, 25.0, 50.0, and 100 mg/L. After 72 hours, the measured levels had been maintained, ranging between 85 and 112% of their nominal values (between 77 and 109% of their starting values. The overall mean measured test levels of monochloropyridinol were 5.89, 13.4, 25.0, 48.7 and 104 mg/L.

The 72-hour E_bC_{50} value was 52.5 mg/L. The NOEC was 13.4 mg/L. The 72-hour E_rC_{50} value was 84.0 mg/L and the NOEC was 25 mg/L. The 72-hour E_yC_{50} value was 49.6 mg/L and the NOEC was 25 mg/L.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Monochloropyridinol

Description: White solid
Lot/Batch no: 4625SJR023-2
Purity: 99.5 %
Stability of test compound: Stable under normal conditions.

2. Vehicle and/or positive control: Vehicle: Treated mains water

3. Test animals –

Species: *Pseudokirchneriella subcapitata* (Strain CAPP 278/4)
Source: Culture Collection of Algae and Protozoa, Scotland
Initial cell count: 1 x 10^4 cells/ml
Acclimation period: Not applicable

Environmental conditions –

Temperature: 21.0 - 25ºC
Photoperiod: 4440-8880 lux continuous white light

B. STUDY DESIGN AND METHODS:

1. In-life dates: 2nd December 2011 – 17th January 2012

2. Experimental treatments:

The study comprised a range finding test and a definitive test with five concentrations, plus an algal nutrient medium control group.

Algae were cultivated in the laboratory under standardised conditions. The test was started by inoculation of a biomass of 1 x 10^4 algal cells per mL test medium. Six control vessels and three test vessels at each treatment level were prepared, plus an additional flask at nominal concentrations of 6.25 and 100 mg/L, which contained test medium but no algal cells. Each replicate contained 100 mL algal suspension in test medium in a 250 mL conical flask. All flasks were loosly capped and incubated in an illuminated orbital incubator. The range finding test employed nomial concentrations of 0.1, 1, 10 and 100 mg/L. After 72 hours, algal growth was inhibited by 93 % at 100 mg/L. The definitive test concentrations, selected based on the results of the range finding test were 6.25, 12.5, 25.0, 50.0, and 100 mg/L.

3. Observations/Sampling:
At approximately 24-hour intervals after the start of the incubation period, samples were taken from each incubated test vessel. Cell densities were measured using a Coulter Z Series Particle Count and Size Analyzer. The estimate of cell numbers in each sample was based on the mean of three consecutive counts corrected for background counts of uninoculated dilution media. The presence of any abnormal cells was also noted during counting.

The test concentrations of monochloropyridinol were measured using a mass spectrrometric method of analysis. At the start of the definitive test, two samples were taken from the freshly prepared control and test media. After 72 hours, the contents of the replicate flask for each group were pooled and further samples taken for analysis. Additional samples were also taken from flasks containing monochloropyridinol at 6.25 and 100 mg/L but with no algal cells in order to obtain information on the extent of adsorption absorption of the test substance by the algal cells. After 72 hours, the measured levels had been maintained, ranging between 85 and 112% of their nominal values (between 77 and 109% of their starting values. The overall mean measured test levels of monochloropyridinol were 5.89, 13.4, 25.0, 48.7 and 104 mg/L.

4. Statistical analysis:

The data were compiled in an Excel spreadsheet and alnalysed using SAS 9.1 (SAS Institute 2002) using mean measured concentrations.

II. RESULTS AND DISCUSSION

Area under the growth curve:
E_bC_{10} (72 h) : 27.6 mg/L (95% confidence limits, 23.6 & 33.3 mg/L)
E_bC_{50} (72 h) : 52.5 mg/L (95% confidence limits, 49.1 & 56.0 mg/L)
No observed effect concentration (NOEC) : 13.4 mg/L
Lowest observed effect concentration (LOEC) : 25.0 mg/L

Average specific growth rate:
E_rC_{10} (0-72 h) : 43.2 mg/L (95% confidence limits, 38.8 & 47.2 mg/L)
E_rC_{50} (0-72 h) : 84.0 mg/L (95% confidence limits, 75.9 & 88.5 mg/L)

No observed effect concentration (NOEC) : 25.0 mg/L
Lowest observed effect concentration (LOEC) : 48.7 mg/L

Yield:
E_yC_{10} (0-72 h) : 27.8 mg/L (95% confidence limits, 20.8 & 41.8 mg/L)
E_yC_{50} (0-72 h): 49.6 mg/L (95% confidence limits, 44.5 & 55.0 mg/L)

No observed effect concentration (NOEC) : 25.0 mg/L
Lowest observed effect concentration (LOEC) : 48.7 mg/L

The experiment is considered valid as cell density in the control cultures increased by at least a factor of 16, the mean coefficient of variation for section-by-section specific growth rates (0-24, 24-48 and 48-72 hours) in the control culture did not exceed 35 %, and the coefficient of variation for average specific growth rates in the replicate control cultures was less than 10% for the whole time period. Therefore, the validity criteria were met.

The mean algal cell concentrations during the test period of 72 hours are given in Table 10.2.2.3-8.

Table 10.2.2.3-8: Mean cell concentrations of *Pseudokirchneriella subcapitata* cultures exposed to Monochloropyridinol during the 72 hours test period

Nominal Concentration (mg /L)	Mean cell concentration (cells/mL)		
	24 h	48 h	72 h
Control	61489	313333	1007744
6.25	62861	310128	999600
12.5	66339	320761	1027256
25	61839	298228	904478
50	49183	191794	539289
100	14639	49828	30611

The influence of Monochloropyridinol on the growth of *Pseudokirchneriella subcapitata* is summarised in Table 10.2.2.3-9.

Table 10.2.2.3-9: Results of the influence of Monochloropyridinol on the growth of *Pseudokirchneriella subcapitata*

Nominal Conc. (mg /L)	Area under curve to 72 h	Growth rate to 72 h	Yield to 72 h	0-24 h	24-48 h	48-72 h
Control	0.0	0.0	0.0	0.0	0.0	0.0
6.25	0.7	0.1	0.8	-1.5	2.3	-0.4

12.5	-2.6	-0.4	-2.0	-4.7	3.8	0.4
25	7.8	2.4	10.3	-0.7	3.8	5.4
50	43.1	13.5	47.0	11.9	17.0	11.1
100	93.6	75.8	97.9	78.9	25.29	142.1

Note: negative values indicate stimulation of algal growth.

III. CONCLUSIONS

After 72 hours of exposure to monochloropyridinol, the E_bC_{50}, E_rC_{50} and E_yC_{50} respectively were 52.5, 84.0 and 49.6 mg/L.

The no observed effect concentration (NOEC) for area under the growth curve was 13.4 mg/L and for growth rate and yield was 25.0 mg/L.

(Podd, 2012d)

KCP 10.2.1/07 Podd (2012e)

Report:	KIIIA 10.2.1/07, Podd, G. (2012e)
Title:	Methoxypyridine: Algal Growth Inhibition Test with *Navicula pelliculosa*
Document No.:	Report No.: DWI0020
Guidelines:	OECD 201
GLP:	Yes (certified laboratory)

Executive summary:
The inhibitory effect of methoxypyridine on the growth of the diatomous alga *Navicula pelliculosa* was determined over a test period of 72 hours. Triplicate algal cultures, with an intial cell density of 1 x 10^4 /mL, were exposed to methoxypyridine dissolved in algal nutrient medium at nominal concentrations of 1.00, 3.20, 10.0, 32.0 and 100 mg/L. After 72 hours, the measured levels had been maintained, ranging between 63 and 80 % of their nominal values (between 78 and 100 % of their starting values). The overall mean measured test levels of methoxypyridine were 0.707, 2.49, 7.20, 22.9 and 80.3 mg/L. The 72-hour E_rC_{50} value was 7.72 mg/L and the NOEC was 2.49 mg/L. The 72-hour E_yC_{50} value was 5.58 mg/L and the NOEC was 2.49 mg/L.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Methoxypyridine
Description: White powder
Lot/Batch no: EPP/VMV 410.E
Purity: 98.2 %
Stability of test compound: Stable under normal conditions.

2. Vehicle and/or positive control: Vehicle: Treated mains water

3. Test animals –
Species: *Navicula pelliculosa* (Strain 1050-3)
Source: SammLung Von Algenkulturen (SAG)
Initial cell count: 1 x 10^4 cells/mL
Acclimation period: Not applicable

Environmental conditions –
Temperature: 21.0 – 25 ºC
Photoperiod: 4440-8880 lux continuous white light

B. STUDY DESIGN AND METHODS:

1. In-life dates: 22nd November 2011 – 5th April 2012
2. Experimental treatments:

The study comprised a range finding test and a definitive test with five concentrations, plus an algal nutrient medium control group.

Algae were cultivated in the laboratory under standardised conditions. The test was started by inoculation of a biomass of 1 x 10^4 algal cells per mL test medium. Six control vessels and three test vessels at each treatment level were prepared. Each replicate contained 100 mL algal suspension in test medium in a 250 mL conical flask. All flasks were loosely capped and incubated in an illuminated orbital incubator. The range finding test employed nomial concentrations of 1, 10 and 100 mg/L. After 72 hours, algal growth was inhibited by 97 % at 10 mg/L, no inhibition occurred at 1 mg/L. The definitive test concentrations, selected based on the results of the range finding test were 1.0, 3.20, 10.0, 32.0 and 100 mg/L.

3. Observations/Sampling:
At approximately 24-hour intervals after the start of the incubation period, samples were taken from each incubated test vessel. Cell densities were measured using a haemacytometer (improved Neubauer). The estimate of cell numbers in each sample was based on the mean of eight consecutive counts. The presence of any abnormal cells was also noted during counting.

The test concentrations of methoxypyridine were measured using a GC mass spectrometric method of analysis. At the start of the definitive test, two samples were taken from the freshly prepared control and test media. After 72 hours, the contents of the replicate flask for each group were pooled and further samples taken for analysis. On each occasion, one of the samples was analysed and the other stored in a freezer in case further analysis was required.

4. Statistical analysis:
The data were compiled in an Excel spreadsheet and alnalysed using SAS 9.1 (SAS Institute 2002) using mean measured concentrations.

II. RESULTS AND DISCUSSION

Average specific growth rate:
E_rC_{50} (0-72 h) : 7.72 mg/L (95 % confidence limits, 7.47 & 9.36 mg/L)
No observed effect concentration (NOEC) : 2.49 mg/L

Yield:
E_yC_{50} (0-72 h): 8.58 mg/L (95 % confidence limits, 4.50 & 6.99 mg/L)
No observed effect concentration (NOEC) : 2.49 mg/L

For the test to be valid, cell density in the control cultures must increase by at least a factor of 16, the mean coefficient of variation for daily growth rates (0-24, 24-48 and 48-72 hours) in control culture must not exceed 35 %, and the coefficient of variation for average specific growth rates in the replicate control cultures must not exceed 10 % for the whole time period.

The variation of the average specific growth rate in replicate control cultures exceeded the criteria for this study type (45.9 %). Due to the physical characteristic of this species of algae and the difficulties associated with determining cell density of an algal species that forms in clumps of cells as it grows, cell counts can vary greatly between samples resuting in a higher coefficient of variation. Therefore, this guideline criterion is not considered appropriate for this species of algae. This was not considered to have affected the determination of endpoints or to have had any impact on the integrity of the study.
On Day 0, the temperature of the control media was slightly below the range stated in the protocol (21 – 24 °C), this was not considered to be significant or to have had any impact on the integrity of the study.
The mean algal cell concentrations during the test period of 72 hours are given in Table 10.2.2.3-10.

Table 10.2.2.3-10: Mean cell concentrations of *Navicula Pelliculosa* cultures exposed to Monochloropyridinol during the 72 hours test period

Nominal Concentration (mg /L)	Mean cell concentration (cells/mL)		
	24 h	48 h	72 h
Control	27292	238125	602083
1.00	21250	202500	532083
3.20	15417	153750	543333
10.00	13333	52083	179583
32.00	7083	8333	2083
100	12500	8583	7917

The influence of Monochloropyridinol on the growth of *Navicula pelliculosa* is summarised in Table 10.2.2.3-11.

Table 10.2.2.3-11: Results of the influence of monochloropyridinol on the growth of *Navicula pelliculosa*

Nominal Concentration (mg /L)	Growth rate to 72 h	Yield to 72 h	0-24 h	24-48 h	48-72 h
Control	0.0	0.0	0.0	0.0	0.0
1.0	3.2	11.8	23.7	-2.9	-2.9
3.2	2.7	9.9	61.3	-6.4	-35.1
10.0	29.6	71.4	85.2	33.9	-37.1
32.0	128.9	101.3	151.0	90.9	206.5
100.0	107.7	100.4	85.0	111.7	121.0

Note: negative values indicate stimulation of algal growth.

III. CONCLUSIONS

After 72 hours of exposure to methoxypyridine the E_rC_{50} and E_yC_{50} were 7.72 and 8.58 mg/L respectively. The no observed effect concentration (NOEC) for the growth rate and yield was 2.49 mg/L.

(Podd, 2012e)

10.2.1/08 Seeland-Fremer and Wydra (2014)

Comments of zRMS:	The study was evaluated and accepted. The study was performed in accordance with GLP requirements and OECD 221 guideline. The validity criteria were met. • doubling time of frond number in control was of 1.5 d. The following endpoints based on frond number were derived: • 7 d E_rC_{50} (growth rate) = 38.3 mg product/L; 7 d NOEC = 0.95 mg product/L; 7 d E_rC_{10} = 2.06 mg product/L • 7 d E_yC_{50}(yield inhibition) = 6.06 mg product/L; 7 d NOEC = 0.95 mg product/L; 7 d E_rC_{10} < 0.95 mg product/L The relevant endpoints will be used in the risk assessment.

The following aquatic plant toxicity study performed on Kinvara is submitted in support of the registration and has not been previously evaluated.

Report:	KIIIA 10.2.1/08, Seeland-Fremer., A & Wydra, V. (2014)
Title:	Toxicity of Kinvara to the Aquatic Plant *Lemna gibba* in a Static Growth Inhibition Test
Document No:	Project Number 89201240
Guidelines:	OECD 221
GLP	Yes (certified laboratory)

Executive Summary:
The freshwater plant *Lemna gibba* was exposed to various concentrations of Kinvara under defined conditions in a static test system. The inhibition of growth in relation to control cultures was determined over a test period of 7 days. The 7-day EC_{50} and NOEC/LOEC values for yield and growth rate based on frond number and dry weight were determined. The 7-day EC_{50} (yield) was calculated to be 6.06 and 15.6 mg Kinvara/L for frond number and dry weight respectively. The 7-day EC_{50} (growth rate) was calculated to be 38.3 and > 100 mg Kinvara/L for frond number and dry weight respectively. The 7 day NOEC (yield) and the LOEC (yield) were determined to be 0.95 and 3.05 mg Kinvara/L for frond number and dry weight respectively. The 7 day NOEC (growth rate) and the LOEC (growth rate) were determined to be 0.95 and 3.05 mg Kinvara/L for frond number and 3.05 and 9.77 mg Kinvara/L for dry weight respectively.

1. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: Kinvara
Description: Yellow amber liquid
Lot/Batch #: 13-3601
Purity: Clopyralid: 27.3 g/L
Fluroxypyr-meptyl: 73.3 g/L
MCPA: 233.6 g/L

Stability of test compound: Stable under normal conditions.

3. Test plants -

Species: *Lemna gibba* G3
Growth stage: 4 fronds
Source: IBACON in-house laboratory culture
Acclimation period: 7 days

Environmental conditions -

Temperature: 23ºC to 25 ºC
Photoperiod: Continuous illumination (8350-9310 lux)

B. STUDY DESIGN AND METHODS:

1. In-life dates: 16th May – 26th May 2014

2. Experimental treatments:
Lemna gibba fronds were cultivated in AAP growth medium for seven days prior to the application of Kinvara. A stock solution of Kinvara was diluted to prepare concentrations of 100, 31.3, 9.77, 3.05 and 0.95 mg test item/L. Test media were freshly prepared just before the introduction of aquatic plants. A control test medium was also used without the addition of Kinvara. Test media was renewed every day during the test.

Tests took place in 250 ml glass flasks, each containing 200 mL test medium. These were maintained at 23 °C to 25 °C. The pH of the media was at the start of the test.

Colonies consisting of 4 fronds were transferred from the inoculums culture. Each test vessel contained a total of 12 fronds. There were 3 replicates per treatment level.

The test vessels were placed in a random order and were repositioned on days 2 and 5 to minimise differences in light intensity.

3. Observations
At test start, frond and colony numbers were recorded. On days 2, 5 and 7 frond numbers and the appearance of colonies were observed. Changes to the dry weight of fronds were determined by comparison to a sample of fronds identical to that used to inoculate the test vessels. Dry weight was determined by plants being collected at the end of the test, rinsed in deionised water and dried to a constant weight at 60 °C. The behaviour of the test item in culture medium was determined by comparison of fresh and aged test media using HPLC.

II. RESULTS AND DISCUSSION

The 7-day endpoints for the study are summarised in Table 10.8.2.1-1

Table 10.8.2.1-1: Influence of Kinvara on the growth of *Lemna gibba*

Parameter	Yield (frond number) [mg test item/L]	Growth rate (frond number) [mg test item/L]	Yield (dry weight) [mg test item/L]	Growth rate (dry weight) [mg test item/L]
EC_{50} (7-day)	6.06	38.3	15.6	> 100
95 % conf. limits	3.91 - 9.26	30.9 - 48.7	10.4 - 23.6	n.d.
EC_{20} (7-day)	1.45	5.62	3.47	16.8
95 % conf. limits	< 0.95 - 2.46	3.86 - 7.49	1.49 - 5.69	10.3 - 23.5
EC_{10} (7-day)	< 0.95	2.06	1.58	5.61
95 % conf. limits	n.d.	1.18 - 3.10	< 0.95 - 3.02	2.37 - 9.36
7-day NOEC	0.95	0.95	3.05	3.05
7-day LOEC	3.05	3.05	9.77	9.77
n.d.: not determinable Values refer to nominal test concentrations				

Values refer to nominal test concentrations

The validity criteria for the study were met. In the control group the doubling time of fronds was 1.5 days (less than 60 hours).

The 7-day EC_{50} (yield) was calculated to be 6.06 and 15.6 mg Kinvara/L for frond number and dry weight respectively. The 7-day EC_{50} (growth rate) was calculated to be 38.3 and > 100 mg Kinvara/L for frond number and dry weight respectively. The 7 day NOEC (yield) and the LOEC (yield) were determined to be 0.95 and 3.05 mg Kinvara/L for frond number and dry weight respectively. The 7 day NOEC (growth rate) and the LOEC (growth rate) were determined to be 0.95 and 3.05 mg Kinvara/L for frond number and 3.05 and 9.77 mg Kinvara/L for dry weight respectively.

Changes to the frond number was the parameter that gave the lowest EC_{50} value. Details of the effect on frond number are given in Table 10.8.2.1-2.

Table 10.8.2.1-2: Mean frond number of *Lemna gibba* during the test period of 7 days

Nominal Conc. (mg test tiem/L)	Flask No.	Frond number after		
		3 days	5 days	7 days
Control	m	46.7	121.3	320.7
0.95		49.0	136.0	296.3

3.05		44.0	104.3	202.7
9.77		40.7	80.3	126.7
31.3		32.0	50.3	69.7
100		24.0	31.0	37.0

The shape of fronds and colonies after the test period of 7 days was not different to those in the control up to and including the nominal test concentration of 0.95 mg test item/L.

At the higher test item concentrations the fronds showed deviations from the control replicates after 7 days; *i.e.* gibbous growth, shortened roots and chlorosis (3.05, 9.77, 31.3 and 100 mg test item/L). Additionally, the fronds were separated at 31.3 and 100 mg test item/L.

III. CONCLUSIONS

Based on the effect on frond numbers, the EC_{50} (yield) for the effect of Kinvara on *Lemna gibba* was determined to be 6.06 mg test item/L.

(Seeland-Fremer., A & Wydra, V. (2014)

10.2.1/09 Wenzel (2016)

Comments of zRMS:	The study was evaluated and accepted. The study was performed in accordance with GLP requirements and OECD 239 guideline. The study fulfilled the validity criteria of the OECD 239, with total shoot length and fresh weight (mean values) of the control plants increasing by more than factor 2 (6.7 and 3.9 times, respect-tively) during the test period. Control plants did not show any visual symptoms of chlorosis and were visibly free from contamination. The mean coefficient of variation for yield based on measurements of shoot weight and length in the control cultures did not exceed 35% (13.0% achieved). The following endpoints based on 14 d growth rate were derived: • total shoot length: E_rC_{50} = 296 µg product/L; NOEC = 30.7 µg product/L; E_rC_{10} = 42.1 µg product/L • fresh weight: E_yC_{50}= 221 µg product/L; NOEC = 30.7 µg product/L; ErC_{10}=30.8 µg product/L. • dry weight: E_rC_{50} > 1087 µg product/L; NOEC = 105 µg product/L; E_rC_{10} was not assessed The following endpoints based on 14 d yield were derived: • total shoot length: E_rC_{50} = 137 µg product/L; NOEC = 30.7 µg product/L; E_rC_{10} = 27.6 µg product/L • fresh weight: E_yC_{50}= 126 µg product/L; NOEC = 9.3 µg product/L; E_rC_{10} < 21.7 µg product/L. • dry weight: E_rC_{50} > 1087 µg product/L; NOEC = 105 µg product/L; E_rC_{10} was not assessed The relevant endpoints will be used in the risk assessment.

The following aquatic plant toxicity study performed on Kinvara is submitted in support of the registration and has not been previously evaluated.

Report:	KIIIA 10.2.1/09, Wenzel (2016)
Title:	Macrophyte, water-sediment toxicity test (OECD 239), Kinvara: Effects on the growth of Myriophyllum spicatum with exposure via the water column
Document No:	BCH-002/4-12/K
Guidelines:	OECD 239
GLP	Yes (certified laboratory)

Executive Summary:
The freshwater plant *Myriophyllum spicatum* was exposed to various concentrations of Kinvara under defined conditions in a sediment water system in accordance with the OECD 239 test guidance. The inhibition of growth in relation to control cultures was determined over a test period of 14 days. The 14-day EC_{50} and NOEC/LOEC values for yield and growth rate based on total shoot length, fresh weight and dry weight.

The 14-day E_rC_{50} were calculated to be 221, 296 and >1087 µg Kinvara/L for fresh weight, total shoot length and dry weight respectively. The 14-day NOEC and LOEC were determined to be 9.3 and 30.7 µg Kinvara/L based on the yield endpoint for fresh weight.

1. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: Kinvara
Description: yellow/amber, clear liquid
Lot/Batch #: 15-5845
Purity: Clopyralid: 27.9 g/L
Fluroxypyr-meptyl: 73.7 g/L
MCPA: 233.2 g/L

Staility of test compound: Stable under normal conditions.

3. Test plants -
Species: *Myriophyllum spicatum*
Source: German Federal Environmental Agency
Acclimation period: 14 days

Environmental conditions -
Temperature: 23 ºC to 25 ºC
Photoperiod: Continuous illumination (8350-9310 lux)

B. STUDY DESIGN AND METHODS:
1. In-life dates: 10th March – 24th March 2016

2. Experimental treatments:
Myriophyllum spicatum from a sediment free stock culture were acclimatised for at least 2 weeks in Smart& Barko growth medium and sediment system in accordance with the OECD guidelines. A stock solution of 1200 µg Kinvara/L was diluted to prepare concentrations of 1200, 380, 38 and 12 µg test item/L. A non-GLP range finder test was conducted to determine these concentrations. The concentrations of MCPA, fluroxypyr-meptyl and clopyralid were all measured to provide mean measured concentrations based on the active substances. A control test medium was also used without the addition of Kinvara.

Tests took place in 2 L glass beackers, each containing three plants and 1.7 L test medium. These were maintained at 18 °C to 22 °C. The pH of the media was at the start of the test. For each concentration 5 replicates were used for biologic testing and 10 replicates were used for the control.

3. Observations
During the experiment visual inspection of plant growth and morphology were observed and unusually observations were recorded on day 7 and 14. In addition to the visual inspection measurements of main shoot length, length of lateral branches, fresh weight and dry weight were recorded.

II. RESULTS AND DISCUSSION

Concentrations of MCPA, fluroxypyr-MHE, fluroxypyr acid and clopyralid were measured for all treatments. The concentrations of fluroxypyr-MHE and fluroxypyr acid were combined and expressed in terms of total acid equivalent of fluroxypyr (the measured fluroxypyr-meptyl concentrations were converted to fluroxypyr-acid equivalents accounting for the molecular weight adjustment (i.e. divided by factor of 1.44) and were used together with the fluroxypyr-acid concentrations to derive the concentrations in terms of fluroxypyr equivalents). Overall the mean measured concentration were 77.5-90.6 % of the nominal concentration for MCPA, 86.4-92.7 % of the nominal concentrations for clopyralid and 65.4-92.1 % of the nominal concentration for fluroxypyr-meptyl (see Table A 1). In order to determine why the measured concentrations were below 80 % for MCPA additional sediment analysis were conducted and it was found that 6-10 % of the original MCPA dose was bound within the sediment. As the stability of the formulation was below 80 % during the course of the study all effects endpoints were reported based on mean measured concentrations.

