REGISTRATION REPORT

Part B
Section 9
Ecotoxicology

Detailed summary of the risk assessment

Product code: ADM.4651.H.1.A (former A18032E)

Product name: NIKITA

Chemical active substances:

Dicamba, 312.5 g/kg

Mesotrione, 150 g/kg

Nicosulfuron, 100 g/kg

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(Authorisation)

Applicant: ADAMA Agan

Submission date: January 2021

MS Finalisation date: March 2022 (initial Core Assessment)

June 2022 (final Core Assessment)

Version history

When	What
22 January 2021	Summary of Alvarez, T. (2019) added as per RMS request
March 2022	Initial zRMS assessment The report in the dRR format has been prepared by the Applicant, therefore all comments, additional evaluations and conclusions of the zRMS are presented in grey commenting boxes. Minor changes are introduced directly in the text and highlighted in grey. Not agreed or not relevant information are ~~struck through and shaded for transparency~~.
June 2022	Final report (Core Assessment updated following the commenting period). Additional information/assessments included by the zRMS in the report in response to comments received from the cMS and the Applicant are highlighted in yellow. Information no longer relevant ~~is struck through and shaded~~.

ADAMA use the code ADM.4651.H.1.A for the formulation but for consistency the former Syngenta code A18032E is used throughout the dRR.

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zRMS comments:

1. In the below assessment for some of non-target species, results of studies belonging to other Applicants (Syngenta, Cheminova and FMC) were used. ADAMA has access to these data from LoA, which in case of studies owned by Cheminova and FMC are valid in all 27 EU Member States and United Kingdom. However, the LoA given by Syngenta is valid only for purposes of authorisation of A18032E in Poland and is not applicable in any other EU country. This is not a problem for the below evaluation, since Poland is indicated as the only cMS in the GAP table. However, in case data owned by Syngenta presented in this report are to be used by other Member States in the course of e.g. mutual recognition process, ADAMA will have to provide respective LoA valid in the country in which the authorisation is sought.

2. Since formulation A18032E is intended to be applied exclusively with the adjuvant, all formulation studies on effects on non-target species were performed with one of the recommended adjuvants Adigor (A12127R) at application rate corresponding to 1.0 L/ha. It is, however, noted that according to GAP, adjuvant Adigor is intended to be applied at 1.0-1.5 L/ha and that also other adjuvants are recommended (Styk or Insert and Olejan). Nevertheless, the efficacy data were analysed and demonstrated that when A18032E (0.4 kg/ha) is applied with Adigor at 1.5 L/ha, no notable increase of effects on target plants is observed comparing to application at 1.0 L/ha. Effects of A18032E with other adjuvants on target plants are lower when compared with application of A18032E with Adigor at 1.0 L/ha. Taking this into account, studies performed with Adigor at 1.0 L/ha are considered sufficient. For details of efficacy assessment, please refer to the Core Assessment, Part B, Section 3.

3. It is noted that A18032E is also recommended to be applied with formulation Efica 960 EC, containing S-metolachlor as the active substance. However, S-metolachlor belongs to different chemical group than dicamba, mesotrion and nicosulfuron and in light of current requirements, no specific risk assessment is required. However, the risk mitigation measures resulting from risk assessment performed in the course of authorisation of Efica 960 EC must be respected and combined with risk mitigation measures identified for A18032E identified in this report. Following risk mitigation measures are indicated on the label of Efica 960 EC and must be taken into account when A18032E is used together with Efica 960 EC:
    - 10 m buffer zone to surface water bodies in order to protect aquatic organisms,
    - 1 m buffer zone in order to protect non-target arthropods and non-target terrestrial plants (i.e. standard buffer).

9 Ecotoxicology (KCP 10)

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	11	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 Mesotrione ~~Dicamba~~ a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha Dicamba ~~Mesotrione~~ a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha Nicosulfuron 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	Poland	Maize (ZEAMX)	F	Annual/perennial grass and broadleaved weeds	Foliar, spraying, overall	BBCH 12-14 Spring	a) 1 b) 1	n.a.	a) 0.4 b) 0.4	a) 60 b) 60	a) 125 b) 125	a) 40 b) 40	80-400	n.a.	Tank-mixed adjuvant needed (e.g. Adigor with 1.0 - 1.5 L/ha, STYK (alternative and exclusive ADAMA name: INSERT) with 0.2 L/ha, Olejan with 1.5 L/ha)	A	A	R	A	A	A	R
2	Poland	Maize (ZEAMX)	F	Annual/perennial grass and broadleaved weeds	Foliar, spraying, overall	BBCH 12 – 14 Spring	a) 1 b) 1	n.a.	a) 0.4 b) 0.4	a) 60 b) 60	a) 125 b) 125	a) 40 b) 40	80-400	n.a.	Application in tank mix with 0.8 L/ha, Efica 960 EC	A	A	R	A	A	A	R
Interzonal uses (use as seed treatment, in greenhouses (or other closed places of plant production), as post-harvest treatment or for treatment of empty storage rooms)																						

1	2	3	4	5	6	7	8	9	10	11	11	11	12	13	14	15	16	17	18	19	20	21
None																						
Minor uses according to Article 51 (field uses)																						
None																						
Minor uses according to Article 51 (interzonal uses)																						
None																						

* 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 equipment (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 importance/restrictions

Several risk assessments of this dRR are based on the worst case GAP for C-EU with a higher application rate and are therefore more conservative compared to the applied GAP in Poland.

zRMS comments:

It is noted that in the GAP table above the application rate for mesotrione and dicamba were displaced. Taking into account the concentration of these compounds in the formulated product (312.5 and 150 g a.s./kg for dicamba and mesotrione, respectively, and application rate of the product at 0.4 kg/ha, the correct application rates of these compounds are:

- 125 g/ha for dicamba,
- 60 g/ha for mesotrione.

Table 9.1-1 was amended accordingly.

The application rate for nicosulfuron is correctly reported.

9.1.1 Overall conclusions

zRMS comments:

Conclusions presented in points 9.1.1.1 to 9.1.1.7 below were checked by the zRMS and amended where necessary.

9.1.1.1 Effects on birds (KCP 10.1.1), Effects on terrestrial vertebrates other than birds (KCP 10.1.2), Effects on other terrestrial vertebrate wildlife (reptiles and amphibians) (KCP 10.1.3)

Birds

The acute and long-term risks of A18032E to birds were assessed from toxicity exposure ratios between toxicity endpoints, estimated from studies with dicamba, mesotrione and nicosulfuron, and maximum residues occurring on food items following applications according to the proposed use pattern. The combined toxicity and risk assessment was also performed.

The risk to birds from exposure via drinking water has also been assessed. Risk of secondary poisoning has not been assessed, as dicamba, mesotrione, nicosulfuron and their relevant metabolites have log P_{OW} <3.0.

The TER values, calculated for recommended scenarios, all exceed the trigger values of 10 for acute risk and 5 for long-term risk, indicating that the risk to birds is acceptable following use of A18032E according to the proposed use pattern. Acceptable combined acute and long-term risk assessment could be concluded. The risk assessment for exposure via drinking water from puddles also showed acceptable risk.

~~The TER values, calculated for recommended scenarios, all exceed the trigger values of 10 for acute risk and 5 for long-term risk (including drinking water), indicating that the risk to birds is acceptable following use of A128032E according to the proposed use pattern.~~

Mammals

The acute and long-term risks of A18032E to mammals were assessed from toxicity exposure ratios between toxicity endpoints, estimated from studies with A18032E, dicamba, mesotrione and nicosulfuron, and maximum residues occurring on food items following applications according to the proposed use pattern. The combined toxicity and risk assessment was also performed.

The risk to mammals from exposure via drinking water has also been assessed. Risk of secondary poisoning has not been assessed, as dicamba, mesotrione, nicosulfuron and their relevant metabolites have log P_{OW} <3.0.

The TER values, calculated for recommended scenarios, all exceed the trigger values of 10 for acute risk, indicating that the acute risk to mammals is acceptable following use of A18032E according to the proposed use pattern. Acceptable combined acute risk could be concluded.

The long-term TER values for dicamba and nicosulfuron, calculated for recommended scenarios, exceed the trigger value of 5, indicating acceptable risk. However, the long-term TER values for mesotrione fall below the trigger of 5 and the combined long-term risk was also unacceptable at Tier 1.

Acceptable long-term risk to mammals from mesotrione could be demonstrated in a refined risk assessment by identifying the brown hare and wood mouse as relevant focal species for the intended use pattern, refining the residue decline of mesotrione in potential food items, and considering the realistic

amount of time spent foraging in early maize fields (PT). Considered refinement options were also sufficient to resolve the combined long-term risk to the relevant focal species.

~~The TER values, calculated for recommended scenarios, all exceed the trigger values of 10 for acute risk, indicating that the acute risk to mammals is acceptable following use of A18032E according to the proposed use pattern. The risk to mammals from exposure via drinking water was also acceptable.~~
~~The long-term TER values for dicamba and nicosulfuron, calculated for recommended scenarios, exceed the trigger value of 5, indicating acceptable risk. However, the long-term TER values for mesotrione fall below the trigger of 5 indicating a potential risk. Acceptable long-term risk to small omnivorous and small herbivorous mammals was demonstrated by identifying the hare and wood mouse as relevant focal species, considering the realistic amount of time spent foraging in early maize fields (PT), and by using experimentally derived foliar dissipation data. A more realistic NOEAEL has been used in the refined assessments, and justification for use of this has been provided.~~

9.1.1.2 Effects on aquatic organisms (KCP 10.2)

The PEC/RAC ratios for all aquatic organisms other than macrophytes using worst-case PEC_{SW} values for A18032E, dicamba, mesotrione, nicosulfuron and their metabolites are below the trigger of 1, indicating acceptable risk for these organisms following use of A18032E according to the proposed use pattern when considering the following mitigation measures as presented in the tables below. Since ADAMA ~~Syngenta~~ proposes that the E_rC_{50} values should be used for macrophyte risk assessment in accordance with the Aquatic Guidance Document, these endpoints have been used to summarise the mitigation below. ~~Mitigations addressing the use of the $E_{b\text{ or }y}C_{50}$ are available in the main text.~~

Table 9.1-2: Aquatic organisms: Overall proposed mitigation measures for A18032E applied at 1 x 0.4 kg/ha in maize (125 g dicamba/ha, 60 g mesotrione/ha and 40 g nicosulfuron/ha)

Test substance	Appl. rate (g/ha)	Organism	A or C	Scenario									
				D1	D2	D3	D4	D5	D6	R1	R2	R3	R4
A18032E	400	Fish	A			- [a]							
Dicamba	132	Fish	A			-	-	-	-	-	-	-	-
Mesotrione	75	Fish	A			-	-	-	-	-	-	-	-
Nicosulfuron	40	Fish	A			-	-	-	-	-	-	-	-
Dicamba	132	Fish	C			-	-	-	-	-	-	-	-
Mesotrione	75	Fish	C			-	-	-	-	-	-	-	-
Nicosulfuron	40	Fish	C			-	-	-	-	-	-	-	-
A18032E	400	Aq inverts	A			- [a]							
Dicamba	132	Aq inverts	A			-	-	-	-	-	-	-	-
Mesotrione	75	Aq inverts	A			-	-	-	-	-	-	-	-
Nicosulfuron	40	Aq inverts	A			-	-	-	-	-	-	-	-
Dicamba	132	Aq inverts	C			-	-	-	-	-	-	-	-
Mesotrione	75	Aq inverts	C			-	-	-	-	-	-	-	-
Nicosulfuron	40	Aq inverts	C			-	-	-	-	-	-	-	-
A18032E	400	Algae	C			- [a]							
Dicamba	132	Algae	C			-	-	-	-	-	-	-	-
Mesotrione	75	Algae	C			-	-	-	-	-	-	-	-
Nicosulfuron	40	Algae	C			-	-	-	-	-	-	-	-
A18032E	400	Macrophytes	C			75% DR; or 5 m SD [a]							
Dicamba	132	Macrophytes	C			-	-	-	-	-	-	-	-
Mesotrione	75	Macrophytes	C			-	-	-	-	-	-	E_rC_{50}: 10 m VFS (L&M) or 5 m VFS (VFSmod)	E_rC_{50}: 10 m VFS (L&M) or 5 m VFS (VFSmod) ~~EAC_{pulse}: none~~
Nicosulfuron	40	Macrophytes	C			-	-	-	-	E_rC_{50}: 10 m VFS (L&M) or 5 m VFS (VFSmod) ~~EAC_{pulse}: none~~ [b]	E_rC_{50}: 20 m VFS (L&M) or 5 m VFS (VFSmod) ~~EAC_{pulse}: none~~ [b]	E_rC_{50}: 5 m VFS (VFSmod) ~~EAC_{pulse}: 10 m VFS (L&M)~~ [b]	E_rC_{50}: 5 m VFS (VFSmod) ~~EAC_{pulse}: none~~ [b]

A = acute, C = chronic
An empty/grey field means that the scenario is not relevant to the crop group

"-"mitigation measures are not required for this scenario
SD = spray drift buffer
VFS (L&M) = vegetative filter strip according with FOCUS Landscape and Mitigation V1 (2007)
DR = drift reducing nozzles
EAC_{pulse} = Environmentally Acceptable Concentration derived from pulsed exposure study
[a] spray drift entry; drift value according to Rautmann at al. (2001)
[b] Considering refined RAC of 2.7 µg a.s./L (based on pulsed exposure studies and potential of recovery)

9.1.1.3 Effects on bees (KCP 10.3.1)

The acute risk of A18032E posed to honeybees following the intended uses in maize was re-assessed by the zRMS in line with indications of SANCO/10329/2002 rev 2 final. Respective hazard quotients were calculated with consideration of acute oral and contact studies with A18032E, dicamba, mesotrione and nicosulfuron and the maximum single application rate of the product (0.4 kg/ha) and corresponding rates of active compounds.

All the calculated hazard quotients were less than the relevant trigger of 50, indicating that the acute oral and contact risk to bees is acceptable following use of A18032E according to the proposed use pattern.