Table A 1: Mean measured and nominal concentrations

Nominal Concentration				Mean Measured (geometric mean day 0-14)		
Kinvara	MCPA	Clopyralid	Fluroxypyr meptyl	~~MCPA~~ Clopyralid	~~Clopyralid~~ MCPA	Fluroxypyr meptyl
Control	Control	Control	Control	<LOQ	<LOQ	<LOQ
12	2.49	0.298	0.788	0.257	1.93	0.695
38	7.88	0.942	2.49	0.874	6.36	1.72
120	24.9	2.98	7.87	2.59	21.8	5.14
380	78.6	9.41	24.9	8.39	70.4	20.4
1200	249	29.7	78.5	25.7	225	72.4

The 14-day effects endpoints are presented on Table A 2 in terms of mean measure concentrations (µg/L). The most sensitive effect was noted for changes in fresh weight with the E_rC_{50} (fresh weight) endpoint being 221 µg formulation/L.

Table A 2: Summary of Results

Measured Effects	Endpoint (µg Kinvara/ L) 14 d mean measured			
	NOEC	LOEC	E_yC_{50}	E_RC_{50}
Total shoot length	105	30.7	137	296
Fresh Weight	30.7	105	126	**221**
Dry Weight	105	340	>1087	>1087

Effects on total shoot length: Effects on total shoot length were concentration dependent. Growth rate was inhibited up to 79.2% and yield up to 91.8% compared to the control. An EC50 value of 296 µg Kinvara/L for growth rate and 137 µg Kinvara/L for yield were determined for total shoot length. The NOEC for both growth rate and yield was 30.7 µg Kinvara/L (Table A3).

Table A 3: Mean percent inhibition and effective concentrations of total shoot length growth rate and yield of *Myriophyllum spicatum* following exposure to the test item for 14 days.

Mean measured conc. [µg Kinvara/L]	Growth rate total shoot length	Yield total shoot length
	Inhibition [%]	
Control	0.00	0.00
9.30	-2.09	-4.34
30.7	1.60	2.19
105	24.4	45.0
340	53.5	73.5
1087	79.2	91.8
	Effective and threshold concentrations [µg Kinvara/L]	
EC_{50} (95% cl)	296 (254 – 340)	137 (106 – 169)
EC_{20} (95% cl)	86.5 (66.3 – 106)	49.8 (32.4 – 66.0)
EC_{10} (95% cl)	42.1 (28.9 – 55.8)	27.6 (15.1 – 40.4)
LOEC	105	105
NOEC	30.7	30.7

- negative values indicate an increase of the observed parameter relative to the controls.

Effects on fresh weight: Effects on fresh weight of the shoots above sediment were concentration dependent. Growth rate was inhibited up to 82.7% and yield up to 90.3% compared to the control. An EC50 value for growth rate of 221 µg Kinvara/L and 126 µg Kinvara/L for yield were determined for fresh weight. The NOEC for growth rate was suggested to be 30.7 µg Kinvara/L while the NOEC for yield was 9.30 µg Kinvara/L (Table A4)

Table A 4: Mean percent inhibition and effective concentrations of fresh weight growth rate and yield in *Myriophyllum spicatum* following exposure to the test item for 14 days.

Mean measured conc. [µg Kinvara/L]	Growth rate fresh weight	Yield fresh weight
	Inhibition [%]	
Control	0.00	0.00
9.30	-2.95	-5.38
30.7	6.72	12.0
105	33.5	47.8
340	62.1	76.7
1087	82.7	90.3
	Effective and threshold concentrations [µg Kinvara/L]	
EC_{50} (95% cl)	221 (174 – 282)	126 (99.4 – 159)
EC_{20} (95% cl)	60.6 (38.3 – 83.4)	39.6 (25.4 – 53.8)
EC_{10} (95% cl)	30.8 (16.3 – 46.9)	21.7 (11.8 – 32.2)
LOEC	105	30.7
NOEC	30.7	9.30

- negative values indicate an increase of the observed parameter relative to the controls.

Effects on dry weight: Effects on dry weight of the shoots above sediment increased concentration dependent at concentrations higher than 105 µg Kinvara/L. Growth rate was inhibited up to 22.0% and yield up to 30.1% compared to the control (Table A5). Due to the inhibition lower than 50%, no ECx values were determined. The NOEC for both growth rate and yield was 105 µg Kinvara/L (Table A5).

Table A 5: Mean percent inhibition and effective concentrations of dry weight growth rate and yield in *Myriophyllum spicatum* following exposure to the test item for 14 days.

Mean measured conc. [µg Kinvara/L]	Growth rate dry weight	Yield dry weight
	Inhibition after 14 days exposure [%]	
Control	0.00	0.00
9.30	-11.1	-18.55
30.7	-4.52	-7.07
105	-4.74	-10.28
340	15.8	23.80
1087	22.0	30.07
	Effective and threshold concentrations [µg Kinvara/L]	
EC_{50} (95% cl)	> 1087	> 1087
EC_{20} (95% cl)	Not determined due to effects below 50%.	
EC_{10} (95% cl)		
LOEC	340	340
NOEC	105	105

- negative values indicate an increase of the observed parameter relative to the controls.

III. CONCLUSIONS

Based on the effect on fresh weight, the E_rC_{50} for the effect of Kinvara on *Myriophyllum spicatum* was determined to be 221 µg test item/L.

(Seeland-Fremer., A & Wydra, V. (2014))

A 2.2.2 KCP 10.2.2 Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms

A 2.2.3 KCP 10.2.3 Further testing on aquatic organisms

A 2.3 KCP 10.3 Effects on arthropods

A 2.3.1 KCP 10.3.1 Effects on bees

A 2.3.1.1 KCP 10.3.1.1 Acute toxicity to bees

A 2.3.1.1.1 KCP 10.3.1.1.1 Acute oral toxicity to bees

10.3.1.1.1/01 Ehmke (2014)

Comments of zRMS:	The study was accepted. The proposed oral LD_{50} (48 hr) >210 µg product/bee and the contact LD_{50} (72 hr) >200 µg product/bee are accepted. This study could be used in risk assessment.

Report:	10.3.1.1/01, A. Ehmke (2014)
Title:	Effects of Kinvara (acute contact and oral) on honey bees (*Apis mellifera* L.) in the laboratory
Document No:	Project Code: 89201035
Guidelines:	OECD 213
GLP	Yes (certified laboratory)

Test Item:	Kinvara (XXXX herbicide 233)
Purity:	27.3 g/L clopyralid, 73.3 g/L fluroxypyr-meptyl 233.6 g/L MCPA
Description:	Yellow/amber liquid
Lot No./Batch No. :	13-3601

Test system

Organism (Species):	Honey bee (*Apis mellifera*)
Study Type:	Acute oral and contact
GLP Status:	GLP
Guidelines followed:	OECD 213 and 214 (1998)
Study design:	Definitive test for acute contact and oral. Duration of the contact test- 72 hours Duration of the oral test-48 hours
Information on bee colony (health etc):	Worker bees; collected from the outer combs of the colony on the morning of use. Queen right, disease free colony.
Observations	Assessment of mortality and sub lethal effects after 4, 24, 48 and 72 hours (last time point for contact test only).
Test concentrations:	Contact test: 0 (control-tap water with 0.5 % Adhasit*), 200 µg product/bee. Oral test: 0 (control-50 % sucrose solution), nominal dose of 200 µg product/bee which is equivalent to a measured dose of 210 µg product/bee. *to improve spreading of the test droplet onto the bees thorax)
No of replicates (test substance and control)	Oral: 5 replicates (10 bees in each- total of 50 bees) Contact: 5 replicates (10 bees in each- total of 50 bees)
Feeding method:	Contact- After application of the test substance, bees were provided with a continuous supply of 50 % sucrose solution. Oral- Bees were fed with 50 % w/v aqueous sucrose solution containing the test item. After <2 hours, the treated feeding tubes were replaced with tubes containing untreated 50% sucrose solution for the remainder of the study.
Environmental conditions:	Temperature: 25 °C Relative humidity: 32-77 % Photoperiod: 24 h darkness, observations made under light.
Test unit:	Stainless steel cages (length 10 cm x height 8.5 cm x width 5.5 cm). At the front of the cages was a removable glass sheet. The bottom was perforated with ventilation holes and the inner walls were lined with filter paper.
Reference substance:	Perfekthion BAS 152 11 I (a.s dimethoate 400 g/l). Oral- 0.06, 0.08, 0.16, 0.31 µg test item/bee Contact-0.10, 0.15, 0.20 and 0.30 µg test item/bee

Methodology

Contact study:

A definitive test consisting of a tap water + 0.5 % Adhasit control and a single concentration of 100 µg product/bee. The test item was dissolved in tap water with 0.5% Adhasit to improve spreading of the test item). For the contact toxicity test, treatments consisted of five replicates of the treatment dose and control, with 10 honeybees per replicate (total 50 bees). Bees were lightly anaesthetised with CO_2 and were dosed by topical application of a 5 µL droplet of the test solution to the thorax of each bee. After application, the bees were returned to the test cages and fed with a 50% sucrose solution *ad libitum*.

Oral study:
A definitive test consisting of a 50 % sucrose solution control and a single concentration of 100 µg product/bee. For the test concentration, the test item was dissolved in a 50 % sucrose solution. All bees were starved for 15 minutes prior to the beginning of the test. Treatments consisted of five replicates for the treatment dose and control, with 10 honeybees per replicate (total 50 bees). The test item was offered in syringes. It is assumed that due to the honeybees' behaviour of sharing the food, that each honeybee consumes the same dose. The test item was left in the cages until it was consumed (<2 hours), after this time the amount of test solution consumed by each replicate was determined by weighing the syringes before and after feeding. When all the test material had been consumed, feeding tubes were replaced by tubes containing untreated 50 % sucrose solution.
Assessments for mortalities and sub lethal effects were carried out at 4, 2 and 48 hours for both tests. For the contact test an additional assessment at 72 hours was also conducted.
Temperature and humidity were monitored throughout and the test was conducted in the dark.

Results

Contact toxicity
After 4 hours, 10 bees were found to be moribund in the test concentration. Affected bees were observed after 24 hours (2 bees), 48 hours (5 bees) and 72 hours (4 bees).

Table 10.3.2.1-1: Acute contact toxicity

Nominal test concentration (µg product/bee)	Mean cumulative mortality (%)			
	4 hours	24 hours	48 hours	72 hours
0 (Control)	0	0	0	4
200	0	14	28	30
LD_{50}	>200 µg product/bee			

Oral toxicity
No behavioural abnormalities were observed during the test.

Table 10.3.2.1-2: Acute oral toxicity

Nominal test concentration (µg product/bee)	Measured test concentration (µg product/bee)	Mean cumulative mortality (%)		
		4 hours	24 hours	48 hours
0 (Control)	0 (Control)	0	0	0
200	210	0	4.0	4.0
LD_{50}		>210 µg product/bee		

The 24-hour contact and oral LD_{50} values for dimethoate technical were 0.17 and 0.14 µg/bee, respectively.

Conclusions
All validity criteria were met for this study. Oral and contact control mortality was <15%. In the positive toxic reference studies the effect levels indicated that the honeybees were responding normally within the test system.

The 48 hour LD_{50} for honeybees exposed to Kinvara in an acute oral test was >210 µg product/bee. The 72 hour LD_{50} for honeybees exposed to Kinvara in an acute contact test was >200 µg product/bee.

10.3.1.1.1/02 Wright (2019)

Comments of zRMS:	The study was accepted. The validity criteria were met: there was a single mortality observed in the control group (1/40 = 2.5%), meeting the control validity criterion (<10% mortality). Mortality in the toxic reference treatment was 97.5% after 24 hours meeting the toxic reference validity criterion (>50% mortality). The deviations from study were noted. They could be acceptable without effects on final results/conclusion from the study. The following endpoints were derived: • oral LD_{50} (48 hr) >512.8 µg product/bee; NOED = 234.6 µg product/bee • contact LD_{50} (48 hr) >1281 µg product/bee; NOED = 1281 µg product/bee .

Reference:	KCP 10.3.1.1.1/02
Report	Kinvara: Acute contact and oral toxicity to bumblebees (*Bombus terrestris*), Emma Wright, 2019, Study number FR/001855-09
Guideline(s):	Yes. OECD 246: Guidelines for the testing of chemicals: Bumblebee, acute contact toxicity test 2017 OECD 247: Guidelines for the testing of chemicals: Bumblebee, acute oral toxicity test 2017
Deviations:	Yes *1. Contact control dosing procedure*: control bees were gassed but not dosed with any test solution; 10 wetting agent control and 10 undosed control bees were gassed together in each replicate. This deviation from the study plan is not thought to impact on the integrity or outcome of the study as all the bees were still gassed for the same amount of time (~ 2 minutes after the gas had taken effect) which was long enough to ensure the wetting agent control bees were suitably anaesthetised for dosing. Additionally, all the bees were checked to be fully recovered following dosing. *2. Test item dosing solutions*: dosing solutions for the oral test were made up directly in 50 % w/v sucrose solution. This deviation is not considered to have had any impact on the integrity or outcome of the study as the solubility of the test item was assessed through a separate non-regulatory solubility study in addition to the dosed feed solutions being visually assessed to be homogenous in the sucrose solution and so a water or solvent stock was not required. *3. Samples taken for dose verification*: Six aliquots 0.5 mL of each solution was collected and weighed instead of 1.0 mL. The 0.5 mL samples were suitable volumes for analysis and so this had no impact on the integrity or outcome of the study. *4. Oral test environmental conditions*: oral test the environmental chamber was initially set at 33 °C and re-set to the required 25 °C on the 30th July. The maximum deviation was 8 °C for 48 hours before dosing. As the undosed and toxic reference control bees responded normally this temperature deviation is not thought to have impacted on the integrity or outcome of the study. *5. Oral test – starvation period*: bees were starved for ~ 4 - 5 hours. As the bees were all visually assessed to be healthy and active when the dosed feed was given this increased starvation period did not negatively impact on the

integrity or outcome of the study and will have encouraged the bees to consume more of the test feed over the feeding period.
6. Sample storage: The freezer the samples were stored in had a single deviation from this storage requirement which lasted for ~1 hour with a maximum temperature recorded at -17.1 °C (instead of the requested -18 °C). As this deviation is so short and by less than 1 °C of the required temperature it had no impact on the samples.

GLP:	Yes
Acceptability:	Yes

Materials and methods

1. ***Test Material*:** Kinvara
   - **Description:** Micro Emulsion (ME) composed of
     i. MCPA – 226.6g/L
     ii. Fluroxypyr – 49.3 g/L
     iii. Clopyralid – 26.9g/L
   - **Density:** 1.122 g/mL
   - **CAS #:**
     i. MCPA 94-74-6
     ii. Fluroxypyr 69377-81-7
     iii. Clopyralid 1702-17-6
2. ***Vehicle and/or positive control*:** Reference item: Danadim progress (dimethoate). CAS Number: 60-51-5
3. **Test animals -**
   - **Species:** Bumblebee (*Bombus terrestris*)
   - **Age:** Adult worker bumblebees
   - **Source:** Colonies were obtained from commercial suppliers Biobest, Belgium N.V., (obtained through Agralan, UK). The colonies of bees were accepted onto the study following an assessment of their suitability.
   - **Acclimation period:** Overnight
   - **Feeding:** 50% (w/v) aqueous sucrose solution
   - **Housing:** The test units used for both the contact and oral dosing tests were Nicot queen rearing cages with a syringe feeder inserted through one end. The feeders were 2 mL syringes with the tip cut at an angle to enlarge the opening.
   - **Loading:** *Contact Test*: 8 treatments were used which comprised: 5 treatments of formulation Kinvara, 1 treatment of the toxic reference standard (Danadim progress (dimethoate)) and 2 controls (un-dosed (anaesthetised but not dosed) group and wetting (Triton X100) agent only). Each treatment was replicated 3 times and each replicate consisted of 10 honeybees (total 30 bees per dose group).
     Oral test: 7 treatments were used which comprised: 5 treatments of formulation Kinvara, 1 treatment of the toxic reference standard (Danadim progress (dimethoate)) and 1 control (undosed 50 % w/v aqueous sucrose solution). Each treatment consisted in 40 replicates (bees) per dose.
4. ***Environmental conditions* -**

Temperature:	25 ± 2 °C (oral test); 25 ± 8 °C (contact test)
Humidity:	60 ± 20 % (contact and oral test)
Photoperiod:	Dark
In life dates:	10/07/19 – 03/09/19

Study design

The test was performed using adult worker bumblebees (*Bombus terrestris*).

Test item dose rates for both the contact and oral tests were based on the results of pre-study non-regulatory range finding tests and discussion with the sponsor's representative.

A single component a.s. clopyralid was selected for dose verification. Analysis of samples of the dosing solutions confirmed that this active substance was within 20 % of the expected concentrations for both the contact and the oral test and so concentrations are quoted as nominal throughout this report.

All test solutions were prepared and used on the day of dosing.

Contact Test

Bees were dosed with 5 µL of treated/control application solution placed onto the dorsal thorax of each bee. Application solutions were made up in Triton x-100 in demineralised water at 1g/L.

Test item was applied at 5 dose rates: 78.13, 156.3, 312.5, 625.0, and 1281µg formulation/bee. This is equivalent to 15.78, 31.56, 63.13, 126.3, and 258.8 µg MCPA per bee; 3.446, 6.891, 13.78, 27.56, and 56.49 µg fluroxypyr per bee; and 0.0827, 0.1654, 0.3307, 0.6614, and 1.356 µg clopyralid per bee (based on a W/W purity of 20.2 % MCPA, 4.39 % fluroxypyr and 2.40 % clopyralid, respectively, in the formulation Kinvara as stated in the CofA). Each treatment was replicated 3 times and each replicate consisted of 10 bees (total 30 bees per dose group).

Two sets of controls were used in the contact test. One un-dosed (anaesthetised but not dosed) group and one group to which only the wetting (Triton X100) agent was applied.

The toxic reference treated group bees were dosed with 2 µL of the toxic reference dosing solution made up in 1g/L aqueous Triton x-100 which resulted in a single dose rate of 10 µg a.s./bee.

Oral Test

On day 0 healthy bees were assigned to treatment groups in no particular order, to treatment groups of 40 replicates (bees) per dose, and labelled with the treatment group.

After the 4-5 hours starvation period, all bees were offered a dose of 40 µL treated/control diet. Treatments were made up in in 50 % w/v sucrose solution. The test Item was offered at 5 nominal dose rates: 62.5, 125, 250, 500, and 1000 µg formulation/bee.

The mean measured dose consumed by the bees in the test item treated groups, excluding non-feeders (a non-feeder is defined as a bee which consumed less than 80 % of the mean feed uptake for their treatment group), were calculated to be 60.41, 118.0, 234.6, 315.5, 512.8 µg formulation/bee. 12.20, 23.84, 47.40, 63.73, and 103.6 µg MCPA per bee; 2.664, 5.204, 10.34, 13.91, and 22.61 µg fluroxypyr per bee; and 1.450, 2.833, 5.629, 7.572, and 12.31 µg clopyralid per bee (based on a W/W purity of 20.2% MCPA, 4.39 % fluroxypyr and 2.40 % clopyralid, respectively, in the formulation Kinvara as stated in the CofA).

The reference item was offered at a single dose rate of 4 µg a.s./bee which resulted in an average consumption (excluding non-feeders) of 3.552 µg a.s./bee.

One set of controls was used in the oral test. Control bees were offered undosed 50 % w/v aqueous sucrose solution.

Observations

Mortality in the contact test was assessed at 4 (± 30 mins), 24 and 48 hours (±1 hour) after dosing and in the oral toxicity test mortality was assessed at 4 hours (± 30 mins), 24 and 48 hours (± 1 hour) after the time that the test feeders were given. In addition to mortality assessments, assessments of sub-lethal effects were made and numbers of bees stumbling or knocked down were recorded at each time period for both the contact and oral tests. The condition of the bees was defined as follows:

Alive/unaffected - Alive and apparently unaffected

Stumbling/affected - Moving but poorly co-ordinated.

Knocked down/moribund - Alive but immobile, i.e. twitching.

Dead - No longer moving or responding to stimuli.

Any other abnormal behaviour, such as agitation, was also recorded, but only in the toxic reference treatment.

24 and 48-hour mortality rates were the measured toxic endpoints.

As the mortality in the test item treated dose rates for each test did not change significantly (by >10 %) between 24 and 48 hours, both tests were stopped at 48 hours.

Statistical analyses

For the contact tests the statistical analysis was performed using the mortality data from all test organisms, however, for the oral test only the statistical analysis was carried out using data from for those bees identified as 'feeders' (i.e. those bees which had consumed more than 80 % of the mean feed uptake for their treatment group are reported), non-feeders were excluded from the analysis.

The LOEC/D (lowest observable effect concentration/dose) and NOEC/D (no observable effect concentration/dose) were estimated in R v3.2.2. The LOEC/D and NOEC/D were tested using a step-down test for a trend in proportions, equivalent to a Cochran-Armitage test.

It was not possible to fit a model to the data for either test at any end point to produce estimates of the LDx values with confidence intervals.

Results and Discussion

Contact test

There was no mortality observed in the undosed and triton controls after 48 hours, meeting the control validity criterion (<10 % mortality). Mortality in the toxic reference treatment was 90 % after 48 hours meeting the toxic reference validity criterion (>50 % mortality).

Table 10.3.2.1-3: Percent cumulative mortality of bumblebees in the Control, Toxic Reference and Test Item treated groups over 48 hours – Contact Test (n = 30)

Nominal Dose (µg formulation/bee)	Time (hours)			
	0 (set-up)	4	24	48
Undosed Control	0	0	0	0
Triton Control	0	0	0	0
78.125	0	0	3.3	3.3
156.25	0	0	3.3	6.7
312.5	0	3.3	13.3*	16.7*
625	0	0	0	6.7*
1250	0	0	0	3.3
Dimethoate (10 µg a.s./bee)	0	0	76.7	90.0

*significantly different from control mortality (P<0.05) assessed at 24 and 48 hours for Test Item, toxic reference not assessed.

Oral test

There was a single mortality observed in the control group (1/40 = 2.5 %), meeting the control validity criterion (<10 % mortality). Mortality in the toxic reference treatment was 97.5 % after 24 hours meeting the toxic reference validity criterion (>50 % mortality).

When the non-feeder bees were removed from the data: there was no mortality observed in the control group, meeting the control validity criterion (<10 % mortality), and mortality in the toxic reference treatment was 100 % after 24 hours meeting the toxic reference validity criterion (>50 % mortality).

Table 10.3.2.1-4: Percent cumulative mortality of bumblebees in the Control, Toxic Reference and Test Item treated groups, excluding non-feeders, over 48 hours – Oral Test

Nominal Dose Consumed (µg formulation/bee)	Time (hours)				
	n	0 (set-up)	4	24	48
Control	39	0	0	0	0
62.5	33	0	0	0	0
125	30	0	0	0	0
250	28	0	0	0	0
500	18	0	0	11.1*	11.1*
1000	24	0	0	4.2*	12.5*
Dimethoate (4 µg a.s./bee)	33	0	12.1	100	100

*significantly different from control mortality (P<0.05) assessed at 4, 24 and 48 hours for Test Item, toxic reference not assessed.

The number of non-feeders was high in the test item treated groups, especially at higher doses (22 non-feeders at 500 µg formulation/bee and 16 at 1000 µg formulation/bee). This may indicate an anti-feedant or repellent action of the formulation.

Conclusion

Contact

LDx:

There was less than 50 % mortality observed within the highest dose rate of the test item treated bees at any observation time point thus there was insufficient data to estimate lethal dose X % values (LD10, 20 or 50) statistically. By observation it can be stated that; the contact LD50 is > 1281 µg formulation/bee equivalent to >259 µg a.s MCPA/bee, >56.5 µg a.s fluroxypyr/bee and >1.356 µg a.s clopyralid/bee (the highest dose rate tested in the contact test).

NOED/LOED:

Under the conditions of this study Lowest Observable Effect Dose (LOED) or the No Observable Effect Dose (NOED) was determined using a step down test for a trend in the proportions of bee mortalities. This is equivalent to a Cochran-Armitage test. The NOED was found to be the highest dose tested and the LOED must, therefore, be greater than this.

Table 10.3.2.1-5: Contact test 24 & 48 hour Contact NOEDs & LOEDs

Component*	NOED	LOED
formulation	1281 µg formulation /bee	>1281 µg formulation /bee
MCPA	259 µg a.s. /bee	>259 µg a.s. /bee
Fluroxypyr	56.5 µg a.s. /bee	>56.5 µg a.s. /bee
Clopyralid	1.356 µg a.s. /bee	>1.356 µg a.s. /bee

*

All results for the active substances, MCPA, fluroxypyr and clopyralid are based on the concentrations given on the certificate of analysis

Oral: Excluding non-feeders (bees consuming <80% of the mean uptake for their treatment group):

LDx:

There was less than 50 % mortality observed within the highest dose rate of the test item treated bees at any observation time point thus there was insufficient data to estimate lethal dose X % values (LD10, 20 or 50) statistically. By observation it can be stated that; the oral LD50 is > 512.8 µg formulation/bee equivalent to >103.6 µg a.s MCPA/bee, >22.61 µg a.s fluroxypyr/bee and >12.31 µg a.s clopyralid/bee (the highest dose rate tested in the oral test).

NOED & LOED:

Under the conditions of this study Lowest Observable Effect Dose (LOED) or the No Observable Effect Dose (NOED) was determined using a step down test for a trend in the proportions of bee mortalities. This is equivalent to a Cochran-Armitage test. The NOED was found to be 234.6 µg formulation/bee and the LOED was 315.5 µg formulation/bee.

Table 10.3.2.1-6: Oral test 24 & 48 hour Contact NOEDs & LOEDs

Component*	NOED	LOED
formulation	234.4 µg formulation /bee	315.5 µg formulation /bee
MCPA	47.40 µg a.s. /bee	63.73 µg a.s. /bee
Fluroxypyr	10.34 µg a.s. /bee	13.91 µg a.s. /bee
Clopyralid	5.629 µg a.s. /bee	7.572 µg a.s. /bee

* All results for the active substances, MCPA, fluroxypyr and clopyralid are based on the concentrations given on the certificate of analysis

A 2.3.1.1.2 KCP 10.3.1.1.2 Acute contact toxicity to bees

See previous section.

A 2.3.1.2 KCP 10.3.1.2. Chronic toxicity to bees

Comments of zRMS:	The study was accepted. The validity criteria were met: there were no mortalities in the control group meeting the validity criterion of <15% mortality. In the toxic reference group 100% mortality was observed, meeting the validity criterion of > 50% mortality at day 10. The noted deviations did not affect the final study results. The following endpoints were calculated: LC_{50} = 4.848 g formulation/kg food; LD_{50} = 1224 µg formulation/bee/d LDD_{50} = 124 µg formulation/bee/day

Reference:	KCP 10.3.1.3/01
Report	Kinvara: 10 Day chronic oral toxicity test for adult honey bees (*Apis mellifera* L.), Wilkins, S., 2019, study No FR/001855-10.
Guideline(s):	Yes. OECD 245: Guideline for the Testing of Chemicals: Honey Bee (*Apis mellifera* L.), chronic oral toxicity test (10 Day Feeding Test in the Laboratory) 2017.
Deviations:	Yes *1. K_18 excluded from analysis*: Cage K_18 in the 12500 mg formulation/kg treated group was excluded from analysis due to unexplained high mortality within the first 3 days of dosing following a feeder spill/use of empty feeder. As there was a clear dose response using the remaining cages at each dose rate this exclusion is not thought to impact on the integrity or outcome of the study. *2. Samples taken for dose verification*: Samples were labelled A, B, C, D, E and F instead of 3 A and 3 B samples per treatment. Moreover, for the stock solution there was insufficient volume to allow 6 aliquots of 1 mL to be collected. Therefore, 0.5 mL of the stock solution was taken and weighed. This was discussed with the analytical chemist and the 0.5 mL stock samples were corrected for at the dilution stage of the analysis. The analytical results were satisfactory and so this had no impact on the integrity or outcome of the study. *3. Sample storage*: The samples for chemical analysis were required to be stored at <-18 °C. The freezer the samples were stored in had a single deviation from this storage requirement which lasted for ~1 hour with a maximum temperature recorded at -17.1 °C. As this deviation is so short and by less than 1 °C of the required temperature it had no impact on the samples.
GLP:	Yes.
Acceptability:	Yes

Materials and methods

1. ***Test Material*:** Kinvara

Description:	Micro Emulsion (ME) composed of i. MCPA – 226.6g/L ii. Fluroxypyr – 49.3 g/L iii. Clopyralid – 26.9g/L
Density:	1.122 g/mL
CAS #:	i. MCPA 94-74-6 ii. Fluroxypyr 69377-81-7 iii. Clopyralid 1702-17-6

2. ***Vehicle and/or positive control*:** Reference item: Danadim progress (dimethoate). CAS Number: 60-51-5

3. **Test animals -**

Species:	Honey bee, *Apis mellifera* L.
Age:	Newly emerged adult workers (< 48 hours old)
Source:	Home apiary, FERA National Bee Unit
Acclimation period:	Overnight
Feeding:	Bees in each test unit were given ad libitum access to untreated 50 % w/v aqueous sucrose solution as a food source.
Housing:	The test units consisted of an inverted ventilated plastic deli pot lined with a filter paper. A hole was made in the side wall so that a feeder could be placed and held horizontally, allowing the bees access to the sucrose solution while preventing leakage of the feed solution.
Loading:	The test comprised 1 control groups (Untreated 50 % (w/v) sucrose solution), 5 test item treatment groups (nominal concentrations of 12500, 6250.0, 3125.0, 1562.5 and 781.25 mg formulation/kg 50 % w/v sucrose feed) and 1 toxic reference item group (dimethoate, 1.0 mg a.s./kg 50 % w/v sucrose feed). 3 cages of 10 bees were used for each treatment group.

4. ***Environmental conditions* -**

Temperature:	32.4 to 33.1 °C
Humidity:	55.0 to 65.7 %
Photoperiod:	Dark
In life dates:	10/06/2019 – 30/07/2019

Study design

A test item stock solution was prepared daily for each dosing day in deionised water. A further range of dosing dilutions were prepared from this stock each day in deionised water, creating the daily dosing dilutions. Dosed feed solutions were made up daily by adding 50 µl of the appropriate dosing solution per g of 50 % (w/v) aqueous sucrose solution. The dosed feed was weighed and provided to the bees within 2 hours of the dosing solutions being made up.

The toxic reference stock solutions were made up in deionised water on day 0, and then stored in a refrigerator between 6 + 2 °C for the remainder of the test. Dosed feed solutions were made up every 3-4 days from this stock by adding 50µl of the dosing stock per g of 50 % (w/v) aqueous sucrose solution.

The test item dose rates were chosen based on the results of a separate non-regulatory solubility and range finding study and discussion with the sponsor's representative.

The test was run as a dose response test at five nominal concentrations of 781.25, 1562.5, 3125.0, 6250.0, and 12500 mg formulation/kg 50 % (w/v) aqueous sucrose solution. Each cage of bees was offered approximately 1.5mL treated/control diet each day. The mean measured doses consumed by the bees in the test item treated groups were calculated to be 31.39, 56.29, 103.9, 145.4, and 209.1 µg formulation/bee/day.

The toxic reference item was offered at a rate of 1 mg a.s./kg 50 % (w/v) aqueous sucrose solution. The mean measured dose consumed by the bees in the reference item treated group was calculated to be 0.02 µg a.s./bee/day.

The control group was fed untreated 50 % (w/v) aqueous sucrose solution.

Worker bees that emerged overnight were inserted into test units 10 at a time. Bees in each test unit were given ad libitum access to untreated 50% w/v aqueous sucrose solution as a food source.

3 cages of 10 bees were used for each treatment group.

Observations of mortality and behaviour were recorded at 24-hour intervals (± 120 minutes) after initial set up for 10 days.

The number of live, knocked down, stumbling and dead bees were recorded. The condition of the bees was defined as follows:

Alive - Alive and apparently unaffected.

Stumbling - Moving but poorly co-ordinated.

Knocked down - Alive but immobile, i.e. twitching.

Dead - No longer moving.

Statistical analyses

The LC/LD/LDD10, 20 & 50, were generated in Genstat version 16.1. by fitting regression models to the concentration, total dose/bee and dose/bee/day data.

Results and Discussion

Table 10.3.3.1-1: Mean uptake µg formulation/bee/day over the course of the test

Treatment group	Nominal concentration (mg formulation/kg)	Mean uptake µg formulation/bee/day over the course of the test[a]											
		day 0-1	day 1-2	day 2-3	day 3-4	day 4-5	day 5-6	day 6-7	day 7-8	day 8-9	day 9-10	Total uptake µg/bee[b]	Mean uptake µg/bee/day[c]
formulation	781.25	8.50	16.89	28.18	28.38	23.53	42.48	43.13	45.51	35.21	42.13	313.94	31.39
	1563.0	24.07	26.33	52.94	58.51	60.99	73.89	69.84	63.24	62.01	71.08	562.90	56.29
	3125.0	52.63	65.30	117.10	87.75	117.40	153.40	117.60	111.70	93.06	122.60	1038.54	103.85
	6250.0	94.04	119.80	203.10	152.30	178.50	207.30	171.30	124.00	109.59	94.40	1454.33	145.43
	12500	77.86	120.40	156.40	156.40	267.20	272.40	275.60	306.10	249.70	-	1882.06	209.12
Toxic reference	1.0 mg a.s./kg	0.030	0.012	0.028	0.022	0.02	0.00	0.03	-	-	-	0.14	0.02

[a] calculated average per living bee, (results displayed are rounded results, calculated from the exact data)
[b] sum of uptake of formulation/bee at test end over the course of the 10 day feeding period
[c] mean of uptake of formulation/bee/day at test end over the course of the 10 day feeding period

Table 10.3.3.1-2: Mean percentage mortality in the control, reference and test item treated groups over 10 days (n = 30 except 12500mg formulation/kg where n=20) (Cage K_18 in the 12500 mg formulation/kg treated group was excluded from analysis due to unexplained high mortality within the first 3 days of dosing following a feeder spill/use of empty feeder.)

Treatment group	Nominal Concentration (mg formulation/kg)	Mean dose µg formulation/ bee/day	Mean Percentage Mortality									
			day 1	day 2	day 3	day 4	day 5	day 6	day 7	day 8	day 9	day 10
Water control	0	-	0	0	0	0	0	0	0	0	0	0
Formulation	781.25	31.39	0	0	0.0	0	3.3	3.3	3.3	3.3	3.3	3.3
	1563.0	56.29	0	0	0	0	0	0	0	0	0	0
	3125.0	103.85	0	0	3.3	3.3	3.3	3.3	3.3	6.7	6.7	10.0
	6250.0	145.43	0	0	0	0	3.3	3.3	23.3	36.7	66.7	83.3
	12500	209.12	0	33.3	63.3	73.3	76.7	90.0	96.7	96.7	100	100
Toxic reference	1.0 mg a.s./kg	0.02 µg a.s./bee/day	0	0	3.3	30.0	86.7	96.7	100	100	100	100

Validity of the test

There were no mortalities in the control group meeting the validity criterion of <15 % mortality. In the toxic reference group 100 % mortality was observed, meeting the validity criterion of > 50 % mortality at day 10.

Conclusion

There was 100 % mortality observed within the highest dose rate of the test item treated bees and so it was possible to fit a model to estimate a lethal concentration/dose/dietary dose for 10, 20, and 50 % (LC/D/DD 10, 20, & 50).

Table 10.3.3.1-3: LCx Results (+/- 95% confidence intervals)

Lethal concentration Values	LC_{10}	LC_{20}	LC_{50}
		(mg/kg)	
Formulation	2.83 (1.987 – 3.426)	3.523 (2.846 – 4.074)	4.848 (4.298 – 5.504)
Component a.s.			
MCPA	0.572 (0.401 – 0.692)	0.712 (0.575-0.823)	0.979 (0.868 – 1.11)
Fluroxypyr	0.124 (0.0873 – 0.151)	0.155 (0.125 – 0.179)	0.213 (0.189 – 0.242)
Clopyralid	0.0678 (0.0476 – 0.0821)	0.0845 (0.0682 – 0.0977)	0.116 (0.103 – 0.132)

Table 10.3.3.1-4: LDx Results (+/- 95% confidence intervals)

Lethal dose values	LD_{10}	LD_{20}	LD_{50}
		(µg/bee)	
Formulation	870 (671.5 – 989)	991 (836.9 – 1092)	1224 (1128 – 1315)
Component a.s.			
MCPA	176 (136 – 200)	200 (169 - 221)	247 (228 – 266)
Fluroxypyr	38.2 (29.5 – 43.5)	43.5 (36.8 – 48.0)	53.8 (49.6 – 57.8)
Clopyralid	20.9 (16.1 – 23.7)	23.8 (20.1 – 26.2)	29.3 (27.1 – 31.5)

LDDx Results (+/- 95% confidence intervals)

A 2.3.1.3 KCP 10.3.1.3 Effects on honey bee development and other honey bee

KCP 10.3.1.3/01 Ehmke (2014)

Comments of zRMS:	The study was accepted. The validity criteria were met: *Mean control mortality* of the adult bees from day 0 after application to day 21 differed between 3.3 and 21.7 dead bees per colony. As the overall mean mortality in the control group after application was low (11.6 dead bees/colony/day) and comparable to the situation before treatment (16.9 dead bees/colony/day), this value can be empirically regarded to be within the range of normal mortality levels of colonies of the employed size under field conditions. In addition, a mean of 0.12 dead pupae per colony per day were found during the 21 days post-application period. This value can be considered to represent a biologically typical mean number of dead pupae over a period of 21 days. *Reference Item Mortality*: There was a high number of impacted bee brood, which resulted in 76.4 % mean loss of the initial observed cells (90.9% eggs, 69.3 % young larvae and 68.7 % old larvae stages, respectively). The termination rates of the different brood stages were statistically significantly higher compared to the control. Thus, the reference item values were on an absolute scale sufficiently high to demonstrate the sensitivity of the test system and the validity of the test conditions. Application of the reference item Insegar (0.75 g fenoxycarb/L) did result in an increased number of dead pupae/larvae after application. A mean of 12.0 dead pupae/larvae per day and colony was found between application and test termination (day 21 after feeding). This was statistically significant compared to the value of the control colonies. No deviations to the study plan occurred during the course of this study.

The following honeybee brood feeding test was performed on Kinvara and is provided in support of the assessment and has not been previously evaluated.

Report:	KIIIA1 10.4.6.1/01, A. Ehmke (2014)
Title:	Study on the Effects of Kinvara on Honey Bee Brood (*Apis mellifera L.*) – Brood Feeding Test -
Document No:	Project Code: 89201031
Guidelines:	Oomen P.A., de Ruijter, A. & van der Steen, J., 1992: Method for honey bee brood feeding tests with insect growth-regulating insecticides, OEPP/EPPO Bulletin 22:613-616 (1992) Current recommendations of the AG Bienenschutz (Schmitzer, Lückmann, 2013), "Evaluation and im- provement of the Oomen bee brood test"; Poster Presentation at the SETAC GLB Conference in Essen, Germany, 2013 and the 8th SETAC Europe Special Science Symposium, Brussels, Belgium, 2013.
GLP	Yes (certified laboratory)

Executive summary:
The effects of Kinvara on honey bee brood development was determined at two test concentrations (2.00 L/ha and 3.00 L/ha) using the method of Oomen *et al.* (1992) and current recommendations of the AG Bienenschutz (2013). Three bee colonies were used per treatment group. The test and reference item solutions were mixed with ready-to-use sugar syrup and applied to the bee colonies via a feeding trough. Ontogenesis of a defined number of honey bee eggs, young- and old larvae was observed for a

period of 21 days following the application for each treatment group and colony. This was assessed one day before the application, by selecting one (or several) brood comb(s) of each colony and by taking a digital photo of this (these) brood comb(s). After saving the photo-file on a computer, eggs, young- and old larvae were marked at this first Brood area Fixing Day (BFD0). For each subsequent brood assessment (BFDn), again, the same comb(s) was (were) selected from the respective colony and another digital photo was taken, in order to investigate the progress of brood development. Ontogenesis of the bee brood was observed for a period of 21 days after application (*i.e.* 22 days following BFD 0). Mortality of adult bees and pupae was also assessed.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: Kinvara (XXXX Herbicide 233)

Description: Yellow/Amber liquid

Lot/Batch #: 13-3601

Purity: 233.6 g/L MCPA
73.3 g/L fluroxypyr-meptyl
27.3 g/L clopyralid

Stability of test compound: Not stated.

2. Vehicle and/or positive control: Vehicle: ready-to-use sugar syrup (Apiinvert)
Positive control: Pure sugar syrup (Apiinvert) was used for the controls

3. Test animals -

Species: Worker honey-bees, carnica strain **(***Apis mellifera L.)*

Age: All ages and all stages.

Source: Institute of Organic Industry, Branch Pszczyna, Poland. in-house culture.

Environmental conditions -

Temperature: Natural conditions

Photoperiod: Natural conditions

Relative Air Humidity: Natural conditions

B. STUDY DESIGN AND METHODS:

1. In-life dates: 7th June 2014 –1st July 2014

2. Experimental treatments

Well-fed and queen right colonies were chosen with each colony occupying two boxes with 11 frames each. At the start of the experiment, each colony had 9 - 14 brood combs containing eggs, larvae and capped cells and a sufficient amount of honey and pollen. The colonies contained about 13560 - 18210 adult honey bees.

All colonies were equipped with a dead bee trap at the entrance. All colonies were set up in autumn 2013 and hibernated at the test site and placed at the same location on a meadow. Bees had free access to natural food sources. Due to the season, there were no main flowering, bee attractive crops or flowering weeds in the surrounding area.

One single application was made per colony during the afternoon in order to prevent robbery. 1L contaminated or untreated commercial ready-to-use sugar syrup was used per colony and given to the bees via a feed trough.

Mortatility was assessed from once per day on days 3-21 after application with the dead bees being collected from the dead-bee traps. The collected dead bees were separated during counting into adult worker bees, larvae and pupae.

At simultaneous observation intervals for mortality, sub-lethal effects such as symptoms of poisoning or any abnormal behaviour at the entrance hole in comparison to the control were recorded according to the following categories:

- **m** = moribund
- **a** = affected
- **c** = cramps
- **ap** = apathy
- **ic** = intensive cleaning
- **ag =** aggressiveness

The honey bee brood was assessed at different expected stages during the development, covering one complete development period of the honey bee (*i.e.* 21 days). The development of the bee brood in individually marked cells was observed by photo-documentation of the combs. The failure or incomplete development in individual cells was quantitatively assessed. For the calculation of the brood termination rate, the observed cells were split into 2 categories:

- the bee brood in the observed cell reached the expected brood stage at the different assessment days or was found empty or containing an egg or a small larva after hatch of the adult on BFD +22 = successful development.
- the bee brood in the observed cell did not reach the expected brood stage at one of the assessment days, was empty or food/nectar was stored in the cell during BFD +4 to BFD +16 = termination of the bee brood development.

3. Observations

Mortality and Abnormalities: once per day on days 3-21 after application.

Honey Bee Brood Development: The honey bee brood was assessed at different expected stages during the development, covering one complete development period of the honey bee (*i.e.*21 days).

II. RESULTS AND DISCUSSION

A. FINDINGS

Mortality

During the feeding phase in both treatments with Kinvara (2.00 L/ha and 3.00 L/ha) some dead bees were observed in the feeding troughs. No further direct (acute) toxicity occurred after ingestion of the test item treated sugar syrup. There was no clearly increased mortality in the dead bee traps of the test item treated replicates at any time of the test.

Until test termination, the number of dead worker bees found in the dead bee traps in the colonies of both test item treated groups was comparable to the control group. On each of the assessment days, no statistically significant difference in the number of dead bees in the test item group was detectable when compared to the control values.

Over the entire post-application period from day 0 to day 21, a mean of 7.9 dead bees/colony/day was found in the dead bee traps of the lower rate test item treated colonies (2.00 L/ha). A mean of 6.4 dead bees/colony/day was found in the dead bee traps of the 3.00 L/ha test item treated colonies for the same period. This was in both cases lower compared to a mean of 11.6 dead bees per colony/day, which was found in the control group. A comparison of the overall mean number of dead bees per treatment group for the entire post-application period (day 0 to day 21) did not show a statistically significant difference between the control and both test item treatments.

Table 10.4.6.1-1: Summarised mortality data of the worker bees

	Untreated control			Kinvara [2.00 L/ha]			Kinvara [3.00 L/ha]			Reference Item Insegar [fenoxycarb]		
	dead bees			dead bees			dead bees			dead bees		
time[a]	mean[b]		SD	mean[b]	SD	Statistic	mean[b]	SD	Statistic	mean[b]	SD	Statistic
day -3	15.7	±	10.7	13.3	± 7.0	n.s.	4.0	± 5.3	n.s.	5.3	± 2.5	n.s.

day -2	10.7	±	3.8	5.3	±	6.7	n.s.	3.7	±	3.1	n.s.	6.3	±	4.2	n.s.
day -1	24.3	±	4.0	53.7	±	42.3	n.s.	32.7	±	23.1	n.s.	43.7	±	29.3	n.s.
mean day -3 to day -1 b.a.[c]	16.9	±	6.9	24.1	±	25.9	n.s.	13.4	±	16.6	n.s.	18.4	±	21.8	n.s.
day 0	17.7	±	9.3	10.3	±	13.1	n.s.	4.0	±	4.0	n.s.	20.3	±	11.9	n.s.
day 1	21.7	±	15.9	0.7	±	0.6	n.s.	5.3	±	2.5	n.s.	6.7	±	5.9	n.s.
day 2	4.7	±	3.5	3.3	±	3.2	n.s.	16.0	±	9.2	n.s.	7.7	±	3.5	n.s.
day 3	12.0	±	15.1	13.0	±	13.9	n.s.	11.3	±	5.1	n.s.	4.3	±	4.2	n.s.
day 4	17.3	±	13.1	14.3	±	6.4	n.s.	9.0	±	6.6	n.s.	36.7	±	44.6	n.s.
day 5	8.3	±	6.8	0.0	±	0.0	n.d.	2.7	±	2.3	n.s.	7.3	±	7.1	n.s.
day 6	13.3	±	12.3	1.0	±	1.0	n.s.	5.3	±	6.7	n.s.	6.3	±	4.7	n.s.
day 7	15.0	±	17.3	37.7	±	63.5	n.s.	8.3	±	7.6	n.s.	12.0	±	6.6	n.s.
day 8	12.0	±	11.4	30.0	±	49.4	n.s.	3.0	±	3.6	n.s.	10.0	±	7.8	n.s.
day 9	11.7	±	11.6	0.0	±	0.0	n.d.	4.3	±	4.5	n.s.	4.7	±	4.5	n.s.
day 10	10.7	±	10.0	5.0	±	3.6	n.s.	7.7	±	3.2	n.s.	9.3	±	2.1	n.s.
day 11	10.7	±	5.7	19.0	±	32.9	n.s.	5.7	±	5.1	n.s.	8.0	±	6.0	n.s.
day 12	9.3	±	3.5	0.0	±	0.0	n.d.	3.0	±	3.6	n.s.	6.0	±	5.6	n.s.
day 13	3.3	±	2.5	1.7	±	1.5	n.s.	5.7	±	3.1	n.s.	3.0	±	1.0	n.s.
day 14	5.3	±	2.1	4.7	±	5.7	n.s.	5.0	±	2.0	n.s.	5.3	±	5.5	n.s.
day 15	8.7	±	8.1	5.0	±	2.6	n.s.	4.3	±	2.1	n.s.	12.3	±	13.1	n.s.
day 16	19.7	±	15.5	6.0	±	5.2	n.s.	5.3	±	4.0	n.s.	13.7	±	15.9	n.s.
day 17	12.3	±	7.5	5.0	±	3.5	n.s.	6.3	±	4.5	n.s.	8.3	±	3.8	n.s.
day 18	10.3	±	8.4	2.0	±	1.0	n.s.	4.7	±	4.0	n.s.	7.3	±	5.1	n.s.
day 19	9.3	±	1.2	7.7	±	1.5	n.s.	7.0	±	3.6	n.s.	8.3	±	4.7	n.s.
day 20	13.3	±	8.6	3.0	±	1.0	n.s.	13.3	±	10.0	n.s.	3.7	±	1.5	n.s.
day 21	9.0	±	0.0	5.3	±	4.7	n.d.	3.0	±	1.7	n.d.	8.0	±	7.0	n.d.
daily mean day 0 to day 21 a.a.[d]	**11.6**	**±**	**4.6**	**7.9**	**±**	**9.8**	**n.s.**	**6.4**	**±**	**3.4**	**n.s.**	**9.5**	**±**	**7.2**	**n.s.**

Abnormalities

Mortality of Pupae and Larvae

During the entire period from day 0 following the application until day 21, a mean of 0.03 dead pupae/larvae per day and colony was found in the 2.00 L/ha Kinvara treatment group. Treatment with 3.00 L/ha Kinvara led to a mean of 0.11 dead pupae/larvae per day and colony. In the control group, during the same time period, a mean of 0.12 dead pupae/larvae per day and colony was found. There was no statistically significant difference in the number of dead pupae/larvae between the colonies of both test item groups and the colonies of the control group.

Behavioural Abnormalities

On day 7 following the application 11 affected bees and 3 moribundbees were observed in the dead bee trap of replicate 2 of the 2.00 L/ha Kinvara test item treatment group. Also 3 bees were found to be affected on the same day in the 3.00 L/ha dosing group. As this was the only occurrence of behavioural impairments throughout the test, this single event can be regarded as irrelevant.