~~The acute risk of A18032E to honeybees was assessed from hazard quotients and Exposure Toxicity Ratios (ETRs) following EFSA (2014), estimated from acute oral and contact studies with A18032E, dicamba, mesotrione and nicosulfuron, and exposure rates following application at the maximum single application rate of 0.6 kg A18032E/ha, equivalent to 187.5 dicamba/ha, 90 g mesotrione/ha and 60 g nicosulfuron/ha. All the hazard quotients and Exposure Toxicity Ratios (ETRs) for A18032E are less than the relevant triggers, indicating that the acute oral and contact risk to bees is acceptable following use of A18032E according to the proposed use pattern.~~

~~The chronic adult and larval risk of A18032E to honeybees was assessed from ETRs and toxicity exposure ratios (TERs) following the principles of EFSA (2014), estimated from chronic adult and larval studies with mesotrione, Dicamba and nicosulfuron. All the ETR and TER values are less/greater than respectively the relevant trigger values, indicating that the chronic risk to adult and larval honeybees is acceptable following use of A18032E according to the proposed use pattern.~~

9.1.1.4 Effects on arthropods other than bees (KCP 10.3.2)

At Tier I, the in-field and off-field HQ values for *Typhlodromus pyri* were below the trigger value for the worst-case use scenario (1 x 600 g A18032E/ha in maize) indicating that the risk to non-target arthropods is acceptable following the use of A18032E according to the proposed use pattern.

At Tier I the off-field HQ value for *Aphidius rhopalosiphi* was below the trigger value for the worst-case use scenario (1 x 600 g A18032E/ha in maize) indicating acceptable off-field risk to this species following the use of A18032E according to the proposed use pattern. However, the in-field HQ values for *Aphidius rhopalosiphi* were above the trigger value and required further refinement. The Tier II, extended laboratory studies showed acceptable foliar in-field and off-field effects from foliar applications of A18032E for *Aphidius rhopalosiphi* and *Aloechara bilineata* for the worst-case use scenario (1 x 600 g A18032E/ha in maize).

Overall, the risk to non-target arthropods is therefore acceptable following use of A18032E according to the proposed use pattern with no need for risk mitigation measures.

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

Soil meso- and macrofauna

The ~~acute and~~ long-term risk of A18032E, dicamba, mesotrione and nicosulfuron, and relevant metabolites was evaluated where relevant for earthworms, Collembola and *Hypoaspis*. The risk assessment demonstrated that the risk to non-target soil meso- and macrofauna is acceptable following use of A18032E according to the proposed use pattern.

Soil micro-organisms

All no-effect levels of A18032E, dicamba, mesotrione and nicosulfuron, and relevant metabolites exceeded the relevant PEC_{soil} values, indicating that the risk to soil micro-organisms is acceptable following use of A18032E according to the proposed use pattern.

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

The risk of A18032E to non-target terrestrial plants was assessed from toxicity exposure ratios (TERs) using the A18032E toxicity data from Tier II studies (performed with addition of adjuvant Adigor), and the maximum off-field predicted environmental residues (PERs). TER values, calculated from worst-case endpoints from seedling emergence and vegetative vigour studies with 10 species and a $PER_{off\text{-}field}$ value at 1 m from the treated crop, indicated a potential risk to off-field non-target plants. The risk was refined using a probabilistic risk assessment and considering mitigation with buffers and spray drift reduction technology.

For 1 x 400 g A18032E/ha it was concluded that the risk to non-target plants off-field was acceptable when a non-spray buffer strip of 5 m is considered, or a 1 m buffer with 90% drift reducing nozzles is used.

The risk to terrestrial non-target plants in off-crop areas is therefore acceptable following use of A18032E according to the proposed use pattern when the appropriate mitigation measures are used.

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

Not relevant.

9.1.2 Grouping of intended uses for risk assessment

The following table documents the grouping of the intended uses to support application of the risk envelope approach (according to SANCO/11244/2011).

In the current submission only one single use rate is proposed for Poland, which is a single application of 0.4 kg A18032E/ha in maize (BBCH 12-14).
Also higher application rates are covered by the risk assessments shown within this Dossier, to be compliant with other countries application rates of the Central Zone. The highest use rate will be assessed in all sections except in the aquatic and terrestrial plant risk assessments, where different rates will be assessed in order to identify suitable mitigation specific to the application rate. The different rates will also be used in the mammalian risk assessment in order to be able to differentiate the different levels of higher tier refinement which are required to demonstrate acceptable risk.

Table 9.1-3: Critical use pattern of A18032E grouped according to application rate

Grouping according to application rate			
Group	**Intended uses**	**Relevant use parameters for grouping**	**Relevant parameter or value for sorting**
Exaggerated ~~Maximum~~ rate (to assess all organisms)	Maize	Crop: maize Growth stage: BBCH 12–19 Application rate: 1 x 0.6 kg A18032E/ha: - 187.5 g dicamba/ha (covered by FOCUS modelling at 264 g/ha) - 90 g mesotrione/ha (covered by FOCUS modelling at 100 g/ha) - 60 g nicosulfuron/ha ~~This also covers the proposed split rate application of 0.4 + 0.2 between BBCH 13-17~~[a]	Application rate

Grouping according to application rate			
Group	**Intended uses**	**Relevant use parameters for grouping**	**Relevant parameter or value for sorting**
GAP ~~Lower~~ rate (to assess non-target aquatic and terrestrial plants only in order to define appropriate mitigation requirements)	Maize	Crop: maize Growth stage: BBCH 12–19 Application rate: 1 x 0.45 kg A18032E/ha: - 140.6 g dicamba/ha (covered by FOCUS modelling at 264 g/ha) - 67.5 g mesotrione/ha (covered by FOCUS modelling at 75 g/ha) - 45 g nicosulfuron/ha	Application rate
GAP ~~Lower~~ rate (to assess aquatic organisms and non-target terrestrial plants only in order to define appropriate mitigation requirements)	Maize	Crop: maize Growth stage: BBCH 12–19 Application rate: 1 x 0.4 kg A18032E/ha: - 125 g dicamba/ha (covered by FOCUS modelling at 132 g/ha) - 60 g mesotrione/ha (covered by FOCUS modelling at 75 g/ha) - 40 g nicosulfuron/ha	Application rate

[a] It is worst-case to assume a single application of 0.6 kg/ha as dissipation will occur between the applications when considering a split use

zRMS comments:

The grouping of the intended uses of A18032E in Table 9.1-5 above is agreed by the zRMS.

For groups of organisms for which no acceptable risk could be concluded for the exaggerated application rate of 0.60 kg product/ha, evaluation was based on the application rate of the product intended in Poland (0.40 kg product/ha).

Since the split application is not indicated in the GAP table, it was struck through in Table 9.1-5 above. Furthermore the information regarding the application rates considered in the evaluation has been corrected for clarity.

9.1.3 Consideration of metabolites

A list of metabolites found in environmental compartments is provided below. The need for conducting a metabolite-specific risk assessment in the context of the evaluation of A18032E is indicated in the tables.

Table 9.1-4 Metabolites of dicamba

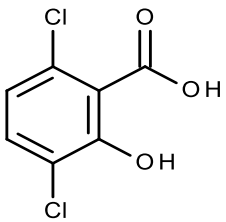
Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?
NOA414746 (DCSA) 3,6-dichloro-2-hydroxybenzoic acid		207	Soil: >10% of a.s. Water: >10% of a.s. Sediment: <5% of a.s	Soil: Yes Water: Yes Sediment: Yes

Table 9.1-5 Metabolites of mesotrione

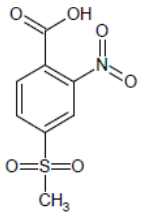
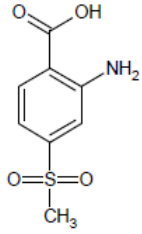
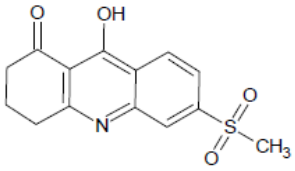
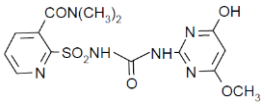
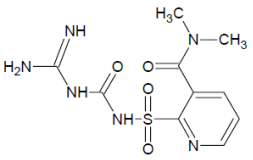
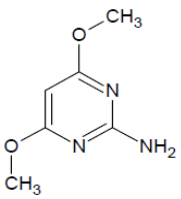
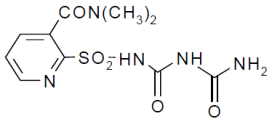
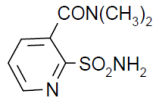
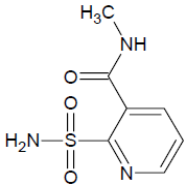
Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?
NOA437130 (MNBA) 4-(methylsulfonyl)-2-nitrobenzoic acid		245	Soil: >10% of a.s (aerobic laboratory degradation and soil photolysis studies) Water: >5% of a.s. in 1 measurement Sediment: <5% of a.s.	Soil: Yes Water: Yes Sediment: Yes
NOA422848 (AMBA) 2-amino-4-(methyl-sulfonyl) benzoic acid		215	Soil: >5% of a.s. in 2 sequential measurements (aerobic laboratory degradation studies and soil photolysis studies) Water: >10% of a.s. Sediment: >5% of a.s. in 2 sequential measurements	Soil: Yes Water: Yes Sediment: Yes
SYN546974 9-hydroxy-6-(methyl-sulfonyl)-3,4-dihydro-acridin-1(2H)-one		291	Soil: - Water: >5% of a.s. in 2 sequential measurements Sediment: >10% of a.s.	Soil: No Water: Yes Sediment: Yes

Table 9.1-6 Metabolites of nicosulfuron

Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?
HMUD 2-{[(4-hydroxy-6-methoxypyrimidin-2-yl)carbamoyl]sulfamoyl}-N,N-dimethylpyridine-3-carboxamide		396.4	Soil: >10% of a.s (aerobic laboratory degradation studies) Water: >10% of a.s. Sediment:>5% of as in 2 sequential measurements	Soil: Yes Water: Yes Sediment: Yes
AUSN 2-[carbaminidoyl-carbamoyl)sulfamoyl]-N,N-dimethylpyridine-3-carboxamide		314.3	Soil: >10% of a.s Water: >5% of a.s. and maximum of formation not yet reached at the end of the study Sediment:<5% of as but maximum of formation not yet reached at the end of the study	Soil: Yes Water: Yes Sediment: Yes
ADMP 4,6-dimethoxypyrimidin-2-amine		155.2	Soil: >5% of as in 2 sequential measurements (field dissipation trial) Water: - Sediment: -	Soil: Yes Water: Yes Sediment: Yes
UCSN 2-[(carbamoyl-carbamoyl)sulfamoyl]-N,N-dimethylpyridine-3-carboxamide		315.3	Soil: >10% of a.s. (aerobic laboratory degradation studies) Water: >5% of a.s. and maximum of formation not yet reached at the end of the study	Soil: Yes Water: Yes Sediment: Yes

Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?
			Sediment:<5% of as	
ASDM N,N-dimethyl-2-sulfamoylpyridine-3-carboxamide		229.2	Soil: >10% of a.s Water: >5% of a.s. and maximum of formation not yet reached at the end of the study Sediment:<5% of as	Soil: Yes Water: Yes Sediment: Yes
MU-466		215.1	Soil: - Water: >0.1 µg/L in the leachate of lysimeter studies Sediment: -	Soil: No Water: No Sediment: No

zRMS comments:

Information regarding metabolites of particular active compounds provided in Tables 9.1-6 to 9.1-8 above is in line with data reported in:

- EFSA Journal 2011;9(1):1965 for dicamba,
- EFSA Journal 2016;14(3):4419 for mesotrione,
- EFSA Scientific Report (2007) 120 for nicosulfuron.

Specific formation fractions and/or maximum occurrence of particular metabolites has been considered in the exposure and risk assessment presented in this report.

9.2 Effects on birds (KCP 10.1.1)

9.2.1 Toxicity data

Avian toxicity studies have been carried out with dicamba, mesotrione and nicosulfuron. There are no potentially relevant metabolites for avian exposure. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on birds of A18032E were not evaluated as part of the EU assessment of dicamba, mesotrione and nicosulfuron. However, the provision of further data on the formulation A18032E is not considered essential, because mammal studies give no indication of higher toxicity from the formulation and the risk to birds from A18032E can be adequately assessed from risk assessment for the individual active substances. The risk to birds from the proposed uses of A18032E will therefore be assessed using the endpoints for dicamba, mesotrione and nicosulfuron.

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process. Justifications are provided below with respect to the conversion of ppm endpoints to mg/kg bw/day endpoints, extrapolation of acute endpoints, calculation of geometric mean endpoints and the evaluation of mixture toxicity.

New data submitted with this application for higher tier risk assessment are listed in Appendix 1 and summarised in Appendix 2.

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

Species	Substance	Exposure system	Results	Reference
Mallard duck (*Anas platyrhynchos*)	Dicamba	Oral 1 d Acute	LD_{50} = 1373 mg a.s./kg bw	EFSA Conclusion 2011 Campbell and Beavers, 1993 SAN837/5221
Bobwhite quail (*Colinus virginianus*)	Dicamba	Oral 1 d Acute	LD_{50} = 216 mg a.s./kg bw	EFSA Conclusion 2011 Campbell et al., 1993 SAN837/5220
~~Proposed refinement of the acute endpoint:~~ Acute geomentric mean of *Anas platyrhynchos* (Mallard duck) and *Colinus virginianus* (Bobwhite quail)			Geomean: 545 mg a.s./kg bw	See section 9.2.1.1
Mallard duck (*Anas platyrhynchos*)	Dicamba	Dietary 8 d Short-term	LD_{50} > 1567 mg a.s./kg bw/d	EFSA Conclusion 2011 Fink, 1977b SAN837/5022
Bobwhite quail (*Colinus virginianus*)	Dicamba	Dietary 8 d Short-term	LD_{50} > 995 mg a.s./kg bw/d	EFSA Conclusion 2011 Fink, 1977a SAN837/5023
Mallard duck (*Anas platyrhynchos*)	Dicamba	Dietary Reproductive toxicity	NOEL = 89 mg a.s./kg bw/d	EFSA Conclusion 2011 Beavers, et al., 1994b SAN837/5205
Bobwhite quail (*Colinus virginianus*)	Dicamba	Dietary Reproductive toxicity	NOEL = 170 mg a.s./kg bw/d	EFSA Conclusion 2011 Beavers et al., 1994a SAN837/5206
Proposed new chronic endpoint: The endpoint used in the long-term risk assessment is LD/10 = 54.5 mg as/kg bw, as this is lower than the NOEC according to EFSA Guidance Document (2009)			54.5 mg a.s./kg bw	See section 9.2.1.1

zRMS comments:

Avian toxicity data for dicamba are in line with the EU agreed endpoints reported in EFSA Journal 2011;9(1):1965.