No behavioural impairments were noted at any time in the colonies treated with the reference item and in the control group until the end of the test, respectively Bee Brood Development.

Strength of Colony

In all colonies the presence of a live and healthy queen or fresh laid eggs as a sign of the presence of the queen was observed.
All stages of brood (eggs, larvae and capped brood) were found during the pre-application check in all colonies in all treatment groups. In addition, also sufficient nectar and pollen stores were found in each colony as an indication of normal behaviour. The mean strength of the colonies per treatment group, two days before application, ranged between 13560 and 18210 adult bees.

Bee Brood Development
Following the assessment of single cells from the egg stage to the successfully hatched worker bee, the mean termination rate in the lower test item group (2.00 L/ha) was with 3.8 % lower compared to the control group (7.3 %). Termination Rate in the higher Kinvara treatment group (3.00 L/ha) was 12.0 %. This slightly increased mean termination rate in this test item group was not statistically significantly different when compared to control.

Comparing the development success of the young larvae after treatment with the test item to the corresponding control values, a very slightly increased mean termination rate in both test item rates (2.00 L/ha and 3.00 L/ha) was observed. When subjecting the data to statistical analysis the difference in both Kinvara rates was found not to be statistically significant.

Also no effect of the test item on old larvae was found: 4.4 % and 4.9 % of the marked old larvae in the 2.00 L/ha and 3.00 L/ha test item colonies had not completed their development, compared to 2.7 % in the control group, which was not statistically significantly different.

There was a high number of impacted bee brood, which resulted in 76.4 % mean loss of the initial observed cells in the reference item treatment group (90.9 % eggs, 69.3 % young larvae and 68.7 % old larvae stages, respectively). The termination rates of the different brood stages were statistically significantly higher compared to the control values.

Table 10.4.6.1-2: Bee Brood Termination Rate

Treatment Group	Eggs	22 days after BFD0				Young Larvae	22 days after BFD0				Old Larvae	22 days after BFD0			
		brood terminated					brood terminated					brood terminated			
	BFD0	#	[%]	mean [%]	statistic	BFD0	#	[%]	mean	statistic	BFD0	#	[%]	mean	statistic
	150	9	6.0			150	4	2.7			150	6	4.0		
Control	150	14	9.3	7.3	-	150	6	4.0	3.3	-	150	3	2.0	2.7	-
	150	10	6.7			150	5	3.3			150	3	2.0		
	150	4	2.7			150	7	4.7			150	4	2.7		
Kinvara	150	4	2.7	3.8	n.s.	150	4	2.7	4.4	n.s.	150	6	4.0	4.4	n.s.
[2.00 L/ha]	150	9	6.0			150	9	6.0			150	10	6.7		
	150	17	11.3			150	8	5.3			150	14	9.3		
Kinvara	150	6	4.0	12.0	n.s.	150	9	6.0	4.4	n.s.	150	3	2.0	4.9	n.s.
[3.00 L/ha]	150	31	20.7			150	3	2.0			150	5	3.3		
	150	145	96.7			150	90	60.0			150	72	48.0		
Reference Item	150	114	76.0	90.9	*	150	97	64.7	69.3	*	150	105	70.0	68.7	*
	150	150	100.0			150	125	83.3			150	132	88.0		

BFD0 = Brood Fixing Day 0; # = number of terminated cells
Statistic: Student t-test, pairwise comparison, one-sided greater, $\alpha = 0.05$
n.s. = not statistical significant from the control; * = statistical significant from the control

III. CONCLUSIONS

Although the mean termination rate of the eggs in the higher Kinvara rate (3.00 L/ha) was slightly higher in the test item treatment group (12.0 %) when compared to the values of the control group (7.3 %), there was no statistically significant difference. The lower rate of Kinvara (2.00 L/ha) resulted in an egg brood termination rate of 3.8 % and thus was not statistically significant to the control value.

There was also no effect on the development of young larvae after consumption of both test item rates *via* treated sugar solution. The development success of the young larvae in both test item treatment groups resulted in a mean termination rate of 4.4 %, respectively. The termination rate of the young larvae in the control group was 3.3 %. This difference was not statistically significant.

No effect on the development of old larvae was observed after consumption of both test item treated sugar solutions. The mean termination rates of old larvae in the test item treatment groups was 4.4 % and 4.9 % for the lower and higher rate, respectively, compared to 2.7 % in the control group. The val- ues of the termination rates for both test item rates were not statistically significant compared to the control group.

Adult bee mortality following the administration of the contaminated food in both test item treatment groups (2.00 L/ha and 3.00 L/ha Kinvara) was lower (mean of 7.9 and 6.4 dead bees per day) and thus not statistically significantly different when compared to the control group (11.6 dead bees per day).
Nearly no dead larvae and pupae were found in the dead bee traps after treatment with both Kinvara rates and the control group. No difference was found between the Kinvara treatment groups and the control group.

The reference item treatment (Insegar, a.s. = fenoxycarb) resulted in a statistically significant increase of unsuccessful egg-, young- and old larvae development and thus confirmed the sensitivity of the test system and the validity of the test conditions.

Overall, it can be concluded according to the results of this study that the administration of Kinvara fortified sugar syrup at both rates (2.00 L/ha and 3.00 L/ha) to honey bee colonies does neither adversely affect honey bee colonies nor bee brood development..

(A. Ehmke, 2014)

A 2.3.1.4 KCP 10.3.1.4 Sub-lethal effects

A 2.3.1.5 KCP 10.3.1.5 Cage and tunnel tests

A 2.3.1.6 KCP 10.3.1.6 Field tests with honeybees

A 2.3.2 KCP 10.3.2 Effects on arthropods other than Bees

KCP 10.3.2/01 Moll (2014)

Comments of zRMS:	The study was accepted. Validity criteria were met. The 48-hour LR_{50} for *Typhlodromus pyri* was 1567 ~~g a.s./ha~~ mL prod./ha, and This endpoint was used for risk assessment.

The following non-target arthropod studies using artificial substrates performed on Kinvara were provided in support of the assessment and have not been previously evaluated.

Report:	KIIIA1 10.3.2/01, Moll., M.(2014)
Title:	Effects of Kinvara on the Predatory Mite *Typhlodromus pyri* in the Laboratory - Dose Response Test
Document No:	89201063
Guidelines:	Laboratory residual contact test with the predatory mite *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) for the regulatory testing of plant protection products. Blümel *et al.* (2000) SETAC – Guidance document on regulatory testing procedures for pesticides with non-target arthropods. Barrett *et al.* (1994) ESCORT 2 – Guidance document on Regulatory testing and risk assessment procedures for plant protection products with non-target arthropods. Candolfi *et al.* (2001)
GLP	Yes (certified laboratory)

Executive Summary:
The effect of Kinvara on the predatory mite *Typhlodromus pyri* was determined by exposing mites to Kinvara on treated glass surfaces and comparison with a water treated control and a reference item. Test organisms were exposed to freshly dried residues at rates of 37.3, 111, 333, 1000 and 3000 mL product/ha (nominally 0.21, 0.625, 1.87, 5.63 and 16.9g a.i./ha). Perfekthion (BAS 152 11 I) was used as the reference item and water was used as a control. Treatment groups were assessed for mortality on day 3 and day 7. A reproduction test was performed on those treatment groups with a corrected mortality ≤ 50% on day 7. Under worst case laboratory conditions the LR_{50} of Kinvara is 1567 mL product/ha in 200 L water/ha. Reproduction of *T. pyri* was assessed in the control and at 37.3, 111, 333 and 1000 mL product/ha. Reproduction was not affected up to and including 1000 mL product/ha compared to the control.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: Kinvara (MCPA 233/Fluroxypyr 50 / Clopyralid 28) ME

Description: Yellow / Amber liquid

Lot/Batch #: 13-3601

Purity: MCPA 233.6 g/L
Fluroxypyr-meptyl 73.3 g/L
Clopyralid 27.3 g/L

Stability of test compound: Stable under normal conditions

2. Vehicle and/or positive control: Vehicle: Purified water (200 L/ha)
Positive control: Perfekthion (BAS 152 11 I) (Dimethoate 411.7g/l)

3. Test animals -

Species: *Typhlodromus pyri*

Age: Protonymphs < 24 hours old

Source: Ibacon culture originally obtained from a commercial supplier (Katz Biotech AG, An der Birkenpfuhlheide 10, D-15837 Baruth, and Germany).

Acclimatisation period: Mite cultures were maintained under the same conditions as the test conditions

Environmental conditions-

Temperature: 24-25 ºC

Photoperiod: 16 hours light: 8 hours dark (270-690 lux)

B. STUDY DESIGN AND METHODS:

1. In-life dates: 11th March – 13th May 2014

2. Experimental treatments:
Following a preliminary range-finding test, Kinvara was evaluated in a definitive test at five application rates, equivalent to 37.3, 111, 333, 1000 and 3000 mL product/ha (nominally 0.21, 0.625, 1.87, 5.63 and 16.9g a.i./ha). These variants were compared to a control treatment of purified water and a reference

treatment of Perfekthion BAS 152 11 I (nominally 411.7 g/L dimethoate) applied at a rate of 8 mL product/ha correspondin to 40 µL Perfekthion/L. Applications were made at a rate corresponding to 200 L spray liquid/ha. Treatments were applied to glass plates made from pairs of microscope slide cover-slips, joined together so that a small channel remained between them. Once the plates had dried, they were laid on water-saturated tissue paper so that water was drawn into the channel by capillary action. A ring of a sticky, non-drying gel was drawn onto the plates to create an arena in which to confine mites and that was crossed by the water channel. Twenty protonymphal *T. pyri* were placed in each replicate unit, with three replicates (i.e. 60 mites) prepared per treatment. A mixture of pine (*Pinus nigra)* and birch *(Betula sp.)* pollen (3:1) *ad libitum* on the day of the test start and on each assessment day except for the last one *resp.* at least every four days.The survival of the mites was assessed over a 7-day period, by which time they were adults.

3. Observations:
The bioassay was initiated within 1 hour of treatment being applied, i.e. once the residues on the treated glass plates had dried. The mites were exposed to dried residues on treated glass plates. Survival of the mites was assessed after 3 and 7 days. For the reproduction assessment surviving mites from the control and from all test item groups displaying less than 50 % corrected mortality were sexed and the number of eggs per females was recorded at 3 assessment days within one week. The number of living, dead and escaped mites was counted on day 3 and day 7 after test initiation. Dead mites were removed, escaped mites were considered as dead. The sex-ratio for reproduction testing on day 7 was 1 male : 5 females at a minimum. Number of eggs laid and number of live and dead juvenile stages per female was counted and removed afterwards on 3 assessment days from day 7 on with a maximum interval of 3 days up to day 14 (inclusive). Eggs laid until day 7 inclusive were removed from the test arena and were not counted. The reproduction assessment was performed where the corrected mortality (M_{corr}) was ≤ 50 %.

II. RESULTS AND DISCUSSION

All of the validity criteria for the test were met, and the test is considered valid.
Mite mortality at 7 days was 8.3% in the control treatment, compared with 66 %, 43 %, 28 %, 16% and 5 % in the 3000, 1000, 333, 111 and 37.3 mL/ha treatment rates of Kinvara, respectively. In the reference treatment, 100 % (100 % corrected) mortality was recorded at 7 DAT.

The mean number of eggs produced per female was 6.8 in the control, compared with 7.3, 8.1, 8.2 and 5.8 in the 37.3, 111, 333 and 1000 mL/ha treatment rates of Kinvara, respectively. There were no significant effects on the reproductive capacity of the surviving mites at rates up to and including 1000 mL product/ha compared to the control.

The results are presented in Table 10.5.1-1

Table 10.5.1-1: Effects on the mortality and reproduction of *Typhlodromus pyri* after exposure to Kinvara

Treatment Group	Mortaility		Corrected Mortality [%][b]	Escapees [%][b]
control	8.3 ± 7.6		-	1.7 ± 2.9
37.3 mL product/ha	5.0 ± 5.0	n.s.	-3.6	3.3 ± 2.9
111 mL product/ha	16.7 ± 5.8	n.s.	9.1	5.0 ± 5.0
333 mL product/ha	28.3 ± 5.8	*	21.8	5.0 ± 5.0
1000 mL product/ha	43.3 ± 2.9	*	38.2	8.3 ± 7.6
3000 mL product/ha	66.7 ± 2.9	*	63.6	8.3 ± 5.8

Table 10.5.1-2: Mortality and reproduction of the mites

	Rate [1] [mL/ha]	Mortality [2] [%]	Mortality corr. [3] [%]	Reproduction [4] [eggs/female]	Effect on reproduction [5] [%]
Control	0	8.3	--	6.8	--
Kinvara	37.3	5.0 n.s.	-3.6	7.3 n.s.	-7.8
Kinvara	111	16.7 n.s.	9.1	8.1 n.s.	-19.1
Kinvara	333	28.3 *	21.8	8.2 n.s.	-21.9
Kinvara	1000	43.3 *	38.2	5.8 n.s.	14.0
Kinvara	3000	66.7 *	63.6	--	--
Endpoint [6]					
LR_{50} (95 % CL): 1567 mL product/ha (1118 - 2441 mL product/ha)					

1. Application rate in 200 L water/ha
2. Mortality: after 7 days of exposure to spray residues on glass plates (Fisher´s Exact Test, $\alpha = 0.05$; n.s. = not significant, * = significant)
3. Corrected mortality according to Abbott and improvements by Schneider-Orelli; negative values indicate better survivorship compared to control
4. Reproduction: mean number of eggs/female,
5. (Dunnett´s t-test, $\alpha = 0.05$; * = significant, n.s. = not significant)
6. Calculated on the exact raw data; negative values indicate better performance compared to the control
7. LR_{50} was calculated with Probit Analysis; CL = confidence limits

III. CONCLUSIONS

The LR_{50} for *Typhlodromus pyri* exposed to Kinvara in this worst case scenario was found to be 1567 mL Kinvara/ha. Reproduction of *Typhlodromus pyri* was assessed in the control and at 37.3, 111, 333 and 1000mL Kinvara/ha. Reproduction was not affected up to and including 1000 mL Kinvara/ ha compared to the control.

(Moll., M. 2014)

KCP 10.3.2/02 Moll (2014)

Comments of zRMS:	The study was accepted. Validity criteria were met. The 48-hour LR_{50} for *Aphidius rhopalosiphi* was 769 g a.s./ha. This endpoint was used for risk assessment.

Report:	KIIIA1 10.3.2/02, Moll., M.(2014)
Title:	The effects of Kinvara on the Parasitoid *Aphidius rhopalosiphi* in the Laboratory – Dose Response Test
Document No:	89201001
Guidelines:	A laboratory test for evaluating the effects of plant protection products on the parasitic wasp *Aphidius rhopalosiphi* (DeStephani-Perez) (Hymenoptera, Braconidae), Mead-Briggs *et al* (2000) An extended laboratory test for evaluating the effects of plant protection products on the parasitic wasp *Aphidius rhopalosiphi)* Hymenoptera, Braconidae), Mead-Briggs et al (2010) SETAC – Guidance document on regulatory testing procedures for pesticides with non-target arthropods (Barrett *et al.* 1994) ESCORT 2 – Guidance document on regulatory testing and risk assessment procedures for plant protection products with non-target arthropods (Candolfi *et al* 2001)
GLP	Yes (certified laboratory)

Executive Summary:

Aphidius rhopalosiphi adults less than 48 hours old were exposed to freshly dried residues of Kinvara applied to glass test plates. Kinvara was applied at application rates of 188, 375, 750, 1500 and 3000 mL product/ha (i.e. nominally 1.06, 2.11, 4.22, 8.45 and 16.9g a.i/ha). These variants were compared to a control treatment of purified water and a toxic reference treatment of dimethoate (411.7 g/L) applied at a rate of 0.3 mL in 200 L/ha deionised water (corresponding to 1.5 µL Perfekthion/L). Observations of mortality were recorded approximately 2, 24 and 48 hours after test initiation. The number of parasitoids alive, affected, moribund and dead was recorded. Moribund parasitoids were counted as dead. A reproduction test was performed on those treatment groups with a corrected mortality ≤ 50 %.

Under worst case laboratory conditions, the LR_{50} of Kinvara is 769 ml product/ha in 200 L water/ ha. Reproduction of *Aphidius rhopalosiphi* was assessed in the control and at 188, 375 and 750 mL product/ ha. Reproduction was not affected up to and including 750 mL product/ ha compared to the control.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: Kinvara (MCPA 233/ fluroxypyr 50 / clopyralid 28) ME

Description: Amber -Yellow liquid

Lot/Batch #: 13.3601

Purity: MCPA 233.6 g/L
Fluroxypyr-meptyl 73.3 g/L
Clopyralid 27.3 g/L

Stability of test compound: Stable under normal conditions

2. Vehicle and/or positive control: Vehicle: Purified water
Positive control: BASF Perfekthion (Dimethoate 400 g/L)

3. Test animals -

Species: *Aphidius rhopalosiphi*

Age: < 48 hours old

Source: Katz Biotech AG, Dr.Peter Katz, An der Birkenpfuhlheide 10, D-15837, Baruth, Germany

Acclimation period: Approx 2 days under test conditions in hatching chambers

Environmental conditions-

Temperature: 19-24 ºC

Photoperiod: 840 – 1260 lux (acclimitisation, exposure, parasitisation period)
7170 – 12480 lux (post – parasitisation period)

B. STUDY DESIGN AND METHODS:

1. In-life dates: 17th February - 25th march 2014

2. Experimental treatments:

Aphidius rhopalosiphi adults less than 48 hours old were exposed to freshly dried residues of Kinvara applied to glass test units. Four replicates each containing 3 males and 7 females were prepared for each treatment group and the toxic standard. Kinvara was applied at the following application rates: 188, 375, 750, 1500 and 3000 mL product/ha (i.e. nominally 1.06, 2.11, 4.22, 8.45 and 16.9g a.i/ha). Dimethoate was used as a toxic standard and was applied at a rate of 0.3 ml Perfekthion/ha and deionised water was applied as a control. Treatments were applied using a precision sprayer at an application rate of 200 L/ha. Adult mortality was assessed at 2, 24 and 48 hours after application.

At 48 hours, for treatment groups where > 50 % of parasitoids survived, they were removed and their reproductive capacity was assessed by confining females individually, over untreated barley plants infested with the host cereal aphids, *Rhopalosiphum padi.* The females were removed after 24 hours and the aphid-infested plants left for a further 11 – 12 days before the numbers of aphid mummies that had developed were assessed.

2. Observations:
Treatment groups were assessed for mortality 2, 24 and 48 hours after test initiation. The number of parasitiods alive, affected, moribund and dead was recorded.

The number of aphid mummies was counted 11 days after the 24 hour parasitisation period in all replicates where the females were alive after the 24 hours parasitisation period.

II. RESULTS AND DISCUSSION

After 48 hours exposure to Kinvara, the highest observed corrected mortality was 97.4 %. There was 2.5 % mortality in the control group. The 48 hour LR_{50} was calculated to be 769 mL Kinvara/ha.

The results are shown in Table 10.5.1-3.

Table 10.5.1-2 Effects on mortality and reproduction of *Aphidius rhopalosiphi* after exposure to Kinvara

	Rate [1] [mL/ha]	Mortality [2] [%]	Mortality corr. [3] [%]	Reproduction [4] [mummies/female]	Effect on reproduction [5] [%]
Control	0	2.5	--	30.0	--
Kinvara	188	0.0 n.s.	-2.6	35.7 n.s.	-19.0
Kinvara	375	5.0 n.s.	2.6	35.3 n.s.	-17.7
Kinvara	750	52.5 *	51.3	29.7 n.s.	1.0
Kinvara	1500	95.0 *	94.9	--	--
Kinvara	3000	97.5 *	97.4	--	--
Endpoint [6]					
LR_{50} (95 % CL): 769 mL product/ha (674 - 879 mL product/ha)					

1. Application rate in 200 L water/ha
2. Mortality: after 48 hours of exposure to spray residues on glass plates, (Fisher's Exact Test, $\alpha = 0.05$; n.s. = not significant, * = significant)
3. Corrected mortality according to Abbott and improvements by Schneider-Orelli; negative values indicate better survivorship compared to control
4. Reproduction: mean number of parasitised aphids/female, (Dunnett's t-test, $\alpha = 0.05$; n.s. = not significant)
5. Calculated on the exact raw data; negative values indicate better performance compared to the control
6. LR_{50} was calculated with Logit Analysis; CL = confidence limits

II. CONCLUSIONS

Under worst case laboratory conditions, the LR_{50} of Kinvara is 769 ml product/ha in 200 L water/ ha. Reproduction of *Aphidius rhopalosiphi* was assessed in the control and at 188, 375 and 750 mL product/ ha. Reproduction was not affected up to and including 750 mL product/ ha compared to the control.

(Moll.,M 2014)

10.3.2/03 Moll (2017)

Comments of zRMS:	The study was accepted. Validity criteria were met: Control Mortality: 0.0 % Reference Item Mortality: 93.3 % Control Reproduction Rate: 31.2 mummies per female, Under extended laboratory conditions the LR50 of Kinvara is estimated to be greater than 3000 mL product/ha in 400 L water/ha.

Report:	KIIIA1 10.3.2/03, Moll., M.(2017)
Title:	Kinvara: Effects on the Parasitoid *Aphidius rhopalosiphi*, Extended Laboratory Study – Dose Response Test –
Document No:	122621002
Guidelines:	Mead-Briggs *et al.* 2010: An extended laboratory test for evaluating the effects of plant protection products on the parasitic wasp, *Aphidius rhopalosiphi* (Hymenoptera, Braconidae) SETAC – Guidance document on regulatory testing procedures for pesticides with non-target arthropods (Barrett *et al.* 1994) ESCORT 2 – Guidance document on regulatory testing and risk assessment procedures for plant protection products with non-target arthropods (Candolfi *et al* 2001)
GLP	Yes (certified laboratory)

Test system

Test item:	Kinvara
Purity:	29 g/l Clopyralid 74 g/l Fluroxypyr-meptyl (54.1 g/l Fluroxypyr equivalent) 226.3 g/l MCPA
Description:	Yellow/amber liquid
Lot No./Batch No. :	17-9388

Organism (Species):	Parasitic wasp, *Aphidius rhopalosiphi*
Age/Life stage at dosing	<48 hours
Host species	*Rhopalosiphum padi*
Study Type:	12/13 days in total: 1-2 days acclimatisation under test conditions Exposure for 48 hours to barley plants treated with test substance. Fecundity then further assessed over 10/11 days.
GLP Status:	GLP
Guidelines followed:	Mead-Briggs *et al*., (2010)
Parameters observed:	Repellency, mortality and fecundity.
Observation intervals:	Mortality was assessed after 2, 24 and 48 hours. Behaviour of the wasps assessed in the initial 3 hours (observations at 30 minute intervals) for repellency. Fecundity assessed at the end of 10/11 days (termination). Fecundity only performed for treatment groups were corrected mortality was ≤50 %.

Test units	Acclimatisation: glass tubes (15 cm length, diameter 1.5 cm at largest end and 0.5 cm at smallest opening). Exposure- Pots (13 cm diameter) with approximately 9-10 barley seedlings (end leaf growth stage -BBCH 12). All plants trimmed to a uniform high of 12 cm. Approximately 40-70 minutes before treatment the seedling were sprayed with a 10% w/v solution of fructose. Once the test item was applied, the plants were left to dry for 20-35 minutes before introduction of the parasitoids. Plants were enclosed with a clear polyacrylic cylinder with a hole in it for introducing the parasitoids. After introduction the hole was closed with a stopper which had a ventilation tube in it. The opening of the tube was closed with fine mesh gauze. The soil surface was covered with a thin layer of quartz sand before treatment. Fecundity (parasitisation rate)-Pots (13 cm diameter) containing 13-27 barley seedlings (10 days old), infested with 100-150 host aphids (*R. padi)*, enclosed in clear acrylic cylinders (10cm diameter; 30 cm high). The cylinder had 2 holes in it to improve ventilation; these were covered with fine gauze. Another hole was made for introduction of aphids which was closed off with cotton wool. The top of the cylinder was capped with fine gauze. The soil surface was covered with a thin layer of quartz sand.
Number of replicates	Exposure- 6 replicates (each containing 5 female wasps) for the control, test concentrations and toxic reference Fecundity- 20 individuals from each treatment group. For the positive reference substance, only the exposure side of the experiment was performed.
Test concentrations:	0 (water control), 188, 375, 750, 1500 and 3000 ml formulation/ha. All treatments applied in 400 L spray solution/ha.
Environmental conditions:	Exposure and fecundity- Temperature: 21-22 °C Relative Humidity: 78-82 % Photoperiod: 16 hour light:8 hr dark (790-910 lux for acclimatisation and exposure period, 2140-2340 lux for parasitisation period and 8080-14370 lux post-parasitisation period) Exposure units were ventilated with a small pump. Feeding: 10% fructose solution. This was provided on a cotton wool pad during acclimatisation. For the exposure period, 40 minutes-70 minutes before application the seedlings were lightly sprayed with sugar solution and left to dry.
Reference substance:	Perfekthion (nominal 400 g dimethoate/L) applied at a rate 10 ml product/ha in 400 l/ha water.

Methodology

Exposure

In an extended laboratory study, *A. rhopalosiphi* were exposed for 48 hours to an untreated control and to fresh-dried residues of Kinvara applied to barley seedlings at nominal rates of 188, 375, 750, 1500 and 3000 ml formulation /ha. A toxic reference treatment sprayed with Perfekthion (10 ml product/ha) was also included. The control used was water. All treatments, control and toxic reference where applied in a volume equivalent to 400 L water/ha using a laboratory track-sprayer. After spraying, the plants were left to dry for 20-35 minutes before the parasitoids were introduced. The test comprised of 6 replicates of 5 female wasps in each treatment, including the control and toxic reference.
Assessments for adult wasp mortality were carried out approximately 2, 24 and 48 hours after the start of the exposure phase. Observations were made at 30-minute intervals during the first 3 hours of the exposure phase to record any repellency behaviour of the wasps.

Fecundity (parasitisation rate)

If after the exposure phase, the effects on mortality were ≤50 % then the fecundity phase of the test was set up. As effects in all treatment groups were <50 %, after 48 hours 20 female wasps from each

treatment were added to test units containing untreated barley seedlings infested with cereal aphids. The female wasps were left in these units for a further 24 hours, after which they were removed. The remaining aphids were maintained for 10-11 days and then the parasitised aphids (aphid mummies) per female wasp were counted.
For the positive reference substance, only the exposure side of the experiment was performed as sensitivity to the reference treatment is assessed through mortality.

Statistics
Treatment mortality at 48 h was compared to the control using Fisher's Exact Test. For the analysis of the test item data, the Bonferroni correction was applied. Repellency data was tested for normal distribution and homogeneity of variance using the Shapiro-Wilks test and the Levene test. The William t-test was used for comparing the test item repellency data to the control and the student t-test was used for comparing the reference item repellency data to the control. Reproduction data was tested using the Shapiro-Wilks test and the Williams t-test.

Results
Settling of the wasps on the plants during the initial 3 hours period was >30 % for all test groups and therefore no further assessment of settling behaviour was considered to be necessary. The study report does note that in the 3000 mL product/ha treatment group, the settling rate was 54.7 % in the first 3 hours which was statistically significantly lower than in the control (79.3 %) however as >50 % settling was observed it was concluded that a repellent effect was no observed.

No behavioural observations (affected, moribund parasitoids) were noted.

Table 10.5.1-3: Mortality and reproduction of *A. rhopalosiphi* exposed for 48 hours under laboratory conditions to fresh-dried spray deposits of Kinvara applied to barley seedlings

Parameter	Control	application rate (ml formulation/ha)					Toxic standard
		188	375	750	1500	3000	
Fresh-dried spray deposits							
48 hour exposure phase							
Settling (mean %)	79.3	74.7	76.7	69.3	67.3	54.7**	67.3
Corrected mortality (mean %)	0.0	0	3.3	0	0	3.3	93.3*
Reproduction phase							
Mean mummies per female	31.2	41.9	32.3	42.6	30.8	23.0	-
Effect on reproduction (%)	-	-34.5	-3.6	-36.5	1.3	26.4	-

* Statistically significant compared to the control (Bonferroni-Holm Fisher's Exact Test α=0.05).
** Statistically significant compared to the control (Williams t-test α=0.05).
Negative values indicate an increase

It should be noted that although 20 individual females were used in each treatment group for the fecundity phase of the test, only 17, 17 and 18 individuals survived until the end of the fecundity phase in the control, 188 and 1500 treatment groups, respectively. Only females found alive after the 24 hour parasitisation period were included in the calculation of number of mummies produced per female.

Conclusions
The control mortality was ≤10% after 48 hours of exposure. The mean number of mummies produced per female during the fecundity assessment was ≥ 5 in the control. There were no control replicates with 0 mummies.
The LR_{50} from this study is >3000 mL product /ha. The reproductive performance of surviving wasps was not adversely affected by treatment rates up to and including 3000 mL product/ha.

10.3.2/04 Vaughan (2017)

Comments of zRMS:	The study was accepted. Validity criteria were met: In an extended laboratory test to determine the effects of Kinvara on the ladybird beetle *Coccinella septempunctata*, the LR_{50} was estimated to be > 3000 mL product/ha, the highest rate tested. The NOER with respect to beetle survival was 3000 mL product/ha. Kinvara had no unacceptable effects on the reproductive capacity of the surviving test insects at application rates up to and including 3000 mL product/ha. The ER_{50} was therefore estimated to be > 3000 mL product/ha and the NOER with respect to reproduction was 3000 mL product/ha

Report:	KIIIA1 10.3.2/04, Vaughan, R. .(2017)
Title:	XXXX Kinvara- A rate-response extended laboratory test to evaluate the effects of fresh residues on the ladybird beetle *Coccinella septempunctata* L. (Coleoptera: Coccinellidae)
Document No:	BAR-17-1
Guidelines:	Schmuck *et al*. (2000). A laboratory test system for assessing effects of plant protection products on the plant-dwelling insect *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) SETAC – Guidance document on regulatory testing procedures for pesticides with non-target arthropods (Barrett *et al.* 1994) ESCORT 2 – Guidance document on regulatory testing and risk assessment procedures for plant protection products with non-target arthropods (Candolfi *et al* 2001)
GLP	Yes (certified laboratory)

Test system

Test item:	Kinvara
Purity:	Nominal 28 g/l Clopyralid (analysed 29 g/L) Nominal 50 g/l Fluroxypyr (analysed 54.1 g/L) Nominal 223 g/l MCPA (analysed 226.3 g/L)
Description:	Yellow/amber liquid
Lot No./Batch No. :	17-9388

Organism (Species):	Ladybird beetle, *Coccinella septempunctata*
Age/Life stage at dosing	Larvae (3-4 days old)
Study Type:	Fresh-dried- larvae exposed to individual French bean leaves removed from plants treated with test substance. Larvae exposed until they pupated, assessment recorded for up to 14 days. Mortality of pupae and adults then recorded for 8 days. Fecundity assessed over 2 week period.
GLP Status:	GLP
Guidelines followed:	Schmuck *et al.* (2000)
Parameters observed:	Pre-imaginal mortality (larval + pupal + emergence failures), behavioural abnormalities and fecundity.
Observation intervals:	Survival was assessed every 1-3 days. Fecundity was recorded daily.
Number of replicates	Exposure- 40 for the control, test substance and reference substance. Fecundity- 11-15 per treatment For the positive reference substance, only the exposure side of the experiment was performed.
Test concentrations:	0 (control- purified water), 187.5, 375, 750, 1500 and 3000 ml/ha in a volume rate equivalent to 200 L/ha
Environmental conditions:	Exposure- Temperature: 24.1-25.7 °C Relative Humidity: 62-81 % Photoperiod: 16 hour light:8 hours dark (2900-4200 lux) Feeding: Pea aphids *ad libitum* (until pupation)

	Fecundity Temperature: 24-25.9 °C Relative Humidity: 46-80 % Photoperiod: 16 hour light:8 hours dark (2900-4200 lux) Feeding: Pea aphids provided every day
Reference substance:	BAS 152 11 I (400 g/L dimethoate) applied at a rate of 80 ml/ha in 200 Lha water.

Methodology

In an extended laboratory toxicity study, larvae of the ladybird beetle (species *Coccinella septempunctata)* were exposed to leaves removed from fresh treated bean plants. The test item was applied at a nominal rate of 0 (purified water), 187.5, 375, 750, 1500 and 3000 mL/ha in a volume rate equivalent to 200 L/ha. This was applied via a laboratory track sprayer which was calibrated in advance.

The test included a toxic reference treatment with BAS 152 11 I (dimethoate a.s.) applied at a rate of 80 mL/ha in 200 L/ha. In the exposure phase of the study pre-imaginal mortality was assessed. The control, test and reference treatments comprised of 40 replicates. After the exposure phase of the test, fecundity was assessed for each adult beetles.

Exposure

Once the test item residue had dried (within 1 hour), the treated leaves (grown to BBCH 12) were used to line the floor of a test unit comprised of a glass plate with a Perspex sheet on top with a circular hole in it in which an acrylic cylinder was placed. The inner wall of the acrylic cylinder was treated with an aqueous solution of polytetrafluoroethylene to prevent larvae from escaping. The top of the cylinder was closed with nylon netting. Larvae were confined individually in each unit, 40 units for each test concentration. Pea aphids were provided for food and were replenished daily until the larvae had pupated. Pre-imaginal mortality (larval + pupal + emergence failures) was assessed during the initial exposure period every 1-3 days. Larvae exposed until they pupated, assessment recorded for 12-14 days. Mortality of pupae and adults then recorded for 8 days.
Ladybird pupae were transferred into plastic maintenance boxes approximately 2-4 days after their formation. Replicates from the same treatment group were combined. These boxes were lined with dry tissue paper to facilitate cleaning and ventilation was provided. Drinking water was also provided on cotton wool pads and pea aphids were provided on bean plants for food. This diet was also supplemented with bee-collected pollen and was replenished every 2-3 days. Pupae was then assessed every 1-3 days for the number of adult beetles emerged.

Fecundity

The reproduction assessments commenced approximately 4 weeks after the onset of adult emergence. By this point, egg production had been taking place in the maintenance boxes for 14 days. The fecundity phase of the test was only performed for the test concentrations were corrected mortality was < 60 % and were there was still deemed to be a sufficient number of survivors (>5 females). The sex of individuals was determined and male/female pairs were placed in vented, plastic Petri dishes. These males were moved between dishes from the same test concentrations once during the experimental period to ensure that females were confined with either one or two males. These dishes were lined with tissue paper to provide a substrate upon which eggs could be laid. Pea aphids were provided daily for food. Any eggs produced over the 2 weeks were transferred into petri dishes and their viability was checked. Eggs from each female were kept separate. Fecundity was recorded daily.

For the positive reference substance, only the exposure side of the experiment was performed as sensitivity to the reference treatment is assessed through mortality.

Temperature and humidity were recorded hourly throughout the test. Light intensity was recorded for each phase of the experiment.

Statistics

Pre imaginal mortality was compared to the control using Fishers exact test. The results meant that a probit regression analysis to determine an LR_{50} was not deemed appropriate. Based on Schmuck et al (2000), reproductive performance was only evaluated qualitatively.

Results

Table 10.5.1-4: Mortality and reproduction of *C. septempunctata* exposed under laboratory conditions to Kinvara on bean leaves detached from treated plants

Parameter	Control	application rate (mL/ha)					Toxic standard
		187.5	375	750	1500	3000	
7-day mortality phase							
Larval pupating (%)	87.5	90	92.5	87.5	80	87.5	0
Pupae emerging as adults (%)	100	91.7	89.2	100	96.9	97.1	-
Pre-imaginal mortality (%)	12.5	17.5	17.5	12.5	22.5	15	100
Corrected pre-imaginal mortality (%)[1]	-	5.7	5.7	0	11.4	2.9	100
Reproduction phase							
Eggs/female/day	12.9	-	-	8	13.4	10.6	-
Viable eggs/female day	8.3	-	-	3.9	8.9	6.9	-

[1] corrected according to Abbott (1925)

Conclusions

The validity criteria for this study were met. The water control pre-imaginal mortality did not exceed 30 %. The mean number of viable eggs per female per day was ≥ 2 in the water control treatment and pre-imaginal mortality in the positive reference treatment was ≥ 40 %.

Under the conditions of this extended laboratory test, the effects of an application of Kinvara on the survival of *C. septempunctata* were considered to be <50 %, the NOER with regards to mortality was proposed to be 3000 mL product/ha. Therefore the LR_{50} was >3000 mL product/ha.

There were no adverse effects on viable egg production following exposure. The mean viable eggs/female /day was >2 in all test concentrations and therefore Kinvara is not considered to have any impacts on fecundity. The reproductive ER_{50} is > 3000 mL/ha, the NOER with regards to fecundity was proposed to be 3000 mL product/ha.

A 2.4 KCP 10.4 Effects on non-target soil meso- and macrofauna

A 2.4.1 KCP 10.4.1 Earthworms

A 2.4.1.1 KCP 10.4.1.1 Earthworms - sub-lethal effects

10.4.1.1/01 Luhrs (2014)

Comments of zRMS:	The study was evaluated and accepted. The validity criteria were met: • adult mortality 4 weeks: less than10 % (being 0 % after 4 weeks); • number of juveniles per replicate: more than 200; • coefficient of variation of reproduction: less than 30 % (being 7.95%) NOEC = 184 mg a.s./kg d.w. The study was used in risk assessment.

The following sublethal earthworm study performed on Kinvara is provided in support of the assessment.

Report:	KIIIA1 10.4.1.1/01, Luhrs., U. (2014)
Title:	Effects of Kinvara on Reproduction and Growth of Earthworms *Eisenia fetida* in Artificial Soil
Document No:	89201022
Guidelines:	OECD Guideline No. 222 (2004) and ISO 11268-2, 1998
GLP	Yes

Executive Summary:

The chronic (sub-lethal) toxicity of Kinvara to the earthworm *Eisenia fetida* was examined in an artificial soil. Earthworms were dosed at concentrations of 11.5, 23, 46, 92 and 184 mg Kinvara/kg soil dry weight.

For all treatments, the soil moisture content was maintained at 50% (± 10%) of the maximum water-holding capacity throughout the bioassay. Ten adult *E. fetida* (approx. 11 months old, each 304-599 mg fresh weight and with a visible clitellum) were weighed and were placed on the surface of the soil in each arena immediately after treatment application. One day after treatment (DAT), finely ground cattle manure was placed on the soil surface and this food source was replenished weekly for the first four weeks of the bioassay. At 28 DAT the numbers of the original worms still surviving and their fresh weights were recorded. Any apparent change in the behaviour or physical condition of the confined worms was noted. The adult worms were then removed and the test soil and any egg cocoons or juvenile worms were returned to the test chambers. A final supply of finely ground cattle manure was provided on the soil surface. After a further 28 days (i.e. 56 DAT) the number of juvenile worms that had developed in each replicate arena was recorded.

No mortality was observed in any treatment group at 28 days. The NOEC for mortality was determined to be 184 mg test item/ kg soil ie. the highest treatment rate.

The body weight changes were statistically significantly increased compared to the control at the test concentration of 11.5 to 46 mg test item/kg soil. However, this is not considered to be a treatment related effect since at the two higher concentrations no statistically significant weight change could be observed.

The reproduction rates were not statistically significantly different compared to the control up to and including the highest test concentration of 184 mg test item/kg soil. No behavioural abnormalities were observed in any of the treatment groups, while the feeding activity in all the treated groups was comparable to the control.

Taking into account mortality and changes in biomass, behaviour and reproductive capacity, the 'no-observed-effect concentration' (NOEC) for Kinvara was determined to be 184 mg/kg soil dry weight.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material:	Kinvara
Description:	Yellow / Amber liquid
Lot/Batch #:	13-3601
Purity:	MCPA: 233.6 g/L Fluroxypyr-meptyl: 73.3 g/L Clopyralid: 27.3 g/L
Stability of test compound:	Stable under normal conditions. Vehicle: Water
2. Vehicle and/or positive control:	Positive control: Carbendazim
3. Test animals -	
Species:	*Eisenia fetida*
Age:	~ 9 months (individual wet weights of 301-592 mg) and showing a well-developed clitellum
Source:	Bred under standardised conditions in IBACON laboratories in a breeding medium of cattle manure, peat, sand, calcium carbonate and straw, fed with cattle manure, stored at room temperature
Acclimatisation period:	1 day in artificial soil, under test conditions
Environmental conditions	
-	
Temperature:	18 ºC – 22 ºC
Photoperiod:	16 h light, 400 - 800 lux

B. STUDY DESIGN AND METHODS:

1. In-life dates: 16th May 2014 – 21st July 2014

2. Experimental treatments:
Kinvara was mixed with deionised water and appropriate volumes were added to artificial soil (10 % sphagnum peat; 20 % kaolin clay, 69.4 % fine quartz sand, and 0.4 % calcium carbonate) to give concentrations of 11.5, 23, 46, 92 and 184 mg /kg soil dry weight. An untreated control moistened with deionised water was used.

Each test unit was filled with 610 g of the prepared soil (500 g dry weight plus 105 g deionised water plus 5 g food). The height of the soil layer in the containers was approximately 4-5 cm. Test units comprised plastic boxes, tapered towards the bottom with perforated transparent lids.

There were 4 replicates of 10 worms per test unit at each concentration. For the untreated control reference treatment, there were 8 replicates of 10 worms. The treated soil was placed into replicated plastic boxes (n = 4 per test-item treatment, n = 8 for the untreated control), so that it formed a layer approximately 4-5cm in depth. Ten adult *Eisenia fetida* (approx. 11 months old, 301 - 592 mg fresh weight and with a visible clitellum) were weighed and placed on the surface of the soil in each area immediately after treatment application. One day after treatment (DAT), finely ground cattle manure was scattered on the soil surface and this food source was replenished weekly for the first four weeks of the bioassay.

3. Observations:

Mortality and behavioural and morphological abnormalities of the confined worms was assessed 28 days after exposure. Mean body weights for each replicate were determined at study initiation (day 0) and 28 days after exposure. The numbers of juvenile worms and unhatched cocoons in each test arena was assessed a further 28 days after the adults had been removed (i.e. 56 DAT). Juveniles were removed by placing the test units in a water bath at 50 – 60 ºC and counting all emerging worms. In addition the soil of each container was emptied out onto a tray and checked visually for any remaining juvenile worms.

II. RESULTS AND DISCUSSION

No mortality was observed in any treatment group at 28 days. The NOEC for mortality was determined to be 184 mg test item/ kg soil ie. the highest treatment rate.

The body weight changes were statistically significantly increased compared to the control at the test concentration of 11.5 to 46 mg test item/kg soil. However, this is not considered to be a treatment related effect since at the two higher concentrations no statistically significant weight change could be observed.

The reproduction rates were not statistically significantly different compared to the control up to and including the highest test concentration of 184 mg test item/kg soil. No behavioural abnormalities were observed in any of the treatment groups, while the feeding activity in all the treated groups was comparable to the control.

The mean number of juveniles produced per arena was 239 in the untreated control and 237, 243, 214, 232 and 244 in the 11.5, 23, 46, 92 and 184 mg/kg soil treatment rates respectively. Thus, the percentage change in the number of juveniles relative to the untreated control was 99.2 %, 101.7 %, 89.7 % 97 % and 102 % in the 11.5, 23, 46, 92 and 184 mg/kg soil treatment rates respectively. The reproduction rates were not statistically significantly different compared to the control up to and including the highest test concentration of 184 mg test item/kg soil (Bonferroni-Welch t-test, $\alpha = 0.05$, one-sided smaller). The NOEC for reproduction was determined to be 184 mg test item/kg soil

Table 10.6.3-1: Effect of Kinvara on earthworms (*Eisenia fetida*) in a 56-day reproduction study

Kinvara [mg/kg soil dry weight]	**Control**	**11.5**	**23**	**46**	**92**	**184**
Mortality (day 28) [%]	0.0	0.0	0.0	0.0	0.0	0.0
Statistical Significance	-	-	-	-	-	-
Body weight change (day 28) [%]	38.2	43.1	44.3	48.4	39.3	38.1
Statistical Significance [1)]	-	*	*	*	n.s.	n.s.
Mean No. of juveniles (day 56)	239	237	243	214	232	244
Statistical Significance [2)]	-	n.s.	n.s.	n.s.	n.s.	n.s.
Reproduction in [%] of control (day 56)	-	99.2	101.7	89.7	97.0	102.0
Food consumption [g]	25.0	25.0	25.0	25.0	25.0	25.0
Endpoints [mg test item/kg soil dry weight]						
NOEC (day 28 mortality and weight)	**184**					
NOEC (day 56 reproduction)	**184**					

- = not applicable
n.s. = not significantly different compared to the control
* = significantly different compared to the control
[1)] Williams t-test, $\alpha = 0.05$, two-sided for weight changes and one-sided smaller for reproduction

III. CONCLUSIONS

In an earthworm reproduction and growth study with Kinvara, the No Observed Effect Concentration (NOEC) for mortality and weight changes of the earthworm *Eisenia fetida* was determined to be 184 mg test item/kg soil, *i.e.* the highest concentration tested.

(Luhrs, 2014)

A 2.4.1.2 KCP 10.4.1.2 Earthworms - field studies

A 2.4.2 KCP 10.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)

A 2.4.2.1 KCP 10.4.2.1 Species level testing

10.4.2/01 Staube (2017a)

Comments of zRMS:	The submitted study was accepted. The validity criteria were met: • mean mortality of adult females: ≤ 20 %; observed 6.0 %; • mean number of juveniles per replicate: ≥ 50; observed 186 to 238; • coefficient of variation (mean number of juveniles per replicate): ≤ 30 %; calculated 7.5%. The following endpoints were derived: • mortality: NOEC = 1000 mg test item/kg soil d.w. LC_{50} > 1000 mg test item/kg soil d.w. • reproduction: NOEC = 1000 mg test item/kg soil d.w. EC_{50} < 1000 mg test item/kg soil d.w. The study results were used in risk assessment.

Report:	KIIIA1 10.4.2/01, Straube, D. (2017)
Title:	Kinvara: Effects on Reproduction of the Predatory Mite *Hypoaspis aculeifer* in Artificial Soil with 5% Peat
Document No:	122621089
Guidelines:	Guidelines for the testing of chemicals OECD 226 Predatory Mite (*Hypoaspis (Geolaelaps) aculeifer*) reproduction test in soil, adopted July 29, 2016
GLP	Yes

Test material

Test Item:	Kinvara
Lot No.:	17-9388
Description:	Yellow/amber liquid
Purity:	Clopyralid 29 g/l Fluroxypyr-meptyl 74 g/l MCPA 226.3 g/l

Test system

Organism (*Species*):	Predatory Mite *Hypoaspis aculeifer* (adult females)
Study Type:	Reproduction inhibition study
GLP Status:	Yes
Guidelines Followed:	OECD 226 (2016)
Duration of study:	14 days
Parameters measured:	Survival, reproductive output i.e. number of juveniles and morphological differences.
No. of mite per dose group:	4 replicates (10 females in each)

No. of mite per control group:	8 replicates (10 females in each)
Age of test organisms at test initiation:	7 days after reaching the adult stage (28 days after placing in rearing vessels)
Test Concentrations:	0 (deionized water), 31.25, 62.5, 125, 250, 500 and 1000 mg product/kg soil.
Test substrate:	Artificial soil according to OECD 226: containing 74.8% fine quartz sand, 20% kaolin clay, 5% peat. 0.2% $CaCO_3$ was added to adjust pH to 6.0 ±0.5. Maximum water holding capacity: 39% of the dry weight The moisture content of the soil at initiation- 51.4-52.9% of MWHC. The moisture content of the soil at termination- 49.5-51.3% of MWHC. pH at initiation: 5.8 pH at termination: 5.6
Environmental Conditions:	Temperature: 18-22 °C Light: 400-800 lux (16 h light : 8 h dark)
Feeding:	Fed with cheese mites *ad libitum* at test start and on days 3, 5, 7, 10 and 12.
Reference Item:	Perfekthion/BAS 152 11 I (active ingredient; dimethoate, 400 g/L) tested in a recent experiment (November 2016) EC_{50} of 3.97 mg/kg derived.

Methodology

A 14-day exposure test was performed with nominal test concentrations 0 (deionized water), 31.25, 62.5, 125, 250, 500 and 1000 mg product/kg soil. The control was replicated eight times with 10 female mites in each, a total of 80 individuals. Each treatment was replicated four times, with 10 female mites in each, a total of 40 individuals. One additional abiotic replicate (did not contain any mites) was used for each test concentration and the control, to determine pH and moisture content at the end of the test (after 14 days). Both of these parameters were also measured at test initiation.

The artificial soil was moistened to approximately half of the final water content 2 days before application of the test item. The additional water, to achieve maximum WHC was added when applying the test item. The test was diluted in deionised water in order to produce a stock solution. A dilution series was then prepared in order to achieve the required test concentrations. Aliquots were then added to 300 g dry weight of the artificial soil and mixed homogenously. Each vessel contained 20 g ± 1.0 g artificial soil d.w. The soil was added to 100 ml glass vessels before the predatory mites were introduced on top of the soil. After addition of the mites the test vessels were weighed to allow for soil moisture content to be adjusted throughout the duration of the test.

Mites were fed regularly throughout the study with cheese mites. All test chambers were aerated periodically.

After 14 days of incubation the mites were extracted from the soil. Substrate from individual test chambers was poured carefully into Millipore pots attached to plastic containers. These units were placed in a Kempson extractor were they were exposed to temperatures of 25 ºC-30 ºC for 2 days. Mites were collected in a fixing liquid containing glycol and a detergent. Juvenile mites were counted twice using a binocular microscope, adult mites were counted once visually. None of the replicate counts deviated by more than 10 % from their mean value. Mortality of the introduced mites and reproductive output based on the number of juveniles could be calculated. Mites were also observed for differences in morphology after 14 days.

Statistics

Fishers exact binomial test (mortality) and Williams t-test (reproduction).