The geometric mean LD_{50} calculated from the two acute endpoints available for two species is agreed by the zRMS. For details, please refer to point 9.2.1.1 below. However, since procedure of calculation of the geometric mean from the two equivalent studies performed with different species is in line with recommendation of EFSA (2009) for the screening step and Tier 1 risk assessment, it should not be referred to as "endpoint refinement". Such reference has been struck through in Table 9.2-1 above.

Since $LD_{50}/10$ is lower than the lowest NOEL, in line with EFSA (2009), it should be used in the long-term risk assessment.

Table 9.2-2: Endpoints and effect values relevant for the risk assessment for birds - mesotrione

Species	Substance	Exposure system	Results	Reference
Bobwhite quail (*Colinus virginianus*)	Mesotrione	Oral 1 d Acute	LD_{50} >2000 mg a.s./kg bw	EFSA Conclusion 2016 Rodgers, 1995a ZA1296/0535
			Extrapolated: LD_{50} = 3776 mg a.s./kg bw	See section 9.2.1.1
~~Mallard duck~~	~~Mesotrione~~	~~Oral~~ ~~1 d~~ ~~Acute~~	~~LD_{50} >2000 mg a.s./kg bw~~	~~Hubbard et al. 2018~~ ~~ZA1296_10605~~
			~~Extrapolated:~~ ~~LD_{50} = 3228 mg a.s./kg bw~~	~~See section 9.2.1.1~~
Bobwhite quail (*Colinus virginianus*)	Mesotrione	Dietary 8 d Short-term	LC_{50} >5200 mg a.s./kg diet	EFSA Conclusion 2016 Rodgers, 1995b ZA1296/0537
			LDD_{50} >1654 mg a.s./kg bw/d	See section 9.2.1.1
Mallard duck (*Anas platyrhynchos*)	Mesotrione	Dietary Reproductive toxicity	NOEL = 20.6 mg a.s./ kg bw/d (offspring effects on hatching and chick development)	EFSA Conclusion 2016 Johnson, 1997b ZA1296/0538

zRMS comments:

Avian toxicity data presented in Table 9.2-1 are in general in line with EU agreed endpoints reported in EFSA Journal 2016;14(3):4419 for mesotrione.

It is noted that in support of this evaluation the Applicant provided new acute toxicity study with the mallard duck. However, vertebrate toxicity testing must be performed only when crucial for the evaluation. No data gap has been identified in EFSA Journal 2016;14(3):4419 in area of acute toxicity to birds and the study was not required to finalise the risk assessment. In consequence it has not been evaluated as its submission was not justified.

Table 9.2-3: Endpoints and effect values relevant for the risk assessment for birds - nicosulfuron

Species	Substance	Exposure system	Results	Reference
Bobwhite quail (*Colinus virginianus*)	Nicosulfuron	Oral 1 d Acute	LD_{50} >2000 mg a.s./kg bw	EFSA Conclusion 2007 Cummins, 1991b 90/ISK147/1196
			Extrapolated: LD_{50} = 3776 mg a.s./kg bw	See section 9.2.1.1
Mallard duck (*Anas platyrhynchos*)	Nicosulfuron	Oral 1 d Acute	LD_{50} >2000 mg a.s./kg bw	EFSA Conclusion 2007 Cummins, 1991a 90/ISK146/1227
			Extrapolated: LD_{50} = 3776 mg a.s./kg bw	See section 9.2.1.1
Bobwhite quail (*Colinus virginianus*)	Nicosulfuron	Dietary 5 d Short-term	LC_{50} >1603 mg a.s./kg bw/day	EFSA Conclusion 2007 Cummins, 1991d 90/ISK149/1228
Mallard duck (*Anas platyrhynchos*)	Nicosulfuron	Dietary 5 d Short-term	LC_{50} >911 mg a.s./kg bw/d	EFSA Conclusion 2007 Cummins, 1991c 90/ISK148/1229
Japanese quail (*Coturnix japonica*)	Nicosulfuron	Dietary Reproductive toxicity	NOEC = 171 mg a.s./kg bw/d	EFSA Conclusion 2007 Burri, 1999 696060

zRMS comments:

Avian toxicity data for nicosulfuron are in line with the EU agreed endpoints reported in EFSA Scientific Report (2007) 120, 1-91.

In line with indications of EFSA (2009), extrapolation of the acute endpoints was possible since no mortality was observed in the study with bobwhite quail. Extrapolation factor of 1.888 is confirmed to be correct since 10 birds were used in the study.

It is noted that no mortality was observed also in the study with the mallard duck and for this reason extrapolation of an endpoint using factor of 1.888 (10 individuals tested) is relevant also for this species. Respective information has been inserted by the zRMS in Table 9.2-3.

9.2.1.1 Justification for new endpoints

Consideration of acute endpoint for dicamba used in the risk assessment

As two acute oral toxicity studies are available for dicamba, a geometric mean can be calculated following the approach outlined under Point 2.4.1 of the EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009). Before a geometric mean can be calculated we need to ensure that the studies are equivalent in terms of endpoint and in particular the vehicle/solvent used in dosing. Both the studies conducted with the mallard duck by *Campbell & Beavers, 1993* and the bobwhite quail by *Campbell et al., 1993,* were conducted in accordance with Fifra Subdivision E, Section 71-1; dicamba was dosed in a corn oil solvent by oral gavage in both studies. The studies were conducted in accordance with the same guidance documents by the same laboratory therefore the studies are equivalent and it is appropriate to calculate a geometric mean. The geometric mean out of 1373 mg a.s./kg bw and 216 mg a.s./kg bw is 545 mg/kg bw. The geometric mean LD_{50} value of 545 mg/kg bw will be used in the following risk assessment.

zRMS comments:

Calculation of the geometric mean LD_{50} from the two acute endpoints available for two species is agreed by the zRMS since all conditions described ted in EFSA (2009) are met.

Since the geometric mean LD50 of 545 mg a.s./kg bw is higher than the endpoint for most sensitive species (216 mg a.s./kg bw) by less than factor of 10, the geometric mean endpoint may be used in the risk assessment.

New acute endpoint for mesotrione for the Mallard Duck

A new acute oral toxicity study with mesotrione has been conducted with the mallard duck (Hubbard et al. 2018) as it was requested as a base data set requirement for India. A full study summary is presented in the appendix. No mortalities or treatment-related effects were seen in the 2000 mg a.s./kg level tested as a limit test.

zRMS comments:

As already mentioned in point 9.2.1 above, the new acute toxicity study with the mallard duck was not evaluated as no data gap in area of avian toxicity testing was identified in the course of the EU evaluation and EU agreed toxicity data were sufficient to finalise the evaluation. The acute risk assessment for birds is based on extrapolated LD_{50} of 3776 mg a.s./kg bw, in line with endpoints reported in EFSA Journal 2016;14(3):4419.

Consideration of acute endpoints for mesotrione and nicosulfuron used in the risk assessment

In the acute oral mesotrione toxicity study conducted with the bobwhite quail (*Rodgers et al., 1995*) no mortalities were observed and therefore the LD_{50} was reported as >2000 mg/kg bw. Also, in the acute oral nicosulfuron toxicity studies conducted with the bobwhite quail (*Cummins, 1991b*) no mortalities were observed and therefore the LD_{50} was reported as > 2000 mg/kg bw. Under Point 2.1.2 of the EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009) a method has been proposed to extrapolate upwards the LD_{50} value. The extrapolation is carried out assuming a 50% binomial probability bound that mortality could have occurred but had simply been missed by chance in the test. The extrapolation factors are presented in Table 1 of the guidance document and are dependent upon the number of animals tested and whether no, or a single mortality, was observed in the study. The acute toxicity values have been extrapolated and are presented in the table below.

Table 9.2-4: Extrapolation of the acute oral toxicity values for mesotrione and nicosulfuron

Test substance	Study	Test species	Experimental LD_{50} (mg/kg bw)	Number of animals tested	Number of mortalities	Extrapolation factor [a]	Corrected LD_{50} (mg/kg bw)
Mesotrione	Rogers et al., (1995a)	Bobwhite quail	>2000	10	0	1.888	3776
~~Mesotrione~~	~~Hubbard et al. 2018~~	~~Mallard duck~~	~~>2000~~	~~5~~	~~0~~	~~1.614~~	~~3228~~
Nicosulfuron	Cummins, (1991b)	Bobwhite quail	>2000	10	0	1.888	3776
Nicosulfuron	Cummins, (1991a)	Mallard duck	>2000	10	0	1.888	3776

[a] The extrapolation factor is presented in Table 1 of the guidance document (Point 2.1.2)

The extrapolated LD_{50} values of 3776 ~~3228~~ mg/kg bw for mesotrione and 3776 mg/kg bw for nicosulfuron will be used in the subsequent risk assessment ~~as refined endpoints~~.

zRMS comments:

As already mentioned above, the new study on acute toxicity of mesotrione to mallard duck was not evaluated as being not necessary for the risk assessment. All information related to the this new vertebrate study was thus struck through in the text above.

Extrapolation of mesotrione endpoint from the study performed with bobwhite quail does not need to be justified, since the extrapolated endpoint is already reported in EFSA Journal 2016;14(3):4419.

In avian acute studies performed with nicosulfuron 10 birds were used and no mortality was observed. For this reason it is justified to use an extrapolation factor of 1.888, in line with EFSA (2009). This is applicable for both studies, so information on study with mallard duck has been inserted by the zRMS in Table 9.2-4 above for completeness.

Consideration of reproductive endpoint for dicamba used in the risk assessment

According to the EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009), an estimated reproductive endpoint should be obtained by using the acute oral LD_{50} (from a single species or geometric mean) and divided by 10 to obtain an $LD_{50}/10$. This $LD_{50}/10$ is used as an endpoint in the reproductive assessment to take account of the possibility of reproductive impairment due to sub-lethal effects on pair formation and breeding site selection, incubation, parental care of nestlings, and survival of fledgling birds (in accordance with Appendix J of the EFSA Guidance). If the $LD_{50}/10$ is lower than the lowest reproductive endpoint, then this endpoint should be used for the long-term assessment. For dicamba, the $LD_{50}/10$ of 54.5 mg a.s./kg bw is used as an endpoint in the reproductive assessment, since this endpoint is lower than the lowest NOEL from the avian reproduction studies (89 mg a.s./kg bw/d).

zRMS comments:

Since $LD_{50}/10$ (54.5 mg a.s./kg bw/d) is lower than the lowest agreed NOEL (89 mg a.s./kg bw/d) it is justified to use $LD_{50}/10$ in the long-term risk assessment, in line with EFSA (2009).

Consideration of acute mixture toxicity

According to EFSA/2009/1438[1] combined action of several toxicants must be specifically considered in the risk assessment when it is obvious that such exposure situations will occur for animals.
For the assessment of acute effects (mortality), a surrogate LD_{50} can be calculated. The EFSA Guidance Document indicates that the following equation should be used for deriving a surrogate LD_{50} for a mixture of active substances with known toxicity assuming dose additivity:

$$LD_{50}\,(\text{mix}) = \left(\sum_i \frac{X(a.s._i)}{LD_{50}(a.s._i)} \right)^{-1}$$

where:
$X\,(a.s._i)$ = fraction of active substance (i) in the formulation mixture
$LD_{50}\,(a.s._i)$ = acute toxicity for the active substance (i)

The LD_{50} of the mix is summarised in the table below.

[1] European Food Safety Authority; Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA. EFSA journal 2009; 7(12):1438. [139 pp.]

Table 9.2-5: Acute LD_{50} for the mixture of active substances

Test substance	Concentration of active substance in formulation A18032E (g/kg)	Fraction of active substance in the formulation mixture [A]	Acute toxicity endpoint (mg/kg bw)	Fraction of active substance/LD_{50} for the active substance	LD_{50} mix (mg/kg bw)
Dicamba	312.5	0.56	545	0.00103 ~~0.001019~~	872 ~~870~~
Mesotrione	150	0.27	3776 ~~3228~~	0.0000715 ~~0.000083~~	
Nicosulfuron	100	0.18	3776	0.0000477 ~~0.000047~~	
Total	562.5	1	-	0.001147 ~~0.001149~~	

[A] Concentration of an active substance in the formulation, divided by, the total concentration of all active substances in the formulation.

The EFSA Guidance Document (2009) states that if one active substance can be identified where the two quotients "tox per fraction (a.s.)" and "tox per fraction (mix)" deviate by ≤10%, this indicates that this active substance contributes to more than 90% to mixture toxicity. The other component(s) of the mixture then only have a marginal impact on the predicted risk.
The tox per fractions were calculated as given in Appendix B of the EFSA Guidance Document and the results are given in the table below.

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

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

Table 9.2-6: Calculation of tox per fraction quotients

Active substance	Acute LD_{50} (mg a.s./kg bw)	X (a.s.)	LD_{50}/ X (a.s.)	LD_{50} (mix)	$LD_{50\ mix}$/ ∑X (a.s.) (mg a.s./kg bw)	%-Deviation tox per fraction (a.s.) to $LD_{50\ (mix)}$ [a]
Dicamba	545	0.56	981	872 ~~870~~	872 ~~870~~	The deviation is = 981-872 = 109 Then % deviation = (109/872) *100= 12.5% ~~The deviation is = 981-870 = 111~~ ~~Then % deviation = (111/870) *100= 12.7%~~
Mesotrione	3776 ~~3228~~	0.27	14160 ~~12105~~		872 ~~870~~	The deviation is = 14160-872 = 13288 Then % deviation = (13288/872) *100= 1524% ~~The deviation is = 12105-870 = 11235~~ ~~Then % deviation = (11235/870) *100= 1291%~~
Nicosulfuron	3776	0.18	21240		872 ~~870~~	The deviation is = 21240-872 = 20368 Then % deviation = (20368/872) *100= 2334% ~~The deviation is = 21240-870 = 20370~~ ~~Then % deviation = (20370/870) *100= 2341%~~
mix	872 ~~879~~	1	Σ 36381		-	

[a] Please note that these "tox per fraction" quotients themselves have no biological meaning; they are only to be used for comparison

As can be seen from Table 9.2-6, the tox per fractions for dicamba, mesotrione and nicosulfuron deviates from the tox per fraction for the mix by more than 10%, and it cannot therefore be assumed that one of the active substances will drive the short term risk to birds. The acute risk assessment for birds is therefore conducted for dicamba, mesotrione and nicosulfuron separately, and then a mixture risk assessment is performed.

zRMS comments:

The combined acute toxicity assessment above was amended by the zRMS with consideration of the relevant toxicity data. The estimated LD_{50}mix of 872 mg/kg bw was calculated. None of the substances contributes to >90% of the toxicity of the mixture and for this reason respective mixture risk assessment will be performed with consideration of the estimated endpoint.

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).

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the worst-case application rates (187.5 g dicamba/ha, 90 g mesotrione/ha and 60 g nicosulfuron/ha) are used in the risk assessment.

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

The results of the acute and reproductive first-tier risk assessments are summarised in the following tables.

Dicamba

Table 9.2-7: Screening step assessment of the acute and long-term/reproductive risk for birds due to the use of A18032E - dicamba

Active substance		Dicamba					
Acute toxicity (mg/kg bw) **TER criterion**		545 10					
GAP crop, growth stage	**Application rate (g a.s./ha)**	**Crop scenario Growth stage**	**Indicator species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Maize, post-emergence BBCH 12-19	1 x 187.5	Maize	Small omnivorous bird	158.8	1	29.8	18
Reprod. Toxicity (mg/kg bw/d) **TER criterion**		54.5 5					
GAP crop, growth stage	**Application rate (g a.s./ha)**	**Crop scenario Growth stage**	**Indicator species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Maize, post-emergence BBCH 12-19	1 x 187.5	Maize	Small omnivorous bird	64.8	1 × 0.53	6.44	8.5
Tier 1 long-term risk assessment (necessary for combined risk assessment)							
Maize, post-emergence BBCH 12-19	1 x 187.5	Maize, BBCH 10-29	Medium granivorous bird “gamebird”	3.0	1 × 0.53	0.30	182.8
		Maize, BBCH 10-19	Small insectivorous / worm feeding bird “thrush”	5.7	1 × 0.53	0.57	96.2
		Maize, BBCH 10-29	Small omnivorous bird “lark”	10.9	1 × 0.53	1.08	50.3
		Maize, BBCH 10-29	Medium herbivorous / granivorous bird “pigeon”	22.7	1 × 0.53	2.26	24.1
		Maize, BBCH 10-19	Small insectivorous bird “wagtail”	11.3	1 × 0.53	1.12	48.5

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Dicamba metabolites

Metabolite 5-OH dicamba (NOA405873) is a major foliar metabolite present at >10% of applied parent substance in edible crop parts (refer to **M-CA Section 6 supplement**, **residues** and the residues section in the DAR of the previous EU review). As acute oral toxicity studies with rats and available genotoxicity studies with parent and 5-OH dicamba (NOA405873) indicate that the metabolite is not of higher toxicity than the parent compound (refer to **M-CA Section 5 supplement, toxicology** and the toxicology section in the DAR of the previous EU review), it can be concluded that the risk to birds from this metabolite will be covered by the risk assessment for dicamba (**EFSA Scientific Report, 2011**) and no testing is necessary.

Other metabolites are formed at <10% of parent level in edible crop parts and mammalian testing indicates that they are less toxic than the parent, it can be concluded that the risk to birds will be low and no further risk assessment was conducted (**Dicamba; EFSA Scientific Report, 2011**).

zRMS comments:

The avian risk assessment for dicamba is agreed by the zRMS. It is noted that calculations were performed for the exaggerated application rate of 187.5 g a.s./ha, covering the rate of dicamba intended in Poland (125 g a.s./ha).

In line with conclusions taken at the EU level, no risk assessment from plant metabolites of dicamba is required.

Overall, acceptable acute and long-term dietary risk to birds from dicamba used as A18032E in maize may be concluded.

The Tier 1 long-term risk assessment was included by the zRMS in Table 9.2-7 above as being necessary for combined long-term risk assessment (no acceptable long-term risk for the mixture could be concluded based on TER values calculated at the screening step). Calculations were based on unrounded values.

Mesotrione

Table 9.2-8: Screening step assessment of the acute and long-term/reproductive risk for birds due to the use of A18032E - mesotrione

Active substance		Mesotrione					
Acute toxicity (mg/kg bw) **TER criterion**		3776 ~~3228~~ 10					
GAP crop, growth stage	**Application rate (g a.s./ha)**	**Crop scenario Growth stage**	**Indicator species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Maize, post-emergence BBCH 12-19	1 x 90	Maize	Small omnivorous bird	158.8	1	14.3	264 ~~226~~
Reprod. Toxicity (mg/kg bw/d) **TER criterion**		20.6 5					
GAP crop, growth stage	**Application rate (g a.s./ha)**	**Crop scenario Growth stage**	**Indicator species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Maize, post-emergence BBCH 12-19	1 x 90	Maize	Small omnivorous bird	64.8	1 × 0.53	3.09	6.7
Tier 1 long-term risk assessment (necessary for combined risk assessment)							
Maize, post-emergence BBCH 12-19	1 x 90	Maize, BBCH 10-29	Medium granivorous bird “gamebird”	3.0	1 × 0.53	0.14	144
		Maize, BBCH 10-19	Small insectivorous / worm feeding bird “thrush”	5.7	1 × 0.53	0.27	75.7
		Maize, BBCH 10-29	Small omnivorous bird	10.9	1 × 0.53	0.52	39.6

			"lark"				
		Maize, BBCH 10-29	Medium herbivorous / granivorous bird "pigeon"	22.7	1 × 0.53	1.08	19.0
		Maize, BBCH 10-19	Small insectivorous bird "wagtail"	11.3	1 × 0.53	0.54	38.2

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Mesotrione metabolites

Since metabolites are formed at <10% of parent levels in edible crop parts, and mammalian testing indicates that they are less toxic than the parent, it can be concluded that the risk to birds will be low and no further risk assessment was conducted in accordance with the conclusions in the final RAR.

zRMS comments:

The avian risk assessment for mesotrione is agreed by the zRMS. It is noted that calculations were performed for the exaggerated application rate of 90 g a.s./ha, covering the rate of mesotrione ~~dicamba~~ intended in Poland (60 g a.s./ha).

In line with conclusions taken at the EU level, no risk assessment from plant metabolites of mesotrione is required.

Overall, acceptable acute and long-term dietary risk to birds from mesotrione used as A18032E in maize may be concluded.

The Tier 1 long-term risk assessment was included by the zRMS in Table 9.2-8 above as being necessary for combined long-term risk assessment (no acceptable long-term risk for the mixture could be concluded based on TER values calculated at the screening step). Calculations were based on unrounded values.

Nicosulfuron

Table 9.2-9: Screening step assessment of the acute and long-term/reproductive risk for birds due to the use of A18032E - nicosulfuron

Active substance		Nicosulfuron					
Acute toxicity (mg/kg bw) **TER criterion**		3776 10					
GAP crop, growth stage	**Application rate (g a.s./ha)**	**Crop scenario Growth stage**	**Indicator species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Maize, post-emergence BBCH 12-19	1 x 60	Maize	Small omnivorous bird	158.8	1	9.53	400
Reprod. Toxicity (mg/kg bw/d) **TER criterion**		171 5					
GAP crop, growth stage	**Application rate (g a.s./ha)**	**Crop scenario Growth stage**	**Indicator species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Maize, post-emergence BBCH 12-19	1 x 60	Maize	Small omnivorous bird	64.8	1 × 0.53	2.06	83
Tier 1 long-term risk assessment (necessary for combined risk assessment)							
Maize, post-emergence BBCH 12-19	1 x 60	Maize, BBCH 10-29	Medium granivorous bird "gamebird"	3.0	1 × 0.53	0.10	1792
		Maize, BBCH 10-19	Small insectivorous / worm feeding bird "thrush"	5.7	1 × 0.53	0.18	943

		Maize, BBCH 10-29	Small omnivorous bird "lark"	10.9	1 × 0.53	0.35	493
		Maize, BBCH 10-29	Medium herbivorous / granivorous bird "pigeon"	22.7	1 × 0.53	0.72	237
		Maize, BBCH 10-19	Small insectivorous bird "wagtail"	11.3	1 × 0.53	0.36	476

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Nicosulfuron metabolites

As stated in the EFSA conclusion, the avian toxicity of the two major plant metabolites ASDM and AUSN was not tested. The toxicity of ASDM and AUSN to mammals is low (LD_{50} >2000) and also in the tests with earthworms and aquatic organisms no indication was found that the metabolites would have a higher toxicity than nicosulfuron. Given that exposure levels for herbivorous birds and mammals to these metabolites will be lower than that from nicosulfuron (the maximum residue level of the metabolites is not exceeding one quarter of the maximum level of nicosulfuron) and that their avian toxicity is not likely to be greater than that of nicosulfuron, the risk to birds and mammals from exposure to these metabolites is assumed to be covered by the risk assessment for the parent.

zRMS comments:

The avian risk assessment for nicosulfuron is agreed by the zRMS. It is noted that calculations were performed for the exaggerated application rate of 60 g a.s./ha, covering the rate of nicosulfuron ~~dicamba~~ intended in Poland (40 g a.s./ha).

In line with conclusions taken at the EU level, no risk assessment from plant metabolites of nicosulfuron is required.

Overall, acceptable acute and long-term dietary risk to birds from nicosulfuron used as A18032E in maize may be concluded.

The Tier 1 long-term risk assessment was included by the zRMS in Table 9.2-9 above as being necessary for combined long-term risk assessment (no acceptable long-term risk for the mixture could be concluded based on TER values calculated at the screening step). Calculations were based on unrounded values.

Dicamba/mesotrione/nicosulfuron mixture

Acute risk

Table 9.2-10: Screening step assessment of the acute risk for birds due to the use of A18032E – dicamba/mesotrione/nicosulfuron mixture

Active substance		Dicamba/mesotrione/nicosulfuron mixture					
Acute toxicity (mg/kg bw)		872 ~~870~~					
TER criterion		10					
GAP crop, growth stage	**Application rate (g/ha)[A]**	**Crop scenario Growth stage**	**Indicator species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Maize, post-emergence BBCH 12-19	1 x 337.5	Maize	Small omnivorous bird	158.8	1	53.6	16

SV: shortcut value; MAF: multiple application factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

[A] Application rate of dicamba/mesotrione/nicosulfuron mixture is the sum of the active substances i.e. sum of 187.5 g dicamba/ha + 90 g mesotrione/ha + 60 g nicosulfuron/ha = 337.5 g/ha

Chronic risk

For assessment of chronic effects, according to the EFSA guidance, if a given formulation contains several active substances all known to cause similar effects via a similar biochemical mechanism (e.g. aromatase inhibition) and if this type of effect is actually driving the risk assessment, it is thus recommended to perform an assessment for combined effects on a case by case basis.

For A18032E the active ingredient mesotrione (HPPD inhibitor) has a different mode of action in plants compared to the active ingredient nicosulfuron (ALS/AHAS inhibitor), which are both different from the active ingredient dicamba (synthetic auxin), and their toxicity profiles in birds are very different. For mesotrione the NOEL was determined based on effects on hatching and chick development; for nicosulfuron and dicamba no long-term effects were detected at the maximum dosing levels. Consequently, an assessment for combined effects is not required.

zRMS comments:

The combined acute risk assessment provided in Table 9.2-10 is agreed by the zRMS with minor correction of the considered endpoint, which had no impact on the outcome of the calculation.

With regard to the chronic combined risk assessment, the zRMS agrees that all active compounds have different mode of action, however currently this is not sufficient to support acceptable chronic risk for the mixture. In line with the most up-to-date approach in the combined long-term risk assessment, the margin of safety for individual compounds should be considered (the lowest TER_{LT} must be greater than the standard trigger multiplied by the number of active compounds) or TERmix values should be calculated. Since the TER_{LT} values calculated at the screening step for dicamba and mesotrione were <15 (the trigger relevant for 3 compounds in the mixture), the Tier 1 calculations were included by the zRMS in Tables 9.2-7 to 98.2-9 above and TERmix based on the lowest available TER values for individual compounds was calculated in table below. Calculations were based on unrounded values.

Compound						Σ1/TER	Σ1/TER^{-1}	Trigger
Dicamba		Mesotrione		Nicosulfuron				
TER	1/TER	TER	1/TER	TER	1/TER			
24.1 [1)]	0.041	19.0 [1)]	0.053	237 [1)]	0.004	0.098	10.2	5

[1)] Lowest Tier 1 TER for medium herbivore/granivore

The calculated Tier 1 TERmix is above the trigger of 5 demonstrating acceptable long-term combined risk to birds exposed to the mixture of dicamba, mesotrione and nicosulfuron applied as A18032E.

9.2.2.2 Higher-tier risk assessment

Not required.

9.2.2.3 Drinking water exposure

When necessary, the assessment of the risk for birds due to uptake of contaminated drinking water is conducted for a small granivorous bird with a body weight of 15.3 g (*Carduelis cannabina*) and a drinking water uptake rate of 0.46 L/kg bw/d (*cf.* Appendix K of EFSA/2009/1438).

Leaf scenario

Since A18032E is 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

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).

Dicamba

With a K(f)oc of 12.4, dicamba belongs to the group of less sorptive substances. Here, the maximum use rate of 1 x 187.5 g a.s./ha has been used to cover the risk to birds from all intended uses (see Table 9.1-3).