Results

Mortality was observed in the treatment groups but this was not statistically significant compared to the control up to 1000 mg/kg. Reproduction in the treatment groups was not statistically significantly different from the control up to 1000 mg/kg. There was no difference in the morphology of the mites.

Table 10.6.6-4:Effects of Kinvara on mortality and reproduction of *H. aculeifer* following 14-day exposure in artificial soil

Nominal concentration of Kinvara (mg/kg dry soil)	Mean mortality (%)	Mean number of juveniles	% of control (number of juveniles)
Control	6	212	-
31.25	0	208	98
62.5	5	190	89
125	5	210	99
250	5	187	88
500	3	203	96
1000	10	205	97

Table 10.6.6-5: Summary of endpoints

Biological Parameter	NOEC (mg/kg)	EC_{50}	LC_{50}
Adult Survival	1000	-	>1000
Reproduction	1000	>1000	-

Conclusions

The control organisms met the acceptability criteria for mean adult survival (>80 %) and mean number of juveniles per replicate (>50). The coefficient of variation of the control reproduction was less than 30 % (7.5 %) as specified by the study protocol.

The NOEC for adult survival and reproduction was 1000 mg/kg. The LOEC for both parameters was estimated to be >1000 mg/kg. The LC_{50} and EC_{50} were estimated to be >1000 mg/kg.

10.4.2/02 Staube (2017b)

Comments of zRMS:	The submitted study was accepted. The validity criteria were met: • mean adult mortality: ≤ 20 %; observed 9.0 %; • mean number of juveniles per test vessel: ≥ 100; observed: 398 to 727; • coefficient of variation for the mean number of juveniles: < 30 %; observed 21.8%. The following endpoints were derived: • mortality: NOEC ≥ 500 mg test item/kg soil d.w. LC_{50} > 500 mg test item/kg soil d.w. • reproduction: NOEC = 250 mg test item/kg soil d.w. EC_{50} = 883.8 mg test item/kg soil d.w. The study results can be used in risk assessment.

Report:	KIIIA1 10.4.2/02, Staube, D. (2017b)
Title:	Kinvara: Effects on Reproduction of the Collembola Folsomia candida in Artificial Soil with 5% Peat
Document No:	122621016
Guidelines:	OECD-Guideline for test chemical No. 232 “Collembolan Reproduction Test in Soil”
GLP	Yes

Test material

Test Item:	Kinvara
Lot No.:	17-9388
Description:	Yellow/Amber in water emulsion
Purity:	Clopyralid 29 g/L Fluroxypyr-meptyl 74 g/L MCPA 226.3 g/L

Test system

Organism (*Species*):	Folsomia candida (Willem 1902), 9-11 day old juveniles
Study Type:	Reproduction inhibition study
GLP Status:	Yes
Guidelines Followed:	OECD 232 (2016)
Duration of study:	28 days
Parameters measured:	Mortality, behaviour and reproductive output
No. of mite per dose group:	4 replicates (10 females in each)
No. of mite per control group:	8 replicates (10 females in each)
Age of test organisms at test initiation:	9-11 days
Test Concentrations:	0 (deionized water), 31.25, 62.5, 125, 250 and 500 mg product/kg soil.
Test substrate:	Artificial soil according to OECD 226: containing 74.8 % fine quartz sand, 20 % kaolin clay, 5 % peat. 0.2 % $CaCO_3$ was added to adjust pH to 6.0 ±0.5. Maximum water holding capacity: 39% of the dry weight The moisture content of the soil at initiation- 51.4-52.9 % of MWHC. The moisture content of the soil at termination- 49.5-51.3 % of MWHC. pH at initiation: 5.8 pH at termination: 5.6 to 5.7
Environmental Conditions:	Temperature: 18-22 °C Light: 400-800 lux (16 h light : 8 h dark)
Feeding:	On day 0 and day 14 approximately 2 mg of granulated dried yeast was spread over the soil surface
Reference Item:	Boric acid

Methodology

A 28-day exposure test was performed with nominal test concentrations 0 (deionized water), 31.25, 62.5, 125, 250 and 500 product/kg soil. The control was replicated eight times with 10 individuals in each, a total of 80 individuals. Each treatment was replicated four times, with 10 individuals in each, a total of 40 individuals. One additional abiotic replicate (did not contain any mites) was used for each test concentration and the control, to determine pH and moisture content at the end of the test (after 14 days). Both of these parameters were also measured at test initiation.

All test organisms were bred at the test facility and kept under breeding condition until they were introduced to the tests at 9-11 days old. For the treatment’s individuals were transferred using an aspirator into 100 mL glass containers containing 30±1 g artificial soil.

On day 0 and day 14, half a small spatula (~2 mg) of granulated dried yeast was spread over the soil surface. All vessels were ventilated on day 3, 5, 7, 19, 12, 14, 17, 19, 21, 24 and 26 by opening the lid for a short period.

After 28 days the contents of the test container were suspended in water which causes the Collembola to drift to the surface. The suspensions were tinted with dark ink to aid in counting. The adult animals were counted once visually and the juveniles were counted at least twice under binocular microscopes. Additional juvenile counts were conducted when individual counts deviated more than 10 % from the mean value of counts.

Statistics
Mortality data was anlaysed using Fisher's Exact Binomial Test (multiple comparison with Bonferroni Correction, α=0.05). Reproduction data were tested for normal distribution and homogeneity of variance using Kolmogorov-Smirnov test and Cochran's Test (α=0.05).

Results
Mortality was observed in the treatment groups but this was not statistically significant compared to the control up to 500 mg/kg. No abnormal behaviour was observed with the surviving Collembola. Reproduction in the treatment groups was not statistically significantly different from the control up to and including a concentration of 250 mg/kg.

Table 10.6.6-4:Effects of Kinvara on mortality and reproduction of *F. candida* following 28-day exposure in artificial soil

	Mean Mortality (%)	Significant	Number of Juveniles (% of control)	Significant
Control	4		-	
31.25	6	no	101	no
62.5	5	no	104	no
125	5	no	103	no
250	10	no	90	no
500	10	no	73	**yes**

Table 10.6.6-5: Summary of endpoints

Biological Parameter	NOEC (mg/kg)	EC_{50}	LC_{50}
Adult Survival	>500	-	>500
Reproduction	250	883.8	-

Conclusions
The control organisms met the acceptability criteria for mean adult survival (>80%) and mean number of juveniles per replicate (>100). The coefficient of variation of the control reproduction was less than 30% as specified by the study protocol.

The NOEC for adult survival was >500 mg/kg and the NOEC for reproduction was 250 mg/kg.

A 2.4.2.2 KCP 10.4.2.2 Higher tier testing

A 2.5 KCP 10.5 Effects on soil nitrogen transformation

Comments of zRMS:	The submitted study was accepted. The validity criteria were met. No adverse effects on soil nitrogen transformation (measured as NO_3-N-production) of soil microorganisms when applied at 4.5 mg and 22.4 mg test item/kg soil dry weight treatment at the end of the 28-day incubation period were observed. The effect less than 25% was observed at 22.4 mg formulation/kg dw soil.

Report:	KIIIA1 10.5.1/01, Hammesfahr, U.. (2017)
Title:	Kinvara: Effects on the Activity of the Soil Microflora in the Laboratory
Document No:	122621080
Guidelines:	OECD No. 216, "Soil Microorganisms: Nitrogen Transformation Test" adopted January 21, 2000 and OECD No.217, "Soil Microorganisms: Carbon Transformation Test"
GLP	Yes

Test material

Test item:	Kinvara
Active Substance / Content:	29 g/L Clopyralid 74 g/L Fluroxypyr-meptyl (54.1 g/L Fluroxypyr equivalent) 226.3 g/L MCPA
Description:	Yellow/amber liquid
Lot No./Batch No. :	17-9388

Test system

Organism (Species):	Soil micro-organisms
Study Type:	Laboratory study with natural soil, assessed for nitrate formation and microbial respiration
GLP Status:	GLP
Guidelines followed:	OECD guidelines 216 and 217 (2000).
Duration of study:	28 days
Parameters measured:	Nitrogen transformation (measured as NO_3-N production) Carbon transformation (O_2 consumption)
Observation intervals:	0 (after approx 6 hours), 7, 14 and 28 days
Test concentrations:	Control- pure water Low Dose: 4.5 mg formulation/kg soil d.w High Dose: 22.4 mg formulation/kg soil d.w
Test units:	Disposable plastic boxes Soil respiration:1 L (width 0.12 m, depth 0.165 m, height 0.065 m)- filled with soil up to 6 cm Nitrogen transformation:0.5 L (width 0.1 m, depth 0.1m, height 0.065 m)- filled with soil up to 6 cm Soil was loosely filled into the boxes which were covered in perforated lids to enable air exchange.

Toxic reference	Reference item not required for the guideline, but sodium chloride was tested in a GLP study. It was applied to soil at 16 g/kg soil d.w. At day 28, deviation from the control for soil respiration was -64.13 %, for nitrogen transformation deviation from the control was -107.45 %.
Method of test item application	Incorporation into the soil
Environmental conditions:	Temperature: 20 °C ± 2 °C Light: darkness pH soil: 7.0-7.2
Soil properties	Soil source: Agricultural soil from Germany (fallow grassland). Air dried and sieved to 2mm. The soil was stored under pre incubation conditions according to the OECD guidelines for 29 days prior to test initiation. Textural classification: silty sand. All soil characteristics meet those stated in the OECD 216, 217 guideline. Maximum water holding capacity (WHC): 35.5 %

Methodology

In a soil microflora laboratory study, the effects of Kinvara on nitrogen and carbon transformation were investigated in a silty soil. Kinvara was applied to the soil at nominal concentrations of 4.5 and 22.4 mg/kg soil d.w. Three replicate batches were treated and incubated for each test. The control consisted of soil treated with pure water.

At test initiation pH, dry weight and soil moisture were determined. The test item was mixed into pure water and then subsequently mixed into the soil using a laboratory mixer. Additional water was added in order to achieve approximately 55 % WHC (carbon transformation) and 53/54 % WHC (nitrogen transformation) at test initiation in each replicate. Water content was monitored and adjusted at each sampling date throughout the test if required. Throughout the test, the water content ranged from 53-56 % WHC. pH was also determined at test termination on day 28. pH was only measured in one replicate for each treatment group.

Soil nitrogen transformation

Three replicates of 400g soil dry weight for each test concentration and the control. These replicates of soil were enriched with 0.5 % (related to soil dry weight) fine powdered Lucerne green grass meal (C: N ratio 15/1). Samples were incubated in plastic boxes (0.5 L) with perforated lids which prevented loss of moisture but also permitted air exchange.

At day 0 (within 6 hours of test substance addition) and after 7, 14 and 28 days, samples were removed for determination of ammonium, nitrate and nitrite-nitrogen content. Soil extracts were prepared by taking 24-25 g soil and extracting in 100 mL 0.1M KCl solution and agitating for one hour. The supernatant was centrifuged and then frozen. Analysis was performed using an AA3 continuous flow analyzer by comparison of the test item treated soil with a non-treated control soil.

Soil microflora respiration

Three replicates of 800 g of soil (dry weight) were used for each test concentration and the control. Each soil replicate was added to a plastic 1 L box. Each vessel had a lid to prevent moisture loss but permit air exchange.

At day 0 (within 6 hours of test substance addition) and after 7, 14 and 28 days, 100g samples of soil were removed for determination of microbial respiration. Soil respiration was determined after the addition of glucose to the soil (4 g/kg (moist soil) of glucose, 1.6ml of a solution of 250 g glucose/L pure water). This amount of glucose was determined to give the highest respiration rates. The respiration of micro-organisms leads to O_2 consumption and formation of CO_2. This was measured in a BSB sensomat system, glucose samples were incubated at 20 ± 2 °C. The absorption of CO_2 causes low-pressure in the reaction flask, which is in turn compensated with O_2 delivered by a respirometer. The respirometer determines the cumulative oxygen production which corresponds to the O_2 consumption by the microorganisms. This was measured over 24 hours for each sample.

Statistical analysis

The student t-test (paired wise comparison, two sided α=0.05) was used for comparison of treated and control values.

Results

Table 10.7-6: Effects of Kinvara on the Nitrate content in loamy sand soil

Treatment (mg formulation/kg soil d.w)	Mean nitrogen transformation (mg NO_3-N/kg soil d.w) (% inhibition in comparison to the control)			
	Day 0	Day 7	Day 14	Day 28
Control	25.427	18.873	31.951	43.383
4.5	24.898 (-2.08)	18.320 (-2.93)	30.993 (-3.00)	41.584 (-4.15)
22.4	25.219 (-0.82)	22.149 (17.36)*	35.797 (12.04)*	45.776 (5.52)

Negative values (-) = % below the control value.
*statistically significantly different from the control (Student t-test, α=0.05)

The soil nitrate content increased in the control and all test concentration groups over the course of the study (between day 0 and 28). The % inhibition from the control was < 25 % at day 28.

Table 10.7-7: Effects of Kinvara on the Nitrate transformation rate in loamy sand soil

Treatment (mg formulation/kg soil d.w)	Mean nitrogen transformation rate (mg NO_3-N/kg soil d.w/day) (% inhibition in comparison to the control)		
	Day 0-7	Day 7-14	Day 14-28
Control	-0.936	1.868	0.817
4.5	-0.940 (0.43)	1.810 (-3.10)	0.757 (-7.34)
22.4	-0.438 (-53.21)*	1.950 (4.39)	0.713 (-12.73)

Negative values (-) = inhibition

The % inhibition in nitrogen transformation rate was <25 % between days 14-28 and therefore exposure to Kinvara is not considered to give rise to inhibition of nitrogen transformation.

Table 10.7-8: Effects of Kinvara on oxygen consumption rate i.e. carbon transformation rate in soil.

Treatment (mg/kg soil d.w)	O_2 consumption (mg/kg soil d.w/h) (% inhibition compared to control)			
	Day 0	Day 7	Day 14	Day 28
Control	12.812[A]	11.800[B]	12.140	11.303
4.5	12.321 (-3.83)	11.338 (-3.92)	11.905 (-1.94)	11.051 (-2.23)
22.4	12.528 (-2.22)	11.255 (-4.62)[B]	12.053 (-0.72)	11.085 (-1.93)

Negative values (-) = % below the control value.
[A] for replicate 1 the measurement taken on day 1 due to human error
[B] for replicate 3 the measurement was taken on day 9 due to human error

Exposure to Kinvara at both test concentrations, did not give rise to inhibition in oxygen consumption (compared to the control) of greater than 25 % at day 28.

Conclusions

The coefficient of variation in the control replicates did not deviate more than 15 % (6.96 % and 5.06 % deviation being the maximum for the nitrogen and carbon tests, respectively).

The soil carbon and nitrogen transformation rates were within the trigger value of ±25 % set by OECD guidelines 216 and 217 at day 28.

In the most recent test with the positive reference substance, sodium chloride caused carbon transformation effects of -64.13% after 28 days at the concentrations of 16 g/kg soil d.w. Effects on nitrogen transformation were also observed of -107.45 % after 28 days. This demonstrates the sensitivity of the test system.

A 2.6 KCP 10.6 Effects on terrestrial non-target higher plants

A 2.6.1 KCP 10.6.1 Summary of screening data

A 2.6.2 KCP 10.6.2 Testing on non-target plants

10.6.2/01 Butzler and Munz (2014)

Comments of zRMS:	The submitted study was accepted. The validity criteria were met: Germination Rate of the Seeds: 76 - 98% Mean Survival of Control Plants: 100% Growth and Morphology of the Control Plants: The control plants exhibited no visible phytotoxic effects and the plants exhibited only normal variation in growth and morphology for that particular species. The following endpoint was derived: • $ER_{50} \geq$ = 32.9 mL/ha The endpoint ER_{50} will be used in risk assessment. Phytotoxic effects observed were chlorosis (all species except *Lolium perenne* and *Avena sativa*), necrosis (all species except *Lolium perenne* and *Avena sativa*), growth reduction (all species except *Lolium perenne* and *Avena sativa*), abnormal growth of the leaves (all species except *Brassica oleracea, Lolium perenne* and *Avena sativa*) and abnormal growth of the stems (all species except *Lolium perenne, Avena sativa* and *Allium cepa*). The most sensitive species in terms of phytotoxicity was *Lactuca sativa* with an ER_{50} value of 36.0 mL Kinvara/ha followed by *Vicia faba* and *Lycopersicon esculentum* with ER_{50} values of 86.9 and 89.0 mL Kinvara/ha, respectively. They were followed by *Brassica oleracea, Daucus carota* and *Gossypium herbaceum* (ER_{50} value of 161, 158 and 206 mL Kinvara/ha, respectively), which were followed by *Cucumis sativus* with an ER_{50} value of 533 mL Kinvara/ha followed by *Allium cepa* with an ER_{50} value of 2477 mL Kinvara/ha The least sensitive species were *Lolium perenne* and *Avena sativa* for which no ER_{50}-value could be determined. The phytotoxicity results are added at the end of study summary.

The following vegetative vigour study performed on Kinvara is provided in support of the assessment.

Report:	KIIIA1 10.6.2/01, Butzler, R. & Munz, J., (2014)
Title:	Effects of Kinvara on Terrestrial (Non-Target) plants: Vegetative Vigour Test
Document No:	89201087
Guidelines:	OECD Guideline No. 227, 2006
GLP	Yes

Executive Summary:
Kinvara was tested for effects on the vegetative vigour using ten plant species out of nine different plant families at concentrations in the range 1.37 - 3000 mL Kinvara/ha. The ER_{10}, ER_{20}, ER_{50} and NOER

based on fresh weight were determined and observation of mortality and phytotoxicity (chlorosis, necrosis, absnormal growth of leaves and abnormal growth of stems) were also made.
The plants were grown until they had reached the 2 to 4 true leaf stage prior to dosing. Test rates were calculated for a water amount of 200 L/ha and were administered onto the plants using laboratory spraying equipment. 24 plants were tested per rate and species. The concentration of the active ingredients in the stock solutions was verified analytically. The exposure time was 21 days.

The most sensitive species in terms of fresh weight was *Lactuca sativa* with an ER_{50} value of 32.9 mL Kinvara/ha followed by *Brassica oleracea, Lycopersicon esculentum* and *Daucus carota* with ER_{50} values of 68.9, 78.2 and 107 mL Kinvara/ha, respectively. They were followed by *Vicia faba* and *Gossypium herbaceum* (ER_{50} value of 149 and 185 mL Kinvara/ha, respectively), which were followed by *Cucumis sativus* and *Allium cepa* (ER_{50} value of 1394 and 1748 mL Kinvara/ha, respectively). The least sensitive species were *Lolium perenne* and *Avena sativa* with a NOER of 3000 mL Kinvara/ha, an ER_X-value could not be determined.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: Kinvara
Description: Yellow / Amber liquid
Lot/Batch #: 13-3601
Purity: MCPA: 233.6 g/L
Fluroxypyr-meptyl: 73.3 g/L
Clopyralid: 27.3 g/L
Stability of test compound: Stable under normal conditions.
Vehicle: Water
2. Vehicle and/or positive control: Control – Deionised water
3. Test animals -
Species: Monocotyledoneae and Dicotyledoneae
Age: 2 to 4 true leaf stage
Environmental conditions
-
Temperature: 18 ºC – 24.7ºC
Humidity: 52 %-76 %
Photoperiod: 16 hr light, 8 hr dark (6100 to 12730 lux)

B. STUDY DESIGN AND METHODS:

1. In-life dates: Pre-application: 16th May – 30th May 2014 (*Latuca sativa*)
Application:30th May 2014 (*Latuca sativa*)
Pre application: 16th April – 14th May 2014 (all except *Latuca sativa*)
Application: 14th May 2014 (all except *Latuca sativa*)

2. Experimental treatments:
The effect of Kinvara on monocotyledonous and dicotyledonous plant species was tested. 24 plants per species and treatment group were tested (6 pots containing 4 plants were tested for each treatment group). Each pot represented one replicate. The exposure time was 21 days.

The test was performed in a growth chamber under controlled conditions (phtot-period, humidity, temperature etc.). All applications were performed using laboratory spraying equipment, thus, the application was done in an accurate way with reproducible coverage. The following nominal rates were prepared based on a stock solution, 3000, 1000, 333, 111, 37.0, 12.3, 4.12 and 1.37 mL test item/ha.

The fresh weight of the above ground part of all survived plants of a pot was determined 21 days after application. If all plants of a pot were dead the value for this replicate was assessed as "0".
The number of living and dead plants was recorded 7, 14 and 21 days after application. A plant was considered dead if no living tissue could be found on the leaves or shoots. All other plants were considered living.

Visual phytotoxicity (e.g. chlorosis, necrosis, abnormal growth) was recorded 7, 14 and 21 days after application according to EPPO Standard PP 1/135 (3).

Growth stages at the application day and 21 days after application were recorded according to BBCH-Monograph - Growth stages.

3. Observations:
Observations of mortality and phytotoxicity were made:
- Fresh weight: 21 days after application
- No. of living and dead palnts: 7, 14 and 21 days after application
- Visual phytotoxicity: 7, 14 and 21 days after application

II. RESULTS AND DISCUSSION

All validity criteria were met.

The most sensitive species in terms of fresh weight was *Lactuca sativa* with an ER_{50} value of 32.9 mL Kinvara/ha followed by *Brassica oleracea, Lycopersicon esculentum* and *Daucus carota* with ER_{50} values of 68.9, 78.2 and 107 mL Kinvara/ha, respectively. They were followed by *Vicia faba* and *Gossypium herbaceum* (ER_{50} value of 149 and 185 mL Kinvara/ha, respectively), which were followed by *Cucumis sativus* and *Allium cepa* (ER_{50} value of 1394 and 1748 mL Kinvara/ha, respectively). The least sensitive species were *Lolium perenne* and *Avena sativa* with a NOER of 3000 mL Kinvara/ha, an ER_X-value could not be determined.

Statistical significant mortality was observed for *Vicia faba* at 1000 mL Kinvara/ha (54 %) and for *Lactuca sativa* at 111 mL Kinvara/ha (50 %).

Phytotoxic effects observed were chlorosis (all species except *Lolium perenne* and *Avena sativa*), necrosis (all species except *Lolium perenne* and *Avena sativa*), growth reduction (all species except *Lolium perenne* and *Avena sativa*), abnormal growth of the leaves (all species except *Brassica oleracea, Lolium perenne* and *Avena sativa*) and abnormal growth of the stems (all species except *Lolium perenne, Avena sativa* and *Allium cepa*).