Effective application rate (g/ha)*	=	187.5		
Acute toxicity (mg/kg bw)	=	545	quotient =	0.34
Reprod. toxicity (mg/kg bw/d)	=	54.5	quotient =	3.4

* Effective application rate = Maximum application rate x MAF of 1

The resulting ratios fall below the trigger of 50 indicating that further assessment of the acute and long-term risk to birds from drinking water from puddles is not required for dicamba.

Mesotrione

With a K(f)oc of 50 (geomean; the worst-case is 14 in the final EFSA endpoints), mesotrione belongs to the group of less sorptive substances. Here, the maximum use rate of 1 x 90 g a.s./ha has been used to cover the risk to birds from all intended uses (see Table 9.1-3).

Effective application rate (g/ha)*	=	90		
Acute toxicity (mg/kg bw)	=	3776 ~~3228~~	quotient =	0.024 ~~0.028~~
Reprod. toxicity (mg/kg bw/d)	=	20.6	quotient =	4.4

* Effective application rate = Maximum application rate x MAF of 1

The resulting ratios fall below the trigger of 50 indicating that further assessment of the acute and long-term risk to birds from drinking water from puddles is not required for mesotrione.

Nicosulfuron

With a K(f)oc of 20.7, nicosulfuron belongs to the group of less sorptive substances.

Effective application rate (g/ha)*	=	60		
Acute toxicity (mg/kg bw)	=	3776	quotient =	0.016
Reprod. toxicity (mg/kg bw/d)	=	171	quotient =	0.35

* Effective application rate = Maximum application rate x MAF of 1

The resulting ratios fall below the trigger of 50 indicating that further assessment of the acute and long-term risk to birds from drinking water from puddles is not required for nicosulfuron.

zRMS comments:

The drinking water risk assessment for particular active substances provided in tables above is agreed by the zRMS with minor correction of the acute endpoint considered for mesotrione, which had no impact on the outcome of the calculation. It is noted that it was performed for exaggerated application rate corresponding to 600 g product/ha, covering the rate of 400 g product/ha intended in Poland.

Acceptable acute and chronic risk could be concluded at the screening step for all active compounds.

No calculations were provided by the Applicant for the pertinent soil metabolites of all three active compounds. However, the risk would be acceptable since the maximum ratio for metabolites based on the worst case assumptions (10 times toxicity of the parent and parent exposure) would be <50 (worst case trigger assumed, covering also risk from less sorptive metabolites) for the acute and long-term risk. Hence, no further evaluation has been performed.

9.2.2.4 Effects of secondary poisoning

The log P_{OW} value for dicamba is 0.55 – 1.9 (at pH 5.0 – 8.9) and for its metabolite NOA414746 the log P_{OW} value is -0.84 (pH 6.8). The log P_{OW} values of mesotrione and its main metabolites MNBA, AMBA and SYN546974 amount to 0.11, -1.3, 0.32 and 1.62 respectively and thus do not exceed the trigger value of 3. Nicosulfuron has a log P_{OW} value of 0.6 and its major aquatic metabolites ASDM, AUSN and HMUD have log P_{OW} values of < 1.0.

Therefore, risk assessment for effects due to secondary poisoning is not required for dicamba, mesotrione, nicosulfuron and their relevant metabolites.

zRMS comments:

Provided above information is agreed by the zRMS. The evaluation of the risk of secondary poisoning is triggered due to log Pow <3 for all active compounds and their relevant metabolites.

9.2.2.5 Biomagnification in terrestrial food chains

Not relevant.

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

Not relevant.

9.2.4 Overall conclusions

The acute and long-term risks of A18032E to birds were assessed from toxicity exposure ratios between toxicity endpoints, estimated from studies with dicamba, mesotrione and nicosulfuron, and maximum residues occurring on food items following applications according to the proposed use pattern. The combined toxicity and risk assessment was also performed.

The risk to birds from exposure via drinking water has also been assessed. Risk of secondary poisoning has not been assessed, as dicamba, mesotrione, nicosulfuron and their relevant metabolites have log P_{OW} <3.0.

The TER values, calculated for recommended scenarios, all exceed the trigger values of 10 for acute risk and 5 for long-term risk, indicating that the risk to birds is acceptable following use of A18032E according to the proposed use pattern. Acceptable combined acute and long-term risk assessment could be concluded. The risk assessment for exposure via drinking water from puddles also showed acceptable risk.

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

9.3.1 Toxicity data

Mammalian toxicity studies have been carried out with dicamba, mesotrione, nicosulfuron and their relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on mammals of A18032E were not evaluated as part of the EU assessment of dicamba, mesotrione and nicosulfuron. New data submitted with this application are listed in Appendix 1 and summarised in Section 6 (Mammalian Toxicology) of this dossier.

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process. Justifications regarding higher tier refinement endpoints for mesotrione are provided below.

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

Species	Substance	Exposure system	Results	Reference
Rat	Dicamba	Oral 1 d Acute	LD_{50} = 1581 mg a.s./kg bw (female)	EFSA Conclusion 2011 Wazeter and Goldenthal, 1974 SAN837/5096
Rat	Dicamba	Dietary Reproductive toxicity Two-generation study	NOAEL = 150 mg a.s./kg bw/d	EFSA Conclusion 2011 Masters, 1993 SAN837/5213

zRMS comments:

Mammalian toxicity data for dicamba are in line with the EU agreed endpoints reported in EFSA Journal 2011;9(1):1965.

Table 9.3-2: Endpoints and effect values relevant for the risk assessment for mammals - mesotrione

Species	Substance	Exposure system	Results	Reference
Rat	Mesotrione	Oral 1 d Acute	LD_{50} >5000 mg a.s./kg bw	EFSA Conclusion 2016 Robinson, 1994a ZA1296_10372
Rat	MNBA	Oral 1 d Acute	LD_{50} >5000 mg/kg bw	EFSA Conclusion 2016 Robinson, 1996 ZA1296/0088
Rat	AMBA	Oral 1 d Acute	LD_{50} >5000 mg/kg bw	EFSA Conclusion 2016 Lees, 1996a R44276/0001
Rat	Mesotrione	Dietary Reproductive toxicity Three-generation study	NOAEL = 0.3 mg a.s./kg bw/d (decreased litter size in F_2) ~~Refined NOAEL = 1.2 mg a.s./kg bw/d (specific to F_0 and F_1 considering proposed GAP)~~	EFSA Conclusion 2016 Milburn, 1997a ZA1296/0044
Mouse	Mesotrione	Dietary Reproductive toxicity Two-generation study	NOAEL = 10 mg a.s./kg bw/d (reproductive effects)	RAR, B6, 2008 Moxon, 1997a ZA1296/0046
Rat	Mesotrione	Dietary 28d varying exposure study	NOEL = 2.4 mg a.s./kg bw/d (reversible tyrosenimic effects)	RAR, B6, 2008 Lees, 2000 ZA1296/0392

zRMS comments:

Mammalian toxicity data for mesotrione are in line with the EU agreed endpoints reported in EFSA Journal 2016;14(3):4419. Refined NOAEL of 1.2 mg a.s./kg bw/d has been struck through as not agreed (for justification, see point 9.3.1.1 below.

Table 9.3-3: Endpoints and effect values relevant for the risk assessment for mammals - nicosulfuron

Species	Substance	Exposure system	Results	Reference
Rat	Nicosulfuron	Oral 1 d Acute	LD_{50} >5000 mg a.s./kg bw	EFSA Conclusion 2007 Cummins, 1991b 89/ISK127/0913
Mouse	Nicosulfuron	Oral 1 d Acute	LD_{50} >5000 mg a.s./kg bw	EFSA Conclusion 2007 Cummins, 1991a 89/ISK126/0912
Rat	ASDM	Oral 1 d Acute	LD_{50} >5000 mg/kg bw	EFSA Conclusion 2007 Johnson, 1993a 93/ISK195/0591
Rat	AUSN	Oral 1 d Acute	LD_{50} >2000 mg/kg bw	EFSA Conclusion 2007 Allard, 1996a 601863
Rat	Nicosulfuron	Dietary Reproductive toxicity Two-generation study	NOAEL = 3861 mg a.s./kg bw/d (male) NOAEL = 4404 mg a.s./kg bw/d (female)	EFSA Conclusion 2007 Willoughby, 1992 91/ISK130/0054

zRMS comments:

Mammalian toxicity data for nicosulfuron are in line with the EU agreed endpoints reported in EFSA Scientific Report (2007) 120, 1-91.

Table 9.3-4: Endpoints and effect values relevant for the risk assessment for mammals – A18032E

Species	Substance	Exposure system	Results	Reference
Rat	A18032E	Oral 1 d Acute	LD_{50} >2000 mg/kg bw	Matting, 2013 A18032E_10018

zRMS comments:

The study on acute oral toxicity of A18032E to rat has been evaluated and accepted by the zRMS toxicology expert. For details of the evaluation and study summary, please refer to Core Assessment, Part B, Section 6.

9.3.1.1 Justification for new endpoints

Consideration of the long-term endpoint of mesotrione for use in risk assessment

The low NOEL for effects of mesotrione on mammals is unique to the rat, with unremarkable toxicity seen in the mouse, rabbit and dog. For refinement of risk to omnivorous mammals from mesotrione, where the mouse is the focal species for use in maize, the toxicity endpoint from the 2-generation mouse study (71 mg/kg bw/d) can be considered as a refinement. For the rat, the screening-level NOEL reported in the DAR of 0.3 mg/kg bw/day is based on effects in the F2 generation of a three-generation study of continuous exposure (140 days). This was agreed as the EU endpoint since the Expert Meeting stated that the ecological realism of exposure should not be taken into account for hazard characterisation. However, it is clear that exposure to mesotrione from a single application in maize in the spring will be limited to a short time period as the foliar DT_{50} is less than 1 day (North, 2016), and the mean soil DT_{50}

is 34.3 days (non-normalised, EFSA Conclusion 2016). Therefore, a refined ecologically-relevant NOAEL endpoint of 1.2 mg/kg bw/day is proposed for the refined long term risk assessment for herbivorous mammals, based on the findings in the F_0 and F_1 generations of continuous exposure, based on modifications of reproductive and developmental parameters. During the EU review, the RMS reviewer stated: *the ecotoxicity assessment will need to consider whether a reduction in litter size of 6.8% is acceptable for wild populations.* It should be noted that the reduction in litter size was not accompanied by a similar reduction in the numbers of offspring surviving or their development. It is therefore highly unlikely that this minor reduction is either biologically significant, or likely to have an impact at the population level. Furthermore, at the higher dose level of 100 ppm (12.3 mg/kg/day) the litter size was only marginally reduced and not significantly different compared to control values, indicating that the effect at 1.2 mg/kg bw/d is likely to be incidental. It is also considered highly unlikely that short-term exposure, for example at a critical developmental stage, would have caused the long-term effects seen in the F_2 generation, because similar effects at 1.2 mg/kg bw/d were not seen in the F_1 generation where short-term exposure would similarly have been experienced.

For the mouse, in the EFSA Conclusion, the conclusions are:

- Parental NOAEL: 10 mg/kg (50 ppm) based on increased tyrosine
- Reproductive NOAEL: 10 mg/kg based on reduced number of successful matings
- Offspring NOAEL: 2 mg/kg based on increased testes and kidney weight.

The offspring NOAEL is not relevant to the wild mammal risk assessment as there is no evidence of organ malfunction and there is no impact on survival or reproduction of individuals, so would not lead to a population-level effect. Likewise, the parental NOAEL is again of no relevance to the survival of individuals or to the population, as in the absence of any adverse effect on the animal, it is just a biochemical measurement. However, the reproductive NOAEL could be of relevance to the population-level effects, so this is considered appropriate for the risk assessment (this endpoint is considered highly conservative as there is no dose response for this endpoint or a statistically significant difference).

zRMS comments:

In general, information available in the mesotrione RAR of 2015 (Vol. 3CA, B.6) indicates that in fact, slightly reduced pup survival at 10 ppm (1.2 mg a.s./kg bw/d) was incidental and not treatment related, as at the next higher dose (100 ppm) the pup survival was at the level comparable with control values. For this reason it seems that for purposes of the ecological risk assessment NOAEL of 1.2 mg a.s./kg bw/d could be considered relevant and was actually proposed by the RMS (UK). However, endpoint to be used in the mammalian risk assessment has been discussed during the Pesticides Peer Review experts Meeting 136 in December 2015. The experts decided that NOAEL of 0.3 mg a.s./kg bw/d should be used. As discussion on the EU agreed and already discussed endpoints should not be re-opened at the zonal level, the zRMS is of the opinion that the risk assessment should be based on the NOAEL of 0.3 mg a.s./kg bw/d.

Nevertheless, it is noted that refinement of the endpoint could be possible with the benchmark dose approach, which was not discussed in the course of the EU review, but is considered by scientists to be more reliable way for derivation of an endpoint from mammalian toxicity studies comparing to the standard NOAEL determination. In fact, the guidance of EFSA Scientific Committee on consideration of the benchmark dose approach in the risk assessment (EFSA Journal 2017;15(1):4658) exist, where it is stated that:

The SC confirms that the BMD approach is a scientifically more advanced method compared to the NOAEL approach for deriving a RP, since it makes extended use of dose–response data and it provides a quantification of the uncertainty in the estimated RP resulting from the statistical limitations in the dose–response data.

The BMD approach is for example accepted in Poland when it is the only way to further refine the risk. However, it was internally agreed by the Polish authorities and experts that calculation of $BMDL_{05}$ is required, while some

Member States may consider $BMDL_{10}$ as more relevant. Some Member States may not at all accept endpoints derived using BMD.

The decision on consideration of BMD approach is up to the Applicant. Recommendations of the guidance mentioned above must be followed in case calculations using BMD approach will be submitted.

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/2009/1438).

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the worst-case application rates (187.5 g dicamba/ha, 90 g mesotrione/ha and 60 g nicosulfuron/ha) are used in the risk 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 in the following tables.

Dicamba

Table 9.3-5: Screening step assessment of the acute and long-term/reproductive risk for mammals due to the use of A18032E - dicamba

Active substance		Dicamba					
Acute toxicity (mg/kg bw) **TER criterion**		1581 10					
GAP crop, growth stage	**Application rate (g a.s./ha)**	**Crop scenario**	**Indicator species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Maize, post-emergence BBCH 12-19	1 x 187.5	Maize	Small herbivorous mammal	136.4	1	25.6	62
Reprod. Toxicity (mg/kg bw/d) **TER criterion**		150 5					
GAP crop, growth stage	**Application rate (g a.s./ha)**	**Crop scenario**	**Indicator species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Maize, post-emergence BBCH 12-19	1 x 187.5	Maize	Small herbivorous mammal	72.3	1 × 0.53	7.18	21
Tier 1 long-term risk assessment (necessary for combined risk assessment, intended application rate used for calculations)							
Maize, post-emergence BBCH 12-19	1 x 125	Maize, BBCH 10-19	Small insectivorous mammal “shrew”	4.2	1 × 0.53	0.28	539
		Maize, BBCH 10-29	Small herbivorous mammal “vole”	72.3	1 × 0.53	4.79	31.3
		Maize, BBCH 10-29	Small omnivorous mammal “mouse”	7.8	1 × 0.53	0.52	290

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

Dicamba metabolites

Metabolite 5-OH dicamba (NOA405873) is a major foliar metabolite present at >10% of applied parent substance in edible crop parts (refer to **M-CA Section 6 supplement**, **residues** and the residues section in the DAR of the previous EU review). As acute oral toxicity studies with rats and available genotoxicity studies with parent and 5-OH dicamba (NOA405873) indicate that the metabolite is not of higher toxicity than the parent compound (refer to **M-CA Section 5 supplement, toxicology** and the toxicology section in the DAR of the previous EU review), it can be concluded that the risk to birds from this metabolite will be covered by the risk assessment for dicamba (**EFSA Scientific Report, 2011**) and no testing is necessary.

Other dicamba metabolites are formed at <10% of parent level in edible crop parts and mammalian testing indicates that they are less toxic than the parent, so it can be concluded that the risk to mammals will be low and no further risk assessment was conducted (**Dicamba; EFSA Scientific Report, 2011**).

zRMS comments:

The mammalian risk assessment for dicamba is agreed by the zRMS. It is noted that calculations were performed for the exaggerated application rate of 187.5 g a.s./ha, covering the rate of dicamba intended in Poland (125 g a.s./ha).

In line with conclusions taken at the EU level, no risk assessment from plant metabolites of dicamba is required.

Overall, acceptable acute and long-term dietary risk to birds from dicamba used as A18032E in maize may be concluded.

The Tier 1 long-term risk assessment was included by the zRMS in Table 9.3-5 above as being necessary for combined long-term risk assessment. The intended application rate (125 g a.s./ha) was considered due to the refined risk assessment for mesotrione performed with the target rate (see below). Calculations were based on unrounded values.

Mesotrione

Table 9.3-6: Screening Step assessment of the acute and long-term/reproductive risk for mammals due to the use of A18032E - mesotrione

Active substance		Mesotrione					
Acute toxicity (mg/kg bw) **TER criterion**		>5000 10					
GAP crop, growth stage	**Application rate (g a.s./ha)**	**Crop scenario**	**Indicator species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Maize, post-emergence BBCH 12-19	1 x 90	Maize	Small herbivorous mammal	136.4	1	12.3	>407 ~~>410~~
Reprod. Toxicity (mg/kg bw/d) **TER criterion**		0.3 5					
GAP crop, growth stage	**Application rate (g a.s./ha)**	**Crop scenario**	**Indicator species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Maize, post-emergence BBCH 12-19	1 x 60	Maize	Small herbivorous mammal	72.3	1 × 0.53	2.30	**0.13**

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

Table 9.3-7: First-tier assessment of the long-term/reproductive risk for mammals due to the use of A18032E - mesotrione

Active substance		Mesotrione					
Reprod. Toxicity (mg/kg bw/d) **TER criterion**		0.3 (lowest); ~~1.2 (ecologically relevant NOAEL from exposure to 2 generations)~~ ~~10 (ecologically relevant endpoint for the mouse, where this is the focal species)~~ 5					
GAP crop, growth stage	**Application rate (g a.s./ha)**	**Crop scenario Growth stage**	**Generic Focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Maize, post-emergence BBCH 12-19	1 x 60	Maize BBCH 10-19	Small insectivorous mammal "shrew"	4.2	1.0 × 0.53	0.134	**2.2** ~~9.0~~ ~~75~~
		Maize BBCH 10-29	Small herbivorous mammal "vole"	72.3	1.0 × 0.53	2.30	**0.13** ~~0.52~~ ~~4.3~~
		Maize BBCH 10-29	Small omnivorous mammal "mouse"	7.8	1.0 × 0.53	0.248	**1.2** ~~4.8~~ ~~40~~

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Mesotrione metabolites

Since metabolites are formed at <10% of parent level in edible crop parts and mammalian testing indicates that they are less toxic than the parent, it can be concluded that the risk to mammals will be low and no further risk assessment was conducted in accordance with the conclusions in the final RAR.

zRMS comments:

The screening step and Tier 1 acute and long-term risk assessment for mesotrione based on the EU agreed toxicity data is accepted by the zRMS. The acute risk assessment was based on the exaggerated rate of 90 g a.s./ha, covering the intended rate of 60 g a.s./ha. The long-term risk assessment was performed for the target rate (60 g a.s./ha).

The Tier 1 calculations of the long-term TER values based on the refined toxicity endpoints are not accepted and are thus struck through in Table 9.3-7. Justification for not acceptance of the refined NOAEL is presented in the commenting box in point 9.3.1.1 above.

Overall, on the basis of the screening evaluation, acceptable acute risk may be concluded for the exaggerated intended rate of mesotrione (90 g a.s./ha), covering also intended rate of 60 g a.s./ha.

Potentially unacceptable long-term risk has been demonstrated for all generic focal species. Refinement of the risk is presented in point 9.3.2.2 below.

In line with conclusions taken at the EU level, no risk assessment from metabolites is required.

Nicosulfuron

Table 9.3-8: Screening step assessment of the acute and long-term/reproductive risk for mammals due to the use of A18032E - nicosulfuron

Active substance		Nicosulfuron					
Acute toxicity (mg/kg bw) **TER criterion**		>5000 10					
GAP crop, growth stage	**Application rate (g a.s./ha)**	**Crop scenario**	**Indicator species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Maize, post-emergence BBCH 12-19	1 x 60	Maize	Small herbivorous mammal	136.4	1	8.18	>610
Reprod. Toxicity (mg/kg bw/d) **TER criterion**		3861 5					
GAP crop, growth stage	**Application rate (g a.s./ha)**	**Crop scenario**	**Indicator species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Maize, post-emergence BBCH 12-19	1 x 60	Maize	Small herbivorous mammal	72.3	1 × 0.53	2.30	1679 ~~1700~~
Tier 1 long-term risk assessment (necessary for combined risk assessment, intended application rate used for calculations)							
Maize, post-emergence BBCH 12-19	1 x 40	Maize, BBCH 10-19	Small insectivorous mammal “shrew”	4.2	1 × 0.53	0.09	43363
		Maize, BBCH 10-29	Small herbivorous mammal “vole”	72.3	1 × 0.53	1.53	2519
		Maize, BBCH 10-29	Small omnivorous mammal “mouse”	7.8	1 × 0.53	0.17	23349

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

Nicosulfuron metabolites

As stated in the EFSA conclusion, the toxicity of ASDM and AUSN to mammals is low (LD_{50} >2000) and also in the tests with earthworms and aquatic organisms no indication was found that the metabolites would have a higher toxicity than nicosulfuron. Given that exposure levels for herbivorous birds and mammals to these metabolites will be lower than that from nicosulfuron (the maximum residue level of the metabolites is not exceeding one quarter of the maximum level of nicosulfuron) and that their toxicity is not likely to be greater than that of nicosulfuron, the risk to birds and mammals from exposure to these metabolites is assumed to be covered by the risk assessment for the parent.

zRMS comments:

The mammalian risk assessment for nicosulfuron is agreed by the zRMS. It is noted that calculations were performed for the exaggerated application rate of 60 g a.s./ha, covering the rate of nicosulfuron intended in Poland (40 g a.s./ha).

In line with conclusions taken at the EU level, no risk assessment from plant metabolites of nicosulfuron is required.

Overall, acceptable acute and long-term dietary risk to birds from nicosulfuron used as A18032E in maize may be concluded.

The Tier 1 long-term risk assessment was included by the zRMS in Table 9.3-8 above as being necessary for combined long-term risk assessment. The intended application rate (40 g a.s./ha) was considered due to the refined risk assessment for mesotrione performed with the target rate (see below). Calculations were based on unrounded values.

A18032E - dicamba/mesotrione/nicosulfuron mixture

Acute risk

Table 9.3-9: Screening step assessment of the acute risk for mammals due to the use of A18032E

Product		A18032E					
Acute toxicity (mg/kg bw)		>2000					
TER criterion		10					
GAP crop, growth stage	**Application rate (g A18032E/ha)**	**Crop scenario**	**Indicator species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg A18032E/kg bw/d)**	**TER_a**
Maize, post-emergence BBCH 12-19	600	Maize	Small herbivorous mammal	136.4	1	81.8	>24

SV: shortcut value; MAF: multiple application factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Chronic risk

For assessment of chronic effects, according to the EFSA guidance, if a given formulation contains several active substances all known to cause similar effects via a similar biochemical mechanism (e.g. aromatase inhibition) and if this type of effects is actually driving the risk assessment, it is recommended to perform an assessment for combined effects on a case by case basis.

For A18032E the active ingredients, mesotrione (HPPD inhibitor) has a different mode of action in plants than the active ingredient nicosulfuron (ALS/AHAS inhibitor) which are both different from the active ingredient dicamba (synthetic auxin), and their toxicity profiles in mammals are very different as demonstrated in Section 6. The mammalian toxicity of mesotrione is well understood, with the principal feature of the toxicology being low NOAEL values in the rat (particularly male), due to the induction of tyrosinemia which leads to reversible effects including reduced bodyweight, increased liver and kidney weights and ocular lesions as corneal opacity. The NOAEL is based on effects on litter size and pup survival. The NOAEL for nicosulfuron showed no significant adverse effects on reproductive performance at the top dose level (EFSA Conclusion 2007). For dicamba the NOAEL is based on decreased body weight gain in adults and does' aborting effects in the rabbit teratology study. Whilst a common mode of action is highly unlikely for these different active ingredients, since the effects seen include reduced bodyweight for mesotrione and dicamba, an assessment for combined effects shall be carried out in order to be conservative. Consequently an assessment for combined effects will be conducted and is based on a concentration addition approach. However, please note that the toxicity is clearly driven by the mesotrione content.

In case of concentration addition each substance contributes to the total toxicity of a mixture in proportion to its concentration using the following equation:

$$TER_{combi} = trigger / ((trigger_{<mesotrione>}/TER_{<mesotrione>}) + (trigger_{<active\ substance\ 2>}/TER_{<active\ substance\ 2>})) \text{ etc.}$$

An acceptable risk is expected when $TER_{combi} > trigger$.

In this formula, 'triggers' are the EU triggers.

Table 9.3-10: Tier 1 assessment of the long-term/reproductive risk for mammals due to the use of A18032E in maize: combination risk assessment

Intended use	Maize (BBCH 12-19)				
Application rate (g/ha) **$Trigger_{combi}$** **TER criterion**	187.5 g/ha dicamba 90 g/ha mesotrione 60 g/ha nicosulfuron 5 5				
GAP Crop scenario	**Focal species**	**$TER_{dicamba}$**	**$TER_{mesotrione}$**	**$TER_{nicosulfuron}$**	**TER_{combi}**
Maize, BBCH 10-19	Small insectivorous mammal "shrew"	21	**1.5** [a] 6.0 [b] 50 [c]	1700	**1.4** **4.7** 15
Maize, BBCH 10-29	Small herbivorous mammal "vole"	21	**0.087** [a] **0.35** [b] **2.9** [c]	1700	**0.087** **0.34** **2.5**
Maize, BBCH 10-29	Small omnivorous mammal "mouse"	21	**0.81** [a] **3.2** [b] 27 [c]	1700	**0.78** **2.8** 12

TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.
[a] based on 0.3 mg/kg bw/d (Tier 1 EU agreed endpoint for the rat)
[b] based on 1.2 mg/kg bw/d (ecologically relevant NOAEL from exposure to 2 generations, rat)
[c] based on 10 mg/kg bw/d (ecologically relevant endpoint for the mouse, where this is the focal species)

zRMS comments:

The acute risk assessment for the mixture based on experimentally derived endpoint for A18032E is agreed by the zRMS. It is noted that calculations were performed for the exaggerated application rate of 600 g product/ha, covering the rate of A18032E intended in Poland (400 g product/ha). Acceptable acute dietary risk from the formulation may be concluded.

The combined long-term risk is not agreed by the zRMS since for dicamba and nicosulfuron TER values calculated at the screening sept for small herbivorous mammal were considered, while for mesotrione TER values derived at Tier 1 for all generic focal species were taken into account. Furthermore, Tier 1 risk assessment presented in Table 9.3-7 were calculated for the target rate of mesotrione (60 g a.s./ha) while in calculations presented in Table 9.3-10 TER values obtained for rate of 90 g a.s./ha are presented. Although the Applicants' calculations may be considered as representing worst case, in opinion of the zRMS correct evaluation should be presented, which is provided below. Calculations were based on unrounded values.

Generic focal species	Compound						Σ1/TER	Σ1/TER^{-1}	Trigger
	Dicamba		Mesotrione		Nicosulfuron				
	TER	1/TER	TER	1/TER	TER	1/TER			
Small insectivore	539	0.002	2.2	0.445	43363	0.00002	0.447	**2.24**	5
Small herbivore	31.3	0.032	0.13	7.66	2519	0.0004	7.70	**0.13**	
Small omnivore	290	0.003	1.2	0.827	23349	0.00004	0.83	**1.20**	

Values in **bold** indicate unacceptable risk

All calculated Tier 1 TERmix values are below the trigger of 5 indicating unacceptable risk for all generic focal species exposed to the mixture in the diet. Refinement of the risk is presented in point 9.3.2.2 below.