Table 10.8.1.2-1: Summary of effect rates (based on fresh weight)

	NOER [mL/ha]	LOER [mL/ha]	Statistical Analysis		ER_{10} [mL/ha]	ER_{20} [mL/ha]	ER_{50} [mL/ha]	Statistical Analysis
Brassica oleracea	12.3	37.0	1		25.5	35.8	68.9	4
				lower 95%-cl	6.38	13.4	46.1	
				upper 95%-cl	40.0	51.8	102.1	
				r^2 = 0.859				
Vicia faba	4.12	12.3	1		42.9	65.8	149	4
				lower 95%-cl	16.2	32.7	108	
				upper 95%-cl	66.8	93.3	205	
				r^2 = 0.916				
Lactuca sativa	< 12.3	12.3	2		10.6*	18.1	32.9	4
				lower 95%-cl	6.32*	13.1	27.6	
				upper 95%-cl	14.3	22.2	39.1	
				r^2 = 0.976				
Lycopersicon esculentum	< 4.12	4.12	2		12.0	22.9	78.2	4
				lower 95%-cl	0.64*	3.02*	38.6	
				upper 95%-cl	27.7	44.4	169	
				r^2 = 0.902				

Cucumis sativus	333	1000	3		132	296	1394	4
				lower 95%-cl	52.8	160	1030	
				upper 95%-cl	223	434	2023	
				$r^2 = 0.975$				
Daucus carota	37.0	111	3		13.7	27.8	107	4
				lower 95%-cl	7.63	18.1	84.6	
				upper 95%-cl	20.6	37.9	135	
				$r^2 = 0.978$				

results represent rounded values based on exact data
n.d. not determined due to mathematical reasons or inappropriate data
*the ER_X-value is extrapolated
1 multiple comparison Dunnett's t-test, α = 0.05
2 multiple comparison Williams t-test, α = 0.05
3 multiple comparison Bonferroni-Welch t-test, α = 0.05
4 Probit Analysis, cl = confidence limits

Table 10.8.1.2-2: Summarized results of fresh weight, mortality, phytotoxicity and plant growth

	Treatment Group [mL test item/ha]	Mortality [%]	Mortality Statistics	Fresh Weight [g]	Standard Deviation	Effect* [%]	Fresh Weight Statistics	Phytotoxicity [%]			Growth Stage (BBCH)	Growth Stage (BBCH)
Time of Evaluation				21 DAA				7 DAA	14 DAA	21 DAA	at application	21 DAA
Species												
Brassica oleracea	control	0		18.18	± 2.02	-		0	0	0	12	17-18
	4.12	0	-	19.42	± 1.87	6.8	n.s.[2]	0	0	0	12	17-18
	12.3	0	-	18.23	± 2.97	0.3	n.s.[2]	0	0	1	12	17-18
	37.0	0	-	14.58	± 3.13	-19.8	s.[2]	8	13	12	12	17-18
	111	0	-	4.41	± 1.05	-75.7	s.[2]	28	46	53	12	15-17
	333	0	-	2.02	± 0.47	-88.9	s.[2]	41	68	78	12	12-13
	1000	0	-	1.74	± 0.46	-90.4	s.[2]	45	75	79	12	12-13
Vicia faba	control	0		23.93	± 1.54	-		0	0	0	12-13	36-38
	4.12	0	n.s.[1]	23.38	± 2.08	-2.3	n.s.[2]	1	6	6	12-13	36-38
	12.3	0	n.s.[1]	21.35	± 1.71	-10.8	s.[2]	5	13	13	12-13	35-36
	37.0	0	n.s.[1]	22.57	± 1.85	-5.7	n.s.[2]	11	17	26	12-13	21/35-36
	111	0	n.s.[1]	14.78	± 2.25	-38.2	s.[2]	28	33	45	12-13	21/33
	333	21	n.s.[1]	4.80	± 0.87	-80.0	s.[2]	48	64	83	12-13	13/33
	1000	54	s.[1]	0.72	± 0.40	-97.0	s.[2]	53	88	97	12-13	13/33
Lactuca sativa	control	0		30.37	± 3.14	-		0	0	0	13	19
	12.3	0	n.s.[1]	27.41	± 2.60	-9.7	s.[3]	11	13	12	13	19
	21.4	0	n.s.[1]	19.75	± 0.79	-35.0	s.[3]	22	27	28	13	19
	37	0	n.s.[1]	13.40	± 2.43	-55.9	s.[3]	42	51	53	13	19
	64.2	0	n.s.[1]	7.96	± 2.00	-73.8	s.[3]	55	67	68	13	15-17
	111	50	s.[1]	1.79	± 1.67	-94.1	s.[3]	62	76	92	13	14
Lycopersicon esculentum	control	0		53.43	± 2.35	-		0	0	0	12	16-17/52-53
	4.12	0	n.s.[1]	48.67	± 4.01	-8.9	s.[3]	0	0	2	12	16-17/52-53
	12.3	0	n.s.[1]	45.68	± 3.64	-14.5	s.[3]	3	4	5	12	16-17/52-53
	37.0	0	n.s.[1]	41.75	± 3.58	-21.9	s.[3]	24	18	20	12	16-18
	111	0	n.s.[1]	18.45	± 4.22	-65.5	s.[3]	46	50	62	12	13-14
	333	13	n.s.[1]	9.52	± 2.26	-82.2	s.[3]	62	62	84	12	13
Cucumis sativus	control	0		28.62	± 8.66	-		0	0	0	12	63-65
	37.0	0	-	32.82	± 3.13	14.7	n.s.[4]	1	3	6	12	63-66
	111	0	-	25.72	± 1.63	-10.1	n.s.[4]	7	10	18	12	63-65
	333	0	-	23.15	± 2.86	-19.1	n.s.[4]	33	42	44	12	62-63
	1000	0	-	15.34	± 3.10	-46.4	s.[4]	55	63	64	12	13-14/51-52
	3000	0	-	10.24	± 1.37	-64.2	s.[4]	58	65	81	12	12-13
Daucus carota	control	0		11.92	± 2.62	-		0	0	0	12	16
	4.12	0	n.s.[1]	12.06	± 1.85	1.2	n.s.[4]	0	2	3	12	16
	12.3	0	n.s.[1]	11.17	± 1.71	-6.2	n.s.[4]	6	8	6	12	16
	37.0	0	n.s.[1]	8.93	± 1.11	-25.1	n.s.[4]	8	11	10	12	16
	111	4	n.s.[1]	5.51	± 1.19	-53.8	s.[4]	25	37	34	12	15-16
	333	0	n.s.[1]	2.75	± 0.65	-76.9	s.[4]	53	57	75	12	14
	1000	0	n.s.[1]	1.55	± 0.28	-87.0	s.[4]	61	78	91	12	13
Gossypium herbaceum	control	0		44.44	± 6.35	-		0	0	0	12	23-26
	1.37	0	-	41.16	± 6.64	-7.4	n.s.[3]	0	1	3	12	23-26
	4.12	0	-	38.30	± 4.78	-13.8	s.[3]	5	7	12	12	23-26
	12.3	0	-	36.98	± 3.37	-16.8	s.[3]	6	10	11	12	23-26
	37	0	-	35.01	± 3.54	-21.2	s.[3]	18	28	23	12	24-26
	111	0	-	27.75	± 3.74	-37.6	s.[3]	27	43	47	12	23-25
	333	0	-	15.54	± 2.36	-65.0	s.[3]	51	59	56	12	12
Lolium perenne	control	0		7.89	± 0.48	-		0	0	0	12	23
	1000	0	-	7.04	± 0.68	-10.7	s.[2]	0	0	0	12	23-24
	3000	0	-	7.84	± 0.63	-0.5	n.s.[2]	0	0	0	12	23-24
Avena sativa	control	0		22.96	± 1.85	-		0	0	0	12	20/34
	1000	0	-	22.05	± 1.61	-4.0	n.s.[3]	0	0	0	12	21/34
	3000	0	-	21.78	± 1.54	-5.1	n.s.[3]	0	0	1	12	21/34
Allium cepa	control	0		5.98	± 1.03	-		0	0	0	12	13-14
	12.3	0	n.s.[1]	6.93	± 1.06	16.0	n.s.[4]	0	0	0	12	13-14
	37	0	n.s.[1]	7.17	± 0.50	20.0	n.s.[4]	0	1	2	12	13-14
	111	0	n.s.[1]	7.11	± 0.91	19.0	n.s.[4]	0	1	3	12	13-14
	333	0	n.s.[1]	4.50	± 0.62	-24.7	s.[4]	8	6	9	12	13-14
	1000	0	n.s.[1]	3.66	± 0.35	-38.7	s.[4]	31	32	38	12	13
	3000	4	n.s.[1]	2.42	± 0.55	-59.6	s.[4]	53	58	53	12	12-13

the results represent rounded values calculated on the exact raw data
* : negative values indicate reduction compared to control
DAA days after application; s.: significant; n.s.: not significant
1 pair-wise comparison Fisher's Exact Test, α = 0.05
3 multiple comparison Williams t-test, α = 0.05
4 multiple comparison Bonferroni-Welch t-test, α = 0.05

III. CONCLUSIONS

In a vegetative vigour study with Kinvara, the most sensitive species in terms of fresh weight was *Lactuca sativa* with an ER_{50} value of 32.9 mL Kinvara/ha.

(Butzler, R. & Munz, J., (2014))

Table 14. Phytotoxicity [%], *Brassica oleracea*

Phytotoxicity after 7 days

Species	Pot	Treatment Group [mL test item/ha]						
Brassica oleracea		control	4.12	12.3	37.0	111	333	1000
	1	0	0	0	15	45	50	40
	2	0	0	0	5	20	30	50
	3	0	0	0	5	20	40	50
	4	0	0	0	0	20	40	35
	5	0	0	0	20	30	50	50
	6	0	0	0	5	35	35	45
Mean [%]		0.0	0.0	0.0	8.3	28.3	40.8	45.0
Standard Deviation		± 0.00	± 0.00	± 0.00	± 7.53	± 10.33	± 8.01	± 6.32

Phytotoxicity after 14 days

Species	Pot	Treatment Group [mL test item/ha]						
Brassica oleracea		control	4.12	12.3	37.0	111	333	1000
	1	0	0	0	15	50	75	75
	2	0	0	0	5	50	45	80
	3	0	0	0	10	50	70	80
	4	0	0	0	10	30	75	70
	5	0	0	0	25	50	70	75
	6	0	0	0	10	45	75	70
Mean [%]		0.0	0.0	0.0	12.5	45.8	68.3	75.0
Standard Deviation		± 0.00	± 0.00	± 0.00	± 6.89	± 8.01	± 11.69	± 4.47

Phytotoxicity after 21 days

Species	Pot	Treatment Group [mL test item/ha]						
Brassica oleracea		control	4.12	12.3	37.0	111	333	1000
	1	0	0	0	15	55	80	80
	2	0	0	0	8	60	70	90
	3	0	0	0	8	40	80	80
	4	0	0	3	8	55	80	70
	5	0	0	0	25	60	80	80
	6	0	0	0	8	45	80	75
Mean [%]		0.0	0.0	0.5	12.0	52.5	78.3	79.2
Standard Deviation		± 0.00	± 0.00	± 1.22	± 6.96	± 8.22	± 4.08	± 6.65

Table 17. Phytotoxicity [%], *Vicia faba*

Phytotoxicity after 7 days

Species	Pot	Treatment Group [mL test item/ha]						
Vicia faba		control	4.12	12.3	37.0	111	333	1000
	1	0	0	5	10	35	50	55
	2	0	0	7	10	30	50	50
	3	0	0	5	10	25	50	55
	4	0	2	5	15	25	45	50
	5	0	0	5	10	25	45	50
	6	0	3	5	10	25	45	55
Mean [%]		0.0	0.8	5.3	10.8	27.5	47.5	52.5
Standard Deviation		± 0.00	± 1.33	± 0.82	± 2.04	± 4.18	± 2.74	± 2.74

Phytotoxicity after 14 days

Species	Pot	Treatment Group [mL test item/ha]						
Vicia faba		control	4.12	12.3	37.0	111	333	1000
	1	0	4	10	20	35	60	95
	2	0	6	15	10	20	65	90
	3	0	8	15	20	35	65	90
	4	0	8	15	25	35	60	85
	5	0	4	10	15	35	70	85
	6	0	4	10	10	40	65	85
Mean [%]		0.0	5.7	12.5	16.7	33.3	64.2	88.3
Standard Deviation		± 0.00	± 1.97	± 2.74	± 6.06	± 6.83	± 3.76	± 4.08

Phytotoxicity after 21 days

Species	Pot	Treatment Group [mL test item/ha]						
Vicia faba		control	4.12	12.3	37.0	111	333	1000
	1	0	10	10	25	45	85	99
	2	0	3	20	30	45	80	94
	3	0	6	15	25	45	90	99
	4	0	10	10	30	45	75	94
	5	0	4	8	15	45	85	97
	6	0	4	15	30	45	85	99
Mean [%]		0.0	6.2	13.0	25.8	45.0	83.3	97.0
Standard Deviation		± 0.00	± 3.13	± 4.47	± 5.85	± 0.00	± 5.16	± 2.45

Table 20. Phytotoxicity [%], *Lactuca sativa*

Phytotoxicity after 7 days

Species	Pot	Treatment Group [mL test item/ha]					
Lactuca sativa		control	12.3	21.4	37.0	64.2	111
	1	0	10	20	25	60	65
	2	0	10	20	40	65	60
	3	0	8	25	55	35	60
	4	0	10	20	50	50	65
	5	0	10	15	25	60	60
	6	0	15	30	55	60	60
Mean [%]		0.0	10.5	21.7	41.7	55.0	61.7
Standard Deviation		± 0.00	± 2.35	± 5.16	± 14.02	± 10.95	± 2.58

Phytotoxicity after 14 days

Species	Pot	Treatment Group [mL test item/ha]					
Lactuca sativa		control	12.3	21.4	37.0	64.2	111
	1	0	15	25	30	70	75
	2	0	8	25	55	70	80
	3	0	10	25	50	55	80
	4	0	20	30	60	65	75
	5	0	10	25	50	70	75
	6	0	15	30	60	70	70
Mean [%]		0.0	13.0	26.7	50.8	66.7	75.8
Standard Deviation		± 0.00	± 4.47	± 2.58	± 11.14	± 6.06	± 3.76

Phytotoxicity after 21 days

Species	Pot	Treatment Group [mL test item/ha]					
Lactuca sativa		control	12.3	21.4	37.0	64.2	111
	1	0	8	30	35	70	80
	2	0	10	30	60	70	100
	3	0	10	25	40	60	98
	4	0	20	30	65	65	92
	5	0	5	25	60	75	96
	6	0	20	30	60	70	85
Mean [%]		0.0	12.2	28.3	53.3	68.3	91.8
Standard Deviation		± 0.00	± 6.34	± 2.58	± 12.52	± 5.16	± 7.86

Table 23. Phytotoxicity [%], *Lycopersicon esculentum*

Phytotoxicity after 7 days

Species	Pot	Treatment Group [mL test item/ha]					
Lycopersicon esculentum		control	4.12	12.3	37.0	111	333
	1	0	0	0	25	45	60
	2	0	0	5	30	45	65
	3	0	0	0	20	40	60
	4	0	0	5	20	40	60
	5	0	0	0	20	55	60
	6	0	0	5	30	50	65
Mean [%]		0.0	0.0	2.5	24.2	45.8	61.7
Standard Deviation		± 0.00	± 0.00	± 2.74	± 4.92	± 5.85	± 2.58

Phytotoxicity after 14 days

Species	Pot	Treatment Group [mL test item/ha]					
Lycopersicon esculentum		control	4.12	12.3	37.0	111	333
	1	0	0	0	20	50	65
	2	0	0	8	20	50	65
	3	0	0	0	20	45	60
	4	0	0	8	10	45	60
	5	0	0	0	20	60	60
	6	0	0	6	20	50	60
Mean [%]		0.0	0.0	3.7	18.3	50.0	61.7
Standard Deviation		± 0.00	± 0.00	± 4.08	± 4.08	± 5.48	± 2.58

Phytotoxicity after 21 days

Species	Pot	Treatment Group [mL test item/ha]					
Lycopersicon esculentum		control	4.12	12.3	37.0	111	333
	1	0	0	0	20	60	85
	2	0	0	10	20	65	90
	3	0	8	0	20	55	85
	4	0	0	10	15	55	80
	5	0	5	0	20	75	80
	6	0	0	10	25	60	85
Mean [%]		0.0	2.2	5.0	20.0	61.7	84.2
Standard Deviation		± 0.00	± 3.49	± 5.48	± 3.16	± 7.53	± 3.76

Table 25. Phytotoxicity [%], *Cucumis sativus*

Phytotoxicity after 7 days

Species	Pot	Treatment Group [mL test item/ha]					
Cucumis sativus		control	37.0	111	333	1000	3000
	1	0	0	0	30	60	55
	2	0	0	5	25	55	60
	3	0	0	10	40	55	60
	4	0	5	5	40	60	55
	5	0	0	10	30	45	60
	6	0	0	10	30	55	55
Mean [%]		0.0	0.8	6.7	32.5	55.0	57.5
Standard Deviation		± 0.00	± 2.04	± 4.08	± 6.12	± 5.48	± 2.74

Phytotoxicity after 14 days

Species	Pot	Treatment Group [mL test item/ha]					
Cucumis sativus		control	37.0	111	333	1000	3000
	1	0	0	0	40	65	65
	2	0	0	5	40	65	65
	3	0	15	15	50	60	65
	4	0	5	5	40	65	65
	5	0	0	20	35	55	65
	6	0	0	15	45	65	65
Mean [%]		0.0	3.3	10.0	41.7	62.5	65.0
Standard Deviation		± 0.00	± 6.06	± 7.75	± 5.16	± 4.18	± 0.00

Phytotoxicity after 21 days

Species	Pot	Treatment Group [mL test item/ha]					
Cucumis sativus		control	37.0	111	333	1000	3000
	1	0	0	20	45	75	75
	2	0	0	15	40	65	80
	3	0	25	20	40	55	85
	4	0	10	15	55	75	80
	5	0	0	25	40	40	80
	6	0	0	15	45	75	85
Mean [%]		0.0	5.8	18.3	44.2	64.2	80.8
Standard Deviation		± 0.00	± 10.21	± 4.08	± 5.85	± 14.29	± 3.76

Table 28. Phytotoxicity [%], *Daucus carota*

Phytotoxicity after 7 days

Species	Pot	Treatment Group [mL test item/ha]						
Daucus carota		control	4.12	12.3	37.0	111	333	1000
	1	0	0	0	10	25	50	60
	2	0	0	5	15	25	60	60
	3	0	0	5	0	20	55	65
	4	0	0	20	5	30	50	55
	5	0	0	5	5	25	50	60
	6	0	0	0	10	25	50	65
Mean [%]		0.0	0.0	5.8	7.5	25.0	52.5	60.8
Standard Deviation		± 0.00	± 0.00	± 7.36	± 5.24	± 3.16	± 4.18	± 3.76

Phytotoxicity after 14 days

Species	Pot	Treatment Group [mL test item/ha]						
Daucus carota		control	4.12	12.3	37.0	111	333	1000
	1	0	8	15	10	35	50	75
	2	0	0	5	10	35	70	75
	3	0	0	10	0	30	70	80
	4	0	5	15	20	60	50	70
	5	0	0	5	15	25	50	80
	6	0	0	0	10	35	50	85
Mean [%]		0.0	2.2	8.3	10.8	36.7	56.7	77.5
Standard Deviation		± 0.00	± 3.49	± 6.06	± 6.65	± 12.11	± 10.33	± 5.24

Phytotoxicity after 21 days

Species	Pot	Treatment Group [mL test item/ha]						
Daucus carota		control	4.12	12.3	37.0	111	333	1000
	1	0	10	10	10	40	75	92
	2	0	4	0	4	35	90	85
	3	0	0	10	10	30	75	90
	4	0	4	10	10	55	50	90
	5	0	0	3	15	15	75	92
	6	0	0	4	10	30	85	94
Mean [%]		0.0	3.0	6.2	9.8	34.2	75.0	90.5
Standard Deviation		± 0.00	± 3.95	± 4.40	± 3.49	± 13.20	± 13.78	± 3.08

Table 30. Phytotoxicity [%], *Gossypium herbaceum*

Phytotoxicity after 7 days

Species	Pot	Treatment Group [mL test item/ha]						
Gossypium herbaceum		control	1.37	4.12	12.3	37.0	111	333
	1	0	0	5	5	30	30	55
	2	0	0	5	8	15	20	50
	3	0	0	5	5	25	20	50
	4	0	0	3	5	15	30	50
	5	0	0	5	5	10	30	50
	6	0	0	5	5	15	30	50
Mean [%]		0.0	0.0	4.7	5.5	18.3	26.7	50.8
Standard Deviation		± 0.00	± 0.00	± 0.82	± 1.22	± 7.53	± 5.16	± 2.04

Phytotoxicity after 14 days

Species	Pot	Treatment Group [mL test item/ha]						
Gossypium herbaceum		control	1.37	4.12	12.3	37.0	111	333
	1	0	5	6	8	40	40	65
	2	0	0	6	15	35	40	60
	3	0	0	4	10	40	55	55
	4	0	0	8	5	15	40	60
	5	0	0	10	8	15	40	60
	6	0	0	6	15	25	45	55
Mean [%]		0.0	0.8	6.7	10.2	28.3	43.3	59.2
Standard Deviation		± 0.00	± 2.04	± 2.07	± 4.07	± 11.69	± 6.06	± 3.76

Phytotoxicity after 21 days

Species	Pot	Treatment Group [mL test item/ha]						
Gossypium herbaceum		control	1.37	4.12	12.3	37.0	111	333
	1	0	5	15	10	40	45	65
	2	0	0	15	10	20	45	65
	3	0	0	10	15	20	60	45
	4	0	0	5	10	20	45	55
	5	0	3	15	10	15	45	55
	6	0	9	10	9	20	40	50
Mean [%]		0.0	2.8	11.7	10.7	22.5	46.7	55.8
Standard Deviation		± 0.00	± 3.66	± 4.08	± 2.16	± 8.80	± 6.83	± 8.01

Table 33. Phytotoxicity [%], *Avena sativa*

Phytotoxicity after 7 days

Species	Pot	Treatment Group [mL test item/ha]		
Avena sativa		control	1000	3000
	1	0	0	0
	2	0	0	0
	3	0	0	0
	4	0	0	0
	5	0	0	0
	6	0	0	0
Mean [%]		0.0	0.0	0.0
Standard Deviation		± 0.00	± 0.00	± 0.00

Phytotoxicity after 14 days

Species	Pot	Treatment Group [mL test item/ha]		
Avena sativa		control	1000	3000
	1	0	0	0
	2	0	0	0
	3	0	0	0
	4	0	0	0
	5	0	0	0
	6	0	0	0
Mean [%]		0.0	0.0	0.0
Standard Deviation		± 0.00	± 0.00	± 0.00

Phytotoxicity after 21 days

Species	Pot	Treatment Group [mL test item/ha]		
Avena sativa		control	1000	3000
	1	0	0	0
	2	0	0	0
	3	0	0	0
	4	0	0	0
	5	0	0	3
	6	0	0	0
Mean [%]		0.0	0.0	0.5
Standard Deviation		± 0.00	± 0.00	± 1.22

Table 36. Phytotoxicity [%], *Allium cepa*

Phytotoxicity after 7 days

Species	Pot	Treatment Group [mL test item/ha]						
Allium cepa		control	12.3	37.0	111	333	1000	3000
	1	0	0	0	0	0	20	50
	2	0	0	0	0	5	25	50
	3	0	0	0	0	10	25	50
	4	0	0	0	0	10	55	65
	5	0	0	0	0	10	20	50
	6	0	0	0	0	10	40	55
Mean [%]		0.0	0.0	0.0	0.0	7.5	30.8	53.3
Standard Deviation		± 0.00	± 0.00	± 0.00	± 0.00	± 4.18	± 13.93	± 6.06

Phytotoxicity after 14 days

Species	Pot	Treatment Group [mL test item/ha]						
Allium cepa		control	12.3	37.0	111	333	1000	3000
	1	0	0	0	0	0	15	50
	2	0	0	0	6	5	50	50
	3	0	0	0	0	5	15	60
	4	0	0	0	0	0	45	65
	5	0	0	0	0	10	15	55
	6	0	0	5	2	15	50	65
Mean [%]		0.0	0.0	0.8	1.3	5.8	31.7	57.5
Standard Deviation		± 0.00	± 0.00	± 2.04	± 2.42	± 5.85	± 18.35	± 6.89

Phytotoxicity after 21 days

Species	Pot	Treatment Group [mL test item/ha]						
Allium cepa		control	12.3	37.0	111	333	1000	3000
	1	0	0	6	0	10	40	50
	2	0	0	0	6	8	35	50
	3	0	0	0	3	5	20	50
	4	0	0	0	6	8	45	70
	5	0	0	0	0	10	45	50
	6	0	2	8	3	10	40	45
Mean [%]		0.0	0.3	2.3	3.0	8.5	37.5	52.5
Standard Deviation		± 0.00	± 0.82	± 3.67	± 2.68	± 1.97	± 9.35	± 8.80

10.6.2/02 Butzler and Munz (2014)

Comments of zRMS:	The submitted study was accepted. The validity criteria were met: The following endpoints for most sensitive plant (cabbage) were derived: • ER_{50} > 25.7 mL/ha • NOER = 4.12 mL/ha The endpoint ER_{50} will be used in risk assessment. The observations results are added at the end of study summary.

The following seedling emergence study performed on Kinvara is provided in support of the assessment.

Report:	KIIIA1 10.6.2/02, Butzler, R. & Munz, J., (2014)
Title:	Effects of Kinvara on Terrestrial (Non-Target) plants: Seedling Emergence and Seedling Growth Test
Document No:	89201086
Guidelines:	OECD Guideline No. 228, 2006
GLP	Yes

Executive Summary:
The purpose of this study was to determine a dose dependence of Kinvara on seedling emergence and seedling growth of ten non-target plant species representing nine plant families at concentrations in the range 4.12 - 3000 mL Kinvara/ha. The ER_{10}, ER_{20}, ER_{50} and NOER based on fresh weight were determined and observation of germination, mortality and phytotoxicity (chlorosis, necrosis and growth reduction) were also made.

The seeds were introduced manually into the soil. After sowing the pots were placed on saucers and watered. Test rates were calculated for a water amount of 200 L/ha and were administered onto the plants using laboratory spraying equipment. 30 seeds per species and treatment group were tested. The concentration of the active ingredients in the stock solutions was verified analytically. The exposure time was 14-21 day.

The most sensitive species in terms of fresh weight was *Brassica oleracea* with an ER_{50} value of 25.7 mL Kinvara/ha followed by *Lactuca sativa* with an ER_{50} value of 69.3 mL Kinvara/ha and *Allium cepa* with an ER_{50} value of 73.2 mL Kinvara/ha (ER_{50} value of 61.2 mL Kinvara/ha (control with 5 replicates)). They were followed by *Lycopersicon esculentum, Cucumis sativus* and *Daucus carota* with ER_{50} values of 234, 300 and 214 mL Kinvara/ha, respectively (for *Daucus carota* the ER_{50} value calculated for fresh weight per plant is 268 mL Kinvara/ha). They were followed by *Vicia faba* with an ER_{50} value of 612 mL Kinvara/ha (the ER_{50} value calculated for fresh weight per plant is 632 mL Kinvara/ha). They were followed by *Gossypium herbaceum* and *Avena sativa* (ER_{50} value of 1416 and 2685 mL Kinvara/ha, respectively). The least sensitive species was *Lolium perenne* with a NOER of 3000 mL Kinvara/ha, an ER_X value could not be determined.

II. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: Kinvara
Description: Yellow / Amber liquid
Lot/Batch #: 13-3601
Purity: MCPA: 233.6 g/L
Fluroxypyr-meptyl: 73.3 g/L

	Clopyralid: 27.3 g/L
Stability of test compound:	Stable under normal conditions. Vehicle: Water
2. Vehicle and/or positive control:	Control – Deionised water
3. Test animals -	
Species:	Monocotyledoneae and Dicotyledoneae
Age:	Seedlings
Environmental conditions	
-	
Temperature:	18 ºC – 24.7ºC
Humidity:	60 %-77 %
Photoperiod:	16 hr light, 8 hr dark (5440 to 19930 lux)

B. STUDY DESIGN AND METHODS:

1. In-life dates:	Sowing: 29th April 2014 Application: 30th April 2014

2. Experimental treatments:
The effect of Kinvara on monocotyledonous and dicotyledonous seedlings was tested. 6 pots each containing 5 seeds were tested for each treatment group. Each pot represented one replicate. 30 seeds per species and treatment group were tested. For a given test species, all seeds used in the test were from the same source and lot number. The exposure time was 14 to 21 days after 50 % germination in the control depending on the growth of the seedlings.
The test was performed in a growth chamber under controlled conditions (phtot-period, humidity, temperature etc.). All applications were performed using laboratory spraying equipment, thus, the application was done in an accurate way with reproducible coverage. The following nominal rates were prepared based on a stock solution, 3000, 1000, 333, 111, 37.0, 12.3, 4.12 and 1.37 mL test item/ha.

The fresh weight of the above ground part of all survived plants of a pot was determined 14 or 21 days after 50 % seedling emergence in the control. If all plants of a pot were dead the value for this replicate was assessed as "0".

Germination was checked daily on weekdays until 50% of the control plants had emerged. Further checks were done weekly.

The number of living and dead plants was recorded 7 and 14 days or 7, 14 and 21 days after 50 % seedling emergence in the control. A plant was considered dead if no living tissue could be found on the leaves or shoots. All other plants were considered living.

Visual phytotoxicity (e.g. chlorosis, necrosis, abnormal growth) was recorded weekly according to EPPO Standards after 50 % seedling emergence in the control.

3. Observations:
Observations of germination, mortality and phytotoxicity were made:
- Fresh weight: 14-21 days after application after 50% seedling emergence in the control
- Germination was checked on weekdays until 50% seedling emergence in the control
- No. of living and dead palnts: 7 and 14 or 7, 14 and 21 days after 50% seedling emergence in the control
- Visual phytotoxicity: was recorded after 50% seedling emergence in the control

II. RESULTS AND DISCUSSION

All validity criteria were met.

The most sensitive species in terms of fresh weight was *Brassica oleracea* with an ER_{50} value of 25.7 mL Kinvara/ha followed by *Lactuca sativa* with an ER_{50} value of 69.3 mL Kinvara/ha and *Allium cepa*

with an ER_{50} value of 73.2 mL Kinvara/ha (ER_{50} value of 61.2 mL Kinvara/ha (control with 5 replicates)). They were followed by *Lycopersicon esculentum, Cucumis sativus* and *Daucus carota* with ER_{50} values of 234, 300 and 214 mL Kinvara/ha, respectively (for *Daucus carota* the ER_{50} value calculated for fresh weight per plant is 268 mL Kinvara/ha). They were followed by *Vicia faba* with an ER_{50} value of 612 mL Kinvara/ha (the ER_{50} value calculated for fresh weight per plant is 632 mL Kinvara/ha). They were followed by *Gossypium herbaceum* and *Avena sativa* (ER_{50} value of 1416 and 2685 mL Kinvara/ha, respectively). The least sensitive species was *Lolium perenne* with a NOER of 3000 mL Kinvara/ha, an ER_X value could not be determined.

The germination rate was statistically significant reduced for *Brassica oleracea* at 111 mL Kinvara/ha, for *Lactuca sativa* at 192 mL Kinvara/ha and for *Gossypium herbaceum* at 3000 mL Kinvara/ha. For *Allium cepa* the germination rate was statistically significant reduced at 333 and 1000 mL Kinvara/ha.
Statistically significant mortality was observed for *Vicia faba* at 3000 mL Kinvara/ha (50 %) and for *Cucumis sativus* at 1000 mL Kinvara/ha (47 %).

Phytotoxic effects observed were chlorosis (*Vicia faba, Lycopersicon esculentum* and *Daucus carota*), necrosis (*Brassica oleracea, Vicia faba, Lactuca sativa, Lycopersicon esculentum, Cucumis sativus, Daucus carota, Gossypium herbaceum* and *Allium cepa*) and growth reduction (all species). Additionally mainly *Vicia faba, Lactuca sativa, Lycopersicon esculentum* and *Cucumis sativus* showed abnormal growth of the leaves and mainly *Brassica oleracea, Vicia faba* and *Lactuca sativa* showed abnormal growth of the stems.

Table 10.8.1.3-1: Summary of effect rates (based on fresh weight)

	NOER [mL/ha]	LOER [mL/ha]	Statistical Analysis		ER_{10} [mL/ha]	ER_{20} [mL/ha]	ER_{50} [mL/ha]	Statistical Analysis
Brassica oleracea	4.12	12.3	1		5.14	8.93	25.7	5
				lower 95%-cl	0.41*	1.51*	13.3	
				upper 95%-cl	10.8	16.2	49.2	
				r^2 = 0.904				
Vicia faba	333	1000	2		164	258	612	5
				lower 95%-cl	3.96*	20.0*	281	
				upper 95%-cl	330	457	1334	
				r^2 = 0.863				
Vicia faba (per plant)	333	1000	3		165	262	632	5
				lower 95%-cl	21.4*	62.5	369	
				upper 95%-cl	305	428	1086	
				r^2 = 0.906				
Lactuca sativa	< 12.3	12.3	1		22.6 #	35.3 #	69.3 #	6
				lower 95%-cl	n.d.	n.d.	n.d.	
				upper 95%-cl	n.d.	n.d.	n.d.	
				r^2 = 0.738				
Lycopersicon esculentum	111	333	4		63.0*	98.9*	234	5
				lower 95%-cl	21.1*	45.1*	172	
				upper 95%-cl	101*	142	306	
				r^2 = 0.949				
Cucumis sativus	< 111	111	4		87.9*	134	300	5
				lower 95%-cl	61.4*	103*	264	
				upper 95%-cl	112	161	341	
				r^2 = 0.987				

results represent rounded values based on exact data
n.d. not determined due to mathematical reasons or inappropriate data
* the ER_X-value is extrapolated

The ER_X-value is with reservation ((p(F)= 0.062, α = 0.05).

1 multiple comparison Williams t-test, α = 0.05
1 multiple comparison Bonferroni-Holm U-test, α = 0.05
2 multiple comparison Dunnett's t-test, α = 0.05
3 multiple comparison Bonferroni-Welch t-test, α = 0.05

4 Probit Analysis, cl = confidence limits
5 Weibull Analysis, cl = confidence limits

III. CONCLUSIONS

In a seedling emergence study with Kinvara, the most sensitive species in terms of fresh weight was *Lactuca sativa* with an ER_{50} value of 32.9 mL Kinvara/ha.

(Butzler, R. & Munz, J., (2014))

Table 12. Phytotoxicity [%], *Brassica oleracea*

Phytotoxicity after 7 days

Species	Pot	Treatment Group [mL test item/ha]					
Brassica oleracea		control	4.12	12.3	37.0	111	333
	1	0	0	0	30	98	99
	2	0	0	10	95	80	95
	3	0	0	5	80	95	98
	4	0	0	20	70	95	99
	5	0	0	10	20	98	95
	6	0	5	30	40	95	95
Mean [%]		0.0	0.8	12.5	55.8	93.5	96.8
Standard Deviation		± 0.00	± 2.04	± 10.84	± 30.07	± 6.77	± 2.04

Phytotoxicity after 14 days

Species	Pot	Treatment Group [mL test item/ha]					
Brassica oleracea		control	4.12	12.3	37.0	111	333
	1	0	0	0	25	98	98
	2	0	0	8	98	90	95
	3	0	0	5	40	98	98
	4	0	0	2	50	97	99
	5	0	0	3	25	99	97
	6	0	10	35	20	95	98
Mean [%]		0.0	1.7	8.8	43.0	96.2	97.5
Standard Deviation		± 0.00	± 4.08	± 13.11	± 29.19	± 3.31	± 1.38

Table 17. Phytotoxicity [%], *Vicia faba*

Phytotoxicity after 7 days

Species	Pot	Treatment Group [mL test item/ha]					
Vicia faba		control	37.0	111	333	1000	3000
	1	0	0	10	35	30	97
	2	0	0	10	25	90	95
	3	0	0	15	10	30	97
	4	0	30	5	10	90	97
	5	0	0	15	70	80	97
	6	0	0	8	5	95	90
Mean [%]		0.0	5.0	10.5	25.8	69.2	95.5
Standard Deviation		± 0.00	± 12.25	± 3.94	± 24.38	± 30.73	± 2.81

Phytotoxicity after 14 days

Species	Pot	Treatment Group [mL test item/ha]					
Vicia faba		control	37.0	111	333	1000	3000
	1	0	5	10	40	65	97
	2	0	10	10	25	85	97
	3	0	0	10	30	30	99
	4	0	65	15	20	90	100
	5	0	5	10	70	85	99
	6	0	5	10	10	99	90
Mean [%]		0.0	15.0	10.8	32.5	75.7	97.0
Standard Deviation		± 0.00	± 24.70	± 2.04	± 20.92	± 24.99	± 3.63

Table 21. Phytotoxicity [%], *Lactuca sativa*

Phytotoxicity after 7 days

Species	Pot	Treatment Group [mL test item/ha]					
Lactuca sativa		control	12.3	37.0	64.2	111	192
	1	0	10	10	30	30	50
	2	0	0	0	0	50	70
	3	0	0	0	50	99	70
	4	0	10	5	10	80	99
	5	0	0	0	15	80	97
	6	0	10	5	10	97	70
Mean [%]		0.0	5.0	3.3	19.2	72.7	76.0
Standard Deviation		± 0.00	± 5.48	± 4.08	± 18.00	± 27.30	± 18.73

Phytotoxicity after 14 days

Species	Pot	Treatment Group [mL test item/ha]					
Lactuca sativa		control	12.3	37.0	64.2	111	192
	1	0	0	25	45	50	65
	2	0	0	15	0	70	90
	3	0	0	20	70	99	70
	4	0	10	5	15	85	99
	5	0	0	0	10	85	99
	6	0	0	10	15	96	90
Mean [%]		0.0	1.7	12.5	25.8	80.8	85.5
Standard Deviation		± 0.00	± 4.08	± 9.35	± 26.35	± 18.24	± 14.60

Phytotoxicity after 21 days

Species	Pot	Treatment Group [mL test item/ha]					
Lactuca sativa		control	12.3	37.0	64.2	111	192
	1	0	0	10	10	40	60
	2	0	0	5	0	50	95
	3	0	0	10	60	97	60
	4	0	0	0	10	95	99
	5	0	0	0	5	95	98
	6	0	0	5	10	95	97
Mean [%]		0.0	0.0	5.0	15.8	78.7	84.8
Standard Deviation		± 0.00	± 0.00	± 4.47	± 22.00	± 26.28	± 19.28

Table 24. Phytotoxicity [%], *Lycopersicon esculentum*

Phytotoxicity after 7 days

Species	Pot	Treatment Group [mL test item/ha]					
Lycopersicon esculentum		control	111	192	333	577	1000
	1	0	0	0	15	65	50
	2	0	0	20	0	30	70
	3	0	10	0	10	40	65
	4	0	0	0	10	60	85
	5	0	0	5	30	60	70
	6	0	0	0	30	60	70
Mean [%]		0.0	1.7	4.2	15.8	52.5	68.3
Standard Deviation		± 0.00	± 4.08	± 8.01	± 12.01	± 14.05	± 11.25

Phytotoxicity after 14 days

Species	Pot	Treatment Group [mL test item/ha]					
Lycopersicon esculentum		control	111	192	333	577	1000
	1	0	3	10	20	80	60
	2	0	3	45	3	60	80
	3	0	5	10	10	55	75
	4	0	0	0	30	75	90
	5	0	0	15	85	75	90
	6	0	0	5	60	70	80
Mean [%]		0.0	1.8	14.2	34.7	69.2	79.2
Standard Deviation		± 0.00	± 2.14	± 15.94	± 31.70	± 9.70	± 11.14

Table 28. Phytotoxicity [%], *Cucumis sativus*

Phytotoxicity after 7 days

Species	Pot	Treatment Group [mL test item/ha]					
Cucumis sativus		control	111	192	333	577	1000
	1	0	8	10	40	70	98
	2	0	8	10	20	60	85
	3	0	5	25	30	90	90
	4	0	5	10	30	75	55
	5	0	8	10	30	30	95
	6	0	8	5	30	40	40
Mean [%]		0.0	7.0	11.7	30.0	60.8	77.2
Standard Deviation		± 0.00	± 1.55	± 6.83	± 6.32	± 22.45	± 23.88

Phytotoxicity after 14 days

Species	Pot	Treatment Group [mL test item/ha]					
Cucumis sativus		control	111	192	333	577	1000
	1	0	0	25	55	90	99
	2	0	2	3	15	65	85
	3	0	5	20	3	95	98
	4	0	3	8	55	85	80
	5	0	5	3	55	55	99
	6	0	3	3	50	55	80
Mean [%]		0.0	3.0	10.3	38.8	74.2	90.2
Standard Deviation		± 0.00	± 1.90	± 9.75	± 23.50	± 18.00	± 9.50

Table 33. Phytotoxicity [%], *Daucus carota*

Phytotoxicity after 7 days

Species	Pot	Treatment Group [mL test item/ha]					
Daucus carota		control	4.12	12.3	37.0	111	333
	1	0	0	0	0	4	20
	2	0	0	0	0	3	30
	3	0	0	0	0	5	20
	4	0	25	3	0	5	20
	5	0	0	2	3	5	3
	6	0	0	3	0	3	30
Mean [%]		0.0	4.2	1.3	0.5	4.2	20.5
Standard Deviation		± 0.00	± 10.21	± 1.51	± 1.22	± 0.98	± 9.87

Phytotoxicity after 14 days

Species	Pot	Treatment Group [mL test item/ha]					
Daucus carota		control	4.12	12.3	37.0	111	333
	1	0	0	0	0	0	30
	2	25	0	0	0	0	60
	3	0	0	0	0	3	40
	4	0	25	0	0	15	40
	5	0	0	1	10	5	5
	6	0	0	0	0	5	50
Mean [%]		4.2	4.2	0.2	1.7	4.7	37.5
Standard Deviation		± 10.21	± 10.21	± 0.41	± 4.08	± 5.54	± 18.91

Phytotoxicity after 21 days

Species	Pot	Treatment Group [mL test item/ha]					
Daucus carota		control	4.12	12.3	37.0	111	333
	1	0	0	0	0	0	45
	2	25	0	0	0	0	70
	3	0	5	0	0	0	50
	4	0	25	0	0	5	50
	5	0	0	0	5	3	2
	6	0	0	0	0	0	60
Mean [%]		4.2	5.0	0.0	0.8	1.3	46.2
Standard Deviation		± 10.21	± 10.00	± 0.00	± 2.04	± 2.16	± 23.41

Table 37. Phytotoxicity [%], *Gossypium herbaceum*

Phytotoxicity after 7 days

Species	Pot	Treatment Group [mL test item/ha]						
Gossypium herbaceum		control	12.3	37.0	111	333	1000	3000
	1	0	0	0	0	0	5	80
	2	0	0	0	0	0	70	-
	3	0	0	0	0	5	0	-
	4	0	5	10	0	0	8	80
	5	0	5	0	0	10	0	-
	6	0	0	0	0	0	10	80
Mean [%]		0.0	1.7	1.7	0.0	2.5	15.5	80.0
Standard Deviation		± 0.00	± 2.58	± 4.08	± 0.00	± 4.18	± 27.01	± 0.00

Phytotoxicity after 14 days

Species	Pot	Treatment Group [mL test item/ha]						
Gossypium herbaceum		control	12.3	37.0	111	333	1000	3000
	1	0	0	0	0	0	0	95
	2	0	0	0	0	0	80	80
	3	0	0	0	0	5	3	99
	4	0	3	3	0	0	3	85
	5	0	0	0	0	55	0	95
	6	0	0	0	0	0	3	90
Mean [%]		0.0	0.5	0.5	0.0	10.0	14.8	90.7
Standard Deviation		± 0.00	± 1.22	± 1.22	± 0.00	± 22.14	± 31.96	± 7.12

- could not be evaluated because germination did not occur up to this time

Table 40. Phytotoxicity [%], *Lolium perenne*

Phytotoxicity after 7 days

Species	Pot	Treatment Group [mL test item/ha]			
Lolium perenne		control	333	1000	3000
	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
	4	0	0	0	0
	5	0	0	0	0
	6	0	0	0	0
Mean [%]		0.0	0.0	0.0	0.0
Standard Deviation		± 0.00	± 0.00	± 0.00	± 0.00

Phytotoxicity after 14 days

Species	Pot	Treatment Group [mL test item/ha]			
Lolium perenne		control	333	1000	3000
	1	0	0	5	0
	2	0	0	0	5
	3	0	0	0	5
	4	0	0	0	5
	5	0	0	0	0
	6	0	3	0	5
Mean [%]		0.0	0.5	0.8	3.3
Standard Deviation		± 0.00	± 1.22	± 2.04	± 2.58

Phytotoxicity after 21 days

Species	Pot	Treatment Group [mL test item/ha]			
Lolium perenne		control	333	1000	3000
	1	0	0	3	0
	2	0	0	0	5
	3	0	0	0	15
	4	0	0	0	5
	5	0	0	0	0
	6	0	3	3	0
Mean [%]		0.0	0.5	1.0	4.2
Standard Deviation		± 0.00	± 1.22	± 1.55	± 5.85

Table 43. Phytotoxicity [%], *Avena sativa*

Phytotoxicity after 7 days

Species	Pot	Treatment Group [mL test item/ha]						
Avena sativa		control	12.3	37.0	111	333	1000	3000
	1	0	0	0	0	3	0	3
	2	0	0	0	0	0	0	5
	3	0	0	0	0	0	0	5
	4	0	0	0	0	5	5	5
	5	0	0	0	0	0	5	8
	6	0	0	10	0	2	0	3
Mean [%]		0.0	0.0	1.7	0.0	1.7	1.7	4.8
Standard Deviation		± 0.00	± 0.00	± 4.08	± 0.00	± 2.07	± 2.58	± 1.83

Phytotoxicity after 14 days

Species	Pot	Treatment Group [mL test item/ha]						
Avena sativa		control	12.3	37.0	111	333	1000	3000
	1	0	0	0	0	0	0	5
	2	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	10
	4	0	0	0	0	5	5	10
	5	0	0	0	0	0	5	10
	6	0	0	5	0	3	0	5
Mean [%]		0.0	0.0	0.8	0.0	1.3	1.7	6.7
Standard Deviation		± 0.00	± 0.00	± 2.04	± 0.00	± 2.16	± 2.58	± 4.08

Table 48. Phytotoxicity [%], *Allium cepa*

Phytotoxicity after 7 days

Species	Pot	Treatment Group [mL test item/ha]					
Allium cepa		control	12.3	37.0	111	333	1000
	1	0	0	5	30	90	90
	2	0	0	5	80	90	90
	3	0	0	5	20	90	95
	4	0	0	0	3	90	90
	5	0	0	5	20	85	90
	6	0	0	0	5	90	90
Mean [%]		0.0	0.0	3.3	26.3	89.2	90.8
Standard Deviation		± 0.00	± 0.00	± 2.58	± 28.19	± 2.04	± 2.04

Phytotoxicity after 14 days

Species	Pot	Treatment Group [mL test item/ha]					
Allium cepa		control	12.3	37.0	111	333	1000
	1	0	0	5	25	95	95
	2	0	0	20	90	95	95
	3	0	0	5	30	90	99
	4	0	0	0	0	90	95
	5	20	0	5	5	90	95
	6	0	0	0	5	90	95
Mean [%]		3.3	0.0	5.8	25.8	91.7	95.7
Standard Deviation		± 8.16	± 0.00	± 7.36	± 33.68	± 2.58	± 1.63

Phytotoxicity after 21 days

Species	Pot	Treatment Group [mL test item/ha]					
Allium cepa		control	12.3	37.0	111	333	1000
	1	0	0	5	30	99	98
	2	0	0	20	99	99	99
	3	0	5	0	10	95	99
	4	0	0	0	0	95	97
	5	20	0	20	10	96	99
	6	0	0	0	5	90	98
Mean [%]		3.3	0.8	7.5	25.7	95.7	98.3
Standard Deviation		± 8.16	± 2.04	± 9.87	± 37.35	± 3.33	± 0.82

A 2.6.3 KCP 10.6.3 Extended laboratory studies on non-target plants

A 2.7 KCP 10.7 Effects on other terrestrial organisms (flora and fauna)

A 2.8 KCP 10.8 Monitoring data

Appendix 3 Additional information provided by the applicant (e.g. detailed modelling data)

A 3.1 Sections 9.2.2.3 and 9.3.2.4 - FOCUS Step 2, surface water modelling output for winter cereals

See dRR B8 Section 8.9 for further details.

STEPS 1-2 in FOCUS

FOCUS Surface water Tool for Exposure Preditions Step 2

developed by Michael Klein

Program version:	Version 3.2
Date of this simulation:	25/09/2023, 15:12:35

OVERVIEW ON THE SUBSTANCE SPECIFIC INPUT DATA USED IN THE CALCULATION

Comments: METH_WC_3_S

Active substance:	METH_WC_3_S
Compound for PEC calculation:	METH_WC_3_S
Application rate (g/ha) of a.i.:	150.00
Crop Interception:	average crop cover (20 %)
Application/crop type:	cereals, winter
Number of applications per season:	1
Region and season of application:	South Europe, Mar. - May
Molecular mass of active ingredient (g/mole):	255.00
Molecular mass of calc. compound (g/mole):	211.00
Maximum observed in water/sediment studies (%)	1.00E-02
Maximum observed in soil studies (%)	38.25
DT50 soil (d) parent compound:	13.90
Water solubility (mg/L):	91.00
KOC assessed compound(L/kg):	321.00
KOC parent compound(L/kg):	67.00
DT50 water(d):	1000.00
DT50 sediment (d):	1000.00
DT50 soil (d):	111.11

SCENARIO DATA USED IN THE CALCULATION

Distance to the water body (m):	1.00
Spraydrift (% of application):	2.7590
Runoff + drainage(% of application):	4.00
Ratio of field to water body:	10.00
Water depth (cm):	30.00
Sediment depth (cm):	5.00
Effective sediment depth for sorption (cm):	1.00

Sediment OC (%):	5.00
Sed. bulk density (kg/L):	0.80

RESULTS OF THE CALCULATION

Number of application per season considered for this run:	1
Equilvalent application rate for drift (g/ha):	0.01
Equilvalent application rate for runoff/drainage(g/ha):	37.98
Equilvalent app. rate for runoff/drainage of parent compound(g/ha):	9.93E-03
Loading to water body per drift event(mg/m²):	0.0000
Loading to water body via runoff/drainage (mg/m²):	1.4818
fraction of substance entering water body in water phase:	0.7003
fraction of substance entering water body in sediment:	0.2997
Loading to water body via runoff/drainage of parent substance(mg/m²):	0.0003
fraction of parent substance entering water body in water phase:	0.9180
fraction of parent substance entering water body in sediment:	0.0820
Total Loading to water body via drift (mg/m²):	0.0000 (0.0023%)
Total Loading to water body via water phase(mg/m²):	1.0376 (70.0110%)
Total Loading to water body via sediment phase (mg/m²):	0.4441 (29.9647%)
Total Loading into water phase via Parent's runoff (mg/m²):	0.0003 (0.0202%)
Total Loading into sediment phase via Parent's runoff (mg/m²):	0.0000 (0.0018%)
Maximum PECSW (µg/L):	3.4597
Maximum PECSW occuring on day:	4
Maximum PECsed (µg/kg dry sediment):	11.1054
Maximum PECsed occuring on day:	4

Table: Calculated Concentrations in the water body

	PECsw (µg/L)		PECsed(µg/kg dry sediment)	
Time after max. peak(d)	Actual	TWA	Actual	TWA
0	3.4597	---	11.1054	---
1	3.4573	3.4585	11.0978	11.1016
2	3.4549	3.4573	11.0901	11.0978
4	3.4501	3.4549	11.0748	11.0901
7	3.4429	3.4513	11.0518	11.0786
14	3.4263	3.4429	10.9983	11.0518
21	3.4097	**3.4346**	10.9450	11.0251
28	3.3932	3.4263	10.8921	10.9984
42	3.3604	3.4098	10.7869	10.9454
50	3.3418	3.4004	10.7272	10.9153
100	3.2280	3.3425	10.3618	10.7294