9.3.2.2 Higher-tier risk assessment

A refined risk assessment investigating the potential long-term risk from mesotrione is presented below and additionally in the confidential report referenced below:

Report:	Alvarez T. (2019) Mesotrione: Refined risk assessments for mammals (Central Zone). Syngenta, Jealott's Hill, Bracknell, United Kingdom. Syngenta Unpublished Report (Syngenta File No. A18032E_10335)

The risk assessment has been refined by identification of realistic and relevant focal species in early post-emergent maize, along with associated PT values. Realistic residues and residue dissipation have also been refined. A full justification of all refinements used and studies referenced is provided in ***Alvarez (2019)***. A summary of this information is provided below.

Summary of Alvarez T. (2019):

The TER_{LT} values for the following scenarios do not pass the Tier 1 risk assessment for long-term risk to wild mammals, and therefore require refinement:

Table 9.3.2.2-1: Scenarios requiring refinement of long-term risk (TER_{LT}) to mammals

Active substance	Application rate (g a.s./ha)	Scenario		Tier 1 TER_{LT}
		Crop / growth stage	Generic focal species / diet	
Mesotrione	60	Maize, BBCH 10-19	Small insectivorous mammal "shrew"	**2.2** [a] ~~9.0~~ [b]
		Maize, BBCH 10-29	Small herbivorous mammal "vole"	**0.13** [a] ~~0.52~~ [b]
		Maize, BBCH 10-29	Small omnivorous mammal "mouse"	**1.2** [a] ~~4.8~~ [b]
	~~67.5~~	~~Maize, BBCH 10-19~~	~~Small insectivorous mammal "shrew"~~	~~**2.0** [a]~~ ~~8.0~~ [b]
		~~Maize, BBCH 10-29~~	~~Small herbivorous mammal "vole"~~	~~**0.12** [a]~~ ~~**0.46** [b]~~
		~~Maize, BBCH 10-29~~	~~Small omnivorous mammal "mouse"~~	~~**1.1** [a]~~ ~~**4.3** [b]~~
	~~90~~	~~Maize, BBCH 10-19~~	~~Small insectivorous mammal "shrew"~~	~~**1.5** [a]~~ ~~6.0~~ [b]
		~~Maize, BBCH 10-29~~	~~Small herbivorous mammal "vole"~~	~~**0.087** [a]~~ ~~**0.35** [b]~~
		~~Maize, BBCH 10-29~~	~~Small omnivorous mammal "mouse"~~	~~**0.81** [a]~~ ~~**3.2** [b]~~

[a] Considering the conservative endpoint for mesotrione of 0.3 mg/kg bw/d

~~[b] Considering the ecologically relevant endpoint for mesotrione of 1.2 mg/kg bw/d~~

The Tier 1 TER values are almost all below the trigger value of 5 indicating the need for further refinement. The risk assessment will be refined by identification of realistic and relevant focal species in early post-emergent maize. Also realistic PT values (proportion of an animal's daily diet obtained in treated field), residue values and residue dissipation will be considered.

zRMS comments:

Since in Poland only application rate of 400 g product/ha (corresponding with 60 g mesotrione/ha) is intended, summary of Tier 1 results for rates of 65 and 90 g mesotrione/ha were struck through as not relevant for this assessment. In refinement below only the target application rate will be considered by the zRMS.

Furthermore, results obtained for higher endpoint of 1.2 mg a.s./kg bw/d were struck through since refinement of the endpoint was not agreed by the zRMS (for details, see point 9.3.1.1 above).

Refined long-term risk assessment

1. Ecologically relevant endpoints for wild mammals

Mesotrione

In accordance with the EFSA Guidance Document for bird and mammal risk assessment, the ecological relevance of the mammalian endpoint can be considered in the higher tier risk assessment. During the EU review Expert meeting for mesotrione, the following comments were made regarding the appropriate endpoint for mesotrione:

"In the original assessment of mesotrione the previously agreed Annex I endpoint for reproductive toxicity was 0.3 mg a.s./kg bw/d (2.5 ppm). The endpoint was derived from the multi-generation study on rat by Milburn, 1997 and it was based on the biologically relevant reduction in litter size at ≥10 ppm. Given that:

- the total exposure in multi-generation study is up to 140 days for parents and 50 days for foetuses and offspring ;

- the DT_{50} in soil = 43 days and the DT_{50} on foliage is likely to be low;

- the intended uses envisage a single product application;

the notifier has justified that the exposure in the field of the F2 generation is extremely unlikely. In light of this, in the RAR the RMS proposed a higher NOAEL of 1.2 mg a.s./kg bw/d (10 ppm).

the notifier has justified that the exposure in the field of the F2 generation is extremely unlikely. In light of this, in the RAR the RMS proposed a higher NOAEL of 1.2 mg a.s./kg bw/d (10 ppm)

Discussion:

It was underlined that the observed effects (e.g., litter size and pup survival) could not be unequivocally attributed to tyrosinaemia. With this regard, it was pointed out that, even more in light of this lack of causality, it is not possible to exclude that the observed effects were caused by the short-term exposure to the active substance. Therefore, the meeting agreed not to disregard the effect on the F2 generation. Moreover, the unlikelihood of the exposure in the field was not considered a solid justification, as it was agreed that from a procedural point of view is not correct to take into account the exposure assessment in the hazard characterisation.

Overall the experts agreed to consider relevant the effects on litter size on the F2 generation. Therefore the meeting agreed that the NOAEL of 0.3 mg/kg bw/day should be used in the RA."

Realistic exposure can be considered in the higher tier risk assessment in accordance with the guidance document. It is clear from the soil DT_{50} (<43 days) and the foliar DT_{50} (<24 hours; see below) that realistic exposure after a single application of mesotrione will be limited to a short period of time. This will be considerably shorter than the continuous exposure experienced in the 2-generation rat study (140 days), in which the effects noted at 1.2 mg/kg bw/day were only seen in the second generation of continuous exposure. During the EU review, the RMS reviewer stated: *the ecotoxicity assessment will need to consider whether a reduction in litter size of 6.8% is acceptable for wild populations.* It should be noted that the reduction in litter size was not accompanied by a similar reduction in the numbers of offspring surviving or their development. It is therefore highly unlikely that this minor reduction is either biologically significant, or likely to have an impact at the population level. Furthermore, at the higher dose level of 100 ppm (12.3 mg/kg/day) the litter size was only marginally reduced and not significantly different compared to control values, indicating that the effect at 1.2 mg/kg bw/d is likely to be incidental. It is also considered highly unlikely that short-term exposure, for example at a critical developmental stage, would have caused the long-term effects seen in the F2 generation, because similar effects at 1.2 mg/kg bw/d were not seen in the F1 generation where short-term exposure would similarly have been experienced.

The ecologically relevant NOEAEC of 1.2 mg/kg bw/d will therefore be used to refine the risk assessment for mesotrione.

Further consideration of the toxicological profile of mesotrione:

Mode of action of mesotrione in mammals

Mesotrione has been shown to have a single mode of action (MOA) in mammals, that of inhibition of 4-hydroxyphenyl dioxygenase (HPPD) which is the second enzyme in the catabolic pathway of tyrosine. The consequence of HPPD inhibition is a species- and sex-dependent elevation of tyrosine levels in the plasma (tyrosinaemia), the male rat being most sensitive.

Mesotrione induced tyrosinemia in rats leads characteristic systemic toxicities including reduced bodyweight, increased liver and kidney weights and ocular lesions characterised as corneal opacity. These effects are seen at low dose levels of mesotrione and have been shown to be reversible once mesotrione is excluded from the diet and tyrosine levels return to normal.

In addition, the consequence of severe and sustained tyrosinaemia during reproduction in the rat is a reduction in litter size and in pup survival during the first few days after littering (see ***EU review documentation*** for mesotrione and Lewis and Botham (2013)[2]) The effect on litters in the rat is more pronounced in the second and subsequent generations provided continuous exposure to mesotrione is maintained.

Applicability for the wild mammal risk assessment.

The rat has been shown to be the species most sensitive to the administration of mesotrione, hence the multigeneration study in the rat is the most appropriate study on which to base a chronic risk assessment for wild mammals.

Syngenta has proposed that the nature of the likely exposure to mesotrione, based on a single use per season and a rapid foliar dissipation, means that exposure to mesotrione for longer than 2 weeks per season is very unlikely. Consequently the most appropriate endpoint on which to base the wild mammal risk assessment is that from the overall no observed adverse effect level for reproductive parameters in the first generation of the rat reproduction study (Milburn 1997).

The RMS commented that:

The applicant has proposed a NOAEL of 1.2 mg/kg bw/d (10ppm) for the ecotoxicology risk assessment based on what they have stated is a NOAEL specific to F0 and F1 from the multigeneration rat study. The NOAEL in the DAR was established for effects on litter size across all generations. Consideration of the F1 results indicates a clear effect at dose levels of 2500 ppm. A reduction in litter size by 6.8% is seen in animals treated at 10 ppm and by 11.1% for those treated at 100 ppm when compared to the control group. Litter weight is similarly reduce at these doses, but this is considered to be associated with reduced litter size. A significant reduction in pup survival is seen at 10 ppm but this is not dose-related and is therefore not considered to be of toxicological concern.

Parameter	*Generation*	*Dose Level (ppm)*				
		0	*2.5*	*10*	*100*	*2500*
Gestation length (d)	*F0*	*22.7*	*22.4**	*22.6*	*22,7*	*22.9*
	F1	*22.3*	*22.3*	*22.4*	*22.8***	*22.9***
Litter size (no. pups)	*F1*	*11.7*	*12.4*	*10.9*	*10.3*	*9.2***
Litter weight (g) Day 0	*F1*	*70.4*	*72.2*	*65.9*	*63.4*	*57.1***
Pup survival (%)	*F1*	*92.4*	*89.9*	*85.2***	*89.7*	*77.6***

On this basis an F1 NOAEL of 100 ppm (11.6 mg/kg bw/d) could be considered appropriate.

However, the use of data from the first generation has been questioned and Syngenta should like to respond to the issues raised:

a) The causal link of the effect on litter parameters/pup survival to tyrosine

The RMS commented

[2] Lewis RW and Botham JW (2013) A review of the mode of toxicity and relevance to humans of the triketone herbicide 2-(4-methylsulfonyl-2-nitrobenzoyl)-1,3-cyclohexanedione. Crit Rev Toxicol 43(3) 185-199

The applicant has proposed that the effects are due to tyrosinaemia. However, the data do not definitively support this proposal. In a single generation reproductive study, rats were fed diets either with or without 2500 ppm mesotrione and with or without tyrosine (see section B6.8.2.8 [Williams 1997]***). No effects were seen on litter size although those receiving both mesotrione and tyrosine showed considerably higher plasma tyrosine levels compared to those receiving tyrosine only. In the groups dosed with both mesotrione and tyrosine, there was a marked increase in toxicity (pup survival, percent born dead, total litter loss) with increasing tyrosine dose (see table 6.8.2.8-3). However there was no associated increase in plasma tyrosine levels in animals receiving 1% or 2% tyrosine in addition to 2500 ppm mesotrione compared to those receiving mesotrione alone. It is therefore unclear whether these effects can be attributed only to mesotrione induced tyrosinaemia.***

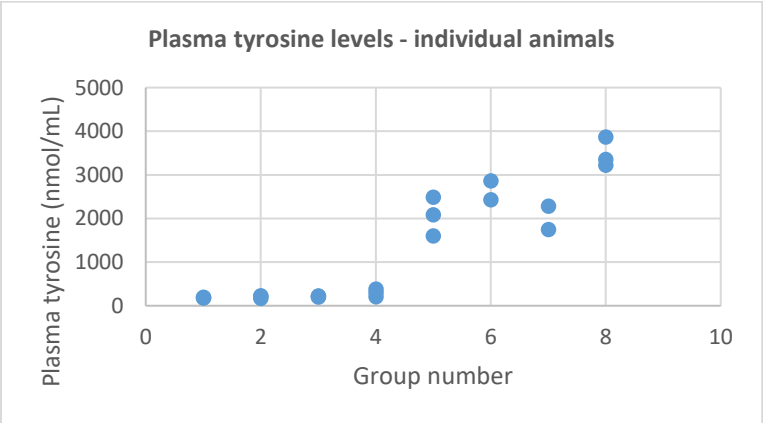
Syngenta response

It is only possible to experimentally elevate plasma concentrations of tyrosine in the rat in the absence of HPPD inhibition if tyrosine is administered in a low protein diet. This was done in adult rats and it was shown that elevated plasma tyrosine concentrations resulted in the characteristic ocular lesions seen after administering mesotrione in the diet for 4 to 6 weeks, linking the ocular lesions seen following mesotrione administration to tyrosinaemia. However, a low protein diet would be incompatible with reproduction, therefore the effect of tyrosine on reproductive parameters was investigated in an exacerbation study. In this study animals were given either 0 (control) or 2500ppm mesotrione with varying levels of tyrosine in the diet.

Exacerbation study design

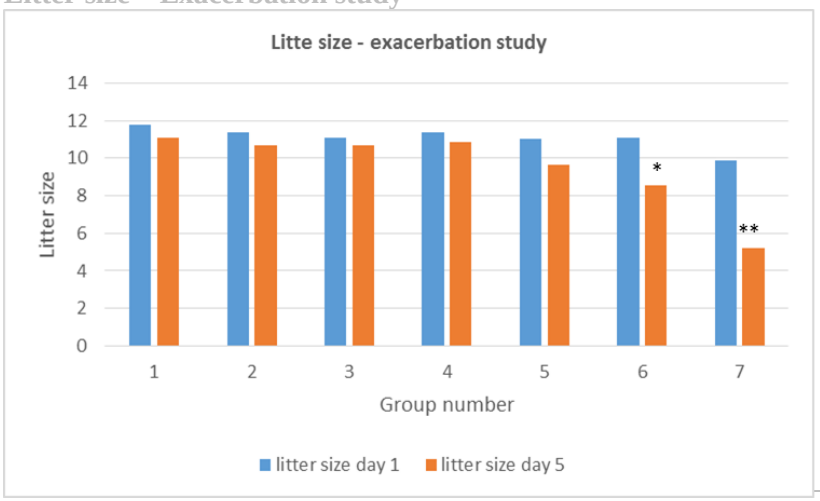
Group	Dietary concentration of ZA1296 (ppm)	Dietary concentration of tyrosine (%)	Numbers of time-mated females
1	0	0	20
2	0	0.5	20
3	0	1.0	20
4	0	2.0	20
5	2500	0	20
6	2500	0.5	20
7	2500	1.0	20
8	2500	2.0	20

Plasma tyrosine was measured in 3 animals/group 48 hours after dosing commenced. The plasma tyrosine levels are shown below



Plasma tyrosine levels are very slightly elevated in group 4 but are significantly elevated only in the presence of HPPD inhibition (Groups 5-8). Overall there is a dose related increase in plasma tyrosine levels. In group 8 toxicity (body weight reduction) was too great to allow the group to complete the study but litter data is available for all other groups:

Litter size – Exacerbation study



*P<0.05 and **p<0.001 (Students t-test 2-sided)

From the data presented, the significant reduction in litter size noted when increasing levels of tyrosine are added to the diet of animal dosed with 2500ppm mesotrione, a dose which completely inhibits HPPD, is accompanied by an increase in systemic tyrosine levels. The correlation is not exact but plasma tyrosine levels were measured only once soon after the start of the study to avoid significant stress on the animals once pregnancy was established and only 3 animals per group were used. It is likely that steady state concentrations of tyrosine had not been established so soon after the start of the study and a closer correlation might have been seen had more animals been used for the plasma analysis. Nonetheless the weight of evidence supports a correlation between increasing impact on litter size/pup survival with increasing tyrosine concentrations. Importantly the dose level of mesotrione in groups 5 to 8 was constant and it can be concluded that the decreased pup survival in groups 5 to 7 cannot therefore be attributed to mesotrione.

b) Likely plasma tyrosine concentrations in rats exposed to mesotrione under field conditions

The RMS commented:

The DAR reported threshold concentrations of 1300 µM plasma tyrosine for effects on pup survival and 1000 µM for effects on reduced litter size (see Volume 1, section 2.6.6). In the dynamic feeding study reported in section 6.8.2.9 [Lees, 2000] ***the results indicate that dosing for 3 days with 100 ppm of mesotrione results in plasma tyrosine levels of in excess of this threshold and that this threshold was exceeded for several days after the dose level was reduced. The plasma data from the dynamic feeding study are from male rats, there is no data on female animals, and although male rats are more sensitive than females, it cannot be discounted that female rats exposed to 100 ppm would not exceed the proposed concentration thresholds for reproductive effects. In female rats, plasma tyrosine levels are found in excess >1000 µM in both a 28-day and a 90 day dietary study (see Sections 6.8.2.1 and 6.8.2.3). On this basis 100 ppm cannot be considered as a NOAEL from the F1 segment of the rat multigeneration study.***

Syngenta response

Male rats have been shown to be more sensitive than females to the effect of HPPD inhibition and a dynamic exposure study (Lees, 2000) was undertaken in this most sensitive sub-population to explore the likely worst case effects on wild mammals of consumption of residues of mesotrione after spraying. The study showed that under exposure conditions mimicking those in the field of decreasing mesotrione, the characteristic ocular lesions seen after exposure to steady state concentrations of ≥1000uM tyrosine in the plasma for 4-6 weeks did not develop. Plasma tyrosine measurements confirmed that under field conditions the steady state levels necessary for the ocular lesions to develop do not occur.

~~Although female rats were not used in this study, tyrosine data are available which confirm that even under conditions in which steady state levels are achieved (90 days continuous dietary dosing) plasma tyrosine concentrations at 100ppm would not exceed the thresholds for reproductive effects proposed in the DAR.~~

~~A 90 day study has been conducted in which plasma tyrosine concentrations were measured in female animals administered various dietary concentrations of mesotrione (Brammer 1997e; EU DAR). The data clearly show that plasma tyrosine concentrations do not exceed 1000uM at dietary concentrations of up to 100ppm mesotrione in female rats.~~

Report:	~~5.8.2.1.2/01: Brammer A, 1997e. ZA1296: 90 Day dose response study in female rats. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Laboratory Report No. CTL/R/1315. 19 November 1997. Unpublished. (Syngenta File No.ZA1296/0382; IAD ZA1296/0373)~~

~~Plasma tyrosine concentrations in female rats given mesotrione in the diet:~~

	~~Dietary level of ZA1296 (ppm)~~							
~~**Parameter**~~	~~**control**~~	~~**1**~~	~~**5**~~	~~**10**~~	~~**50**~~	~~**100**~~	~~**1000**~~	~~**2500**~~
~~Dose received (mg/kg/day)~~	~~-~~	~~0.09~~	~~0.48~~	~~0.95~~	~~4.82~~	~~9.54~~	~~94.83~~	~~236.75~~
~~Plasma tyrosine levels week 13~~	~~127~~	~~147~~	~~219**~~	~~249**~~	~~620**~~	~~836**~~	~~1593**~~	~~1534**~~

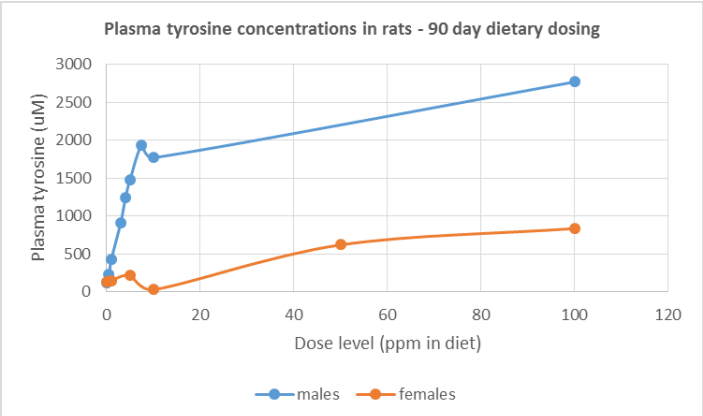
~~In a parallel study with male rats (Brammer 1997d, EU DAR), plasma tyrosine concentrations exceed 1000uM at much lower dietary inclusion rates of mesotrione:~~

Report:	~~5.8.2.1.1/01: Brammer A, 1997d. ZA1296: 90 Day dose response study in male rats. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Laboratory Report No. CTL/R/1304. 19 November 1997. Unpublished. (Syngenta File No.ZA1296//0381; IAD ZA1296/0372)~~

~~Plasma tyrosine concentrations in male rats given mesotrione in the diet:~~

	~~Dietary level of ZA1296 (ppm)~~								
~~**Parameter**~~	~~**control**~~	~~**0.5**~~	~~**1**~~	~~**3**~~	~~**4**~~	~~**5**~~	~~**7.5**~~	~~**10**~~	~~**100**~~
~~Dose received (mg/kg/day)~~	~~-~~	~~0.04~~	~~0.09~~	~~0.27~~	~~0.35~~	~~0.44~~	~~0.67~~	~~0.89~~	~~8.96~~
~~Plasma tyrosine levels week 13~~	~~113~~	~~228*~~	~~431*~~	~~915**~~	~~1241**~~	~~1482**~~	~~1934**~~	~~1771**~~	~~2772**~~

~~The available data show that tyrosine concentrations in females dosed with mesotrione in the diet for 90 day are lower than those in males and that at 100ppm tyrosine concentrations in females do not exceed the lowest threshold suggested for reproductive effects of 1000uM, whereas that threshold is exceeded at 5ppm in males. Plasma tyrosine concentrations reported in the dynamic feeding study, conducted on male rats as the known sensitive sub-population, are not representative of likely tyrosine concentrations in female rats given the same dosing regimen.~~



~~Thus the NOAEC of 1.2 mg/kg bw/d is considered applicable, and conservative for use in the refined risk assessment for mesotrione.~~

Consideration of the predicted exposure pattern

The following graph illustrates the short period of exposure predicted for a herbivorous wild mammal (Brown hare) following application of mesotrione at 105 g a.s./ha, which is representative for the maximum proposed rate of 90g/ha. The 21-day (moving window) time-weighted average exposure is compared to a) the NOEL of 0.3 mg/kg bw/d, and b) the NOEL/5 of 0.06 mg/kg bw/d which takes into account the 5-fold safety factor on the NOEL which is recommended for risk assessment. The initial residue has been calculated using:

Mean initial RUD = RUD x interception factor x FIR/bw

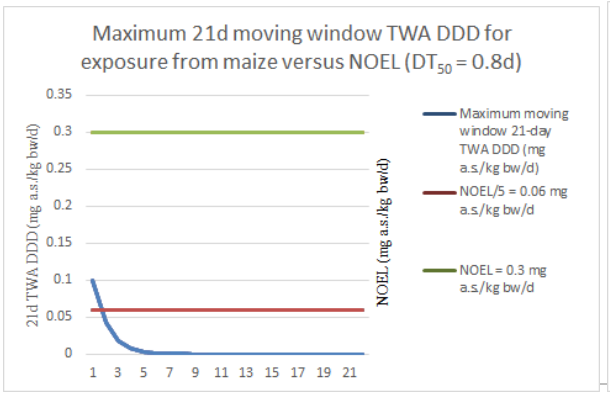
The following variables have been considered:

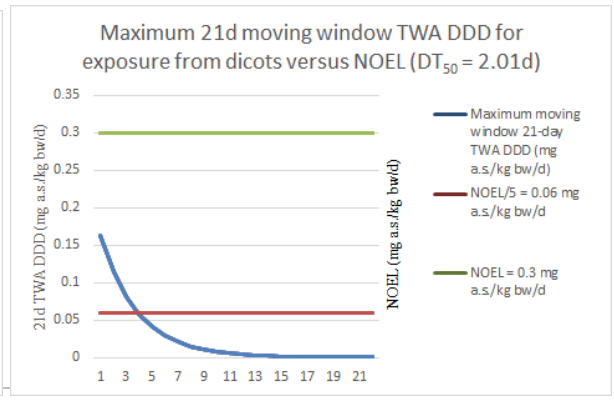
Diet item	RUD	Interception	Hare weight	FIR/bw [c]	Mean initial RUD [d]	Application rate	Mean initial residue DDD [e]	DT_{50}
Maize (grasses/ cereals)	54.2 [a] mg/kg	1 [b]	3800g [a]	0.32	17.34	0.105 kg	1.82 mg a.s./kg bw/day	0.8 days (worst case) 0.61 (geomean)
Dicot weeds (non-grass herbs)	28.7 [a] mg/kg	1 [b]	3800g [a]	0.39	11.19	0.105 kg	1.18 mg a.s./kg bw/day	2.05 days (geomean)

[a] Default from EFSA Bird and Mammal Guidance
[b] No interception – worst case
[c] For full details of calculation of FIR/bw please see sections below
[d] For example, the mean initial RUD = 54.2 x 1 x 0.32 = 17.886 mg/kg bw/d
[e] For example, mean initial residue DDD after application of 105g/ha = 0.105 x 17.34 = 1.82 mg/kg bw/d

The mean initial residue is then used to calculate the 21d moving window time-weighted average exposure. The moving window approach is used as this is worst-case. The dissipation is calculated considering a simple first order (SFO) dissipation which is applicable, considering the results of the residue dissipation studies discussed below.

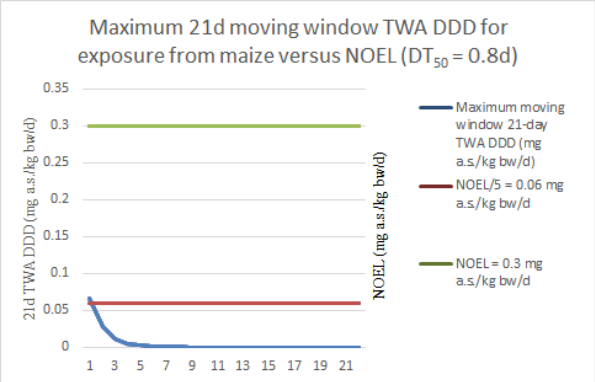
For 105g mesotrione:

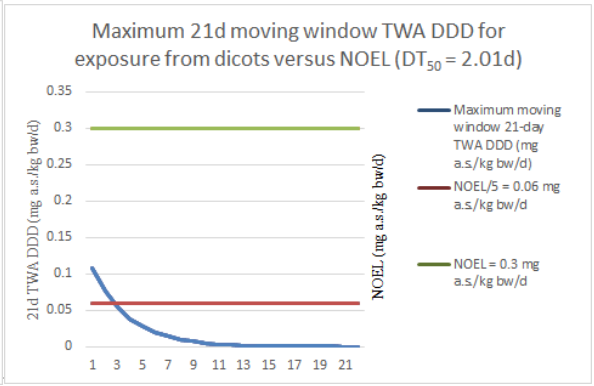




The 21d TWA residue is less than 0.06 mg/kg bw/d indicating acceptable risk within 2 days after application (DT_{50} = 0.8d) for maize, or within 4 days for residues on dicots (DT_{50} = 2.05d), clearly demonstrating that exposure is limited to a very short time period.

~~1 x 70g/ha:~~





~~The 21d TWA residue is less than 0.06 mg/kg bw/d indicating acceptable risk within 1 day after application (DT_{50} = 0.8d) for maize, or within 3 days for residues on dicots (DT_{50} = 2.05d), clearly demonstrating that exposure is limited to a very short time period.~~
~~Hares are nocturnal and mesotrione will only be sprayed during the day. Combined with the disturbance caused by spraying, this will mean that there will be a gap of some hours between spraying and consumption of diet during which significant reduction of residues will occur. Hares (as herbivores) will take several hours/most of the night to consume their daily FIR. Thus the F1 endpoint is clearly relevant (i.e. NOAEL = 1.2 mg/kg bw) since exposure is limited to a very short time period, and furthermore a low risk can be demonstrated within 2-3 days after application for single applications.~~

zRMS comments:

Refinement of the endpoint was not agreed by the zRMS and in the risk refinement below the EU agreed NOAEL of 0.3 mg mesotrione/kg bw/d will be used by the zRMS. Please refer to point 9.3.1.1 for discussion on the Applicants' proposal regarding endpoint refinement.

Above discussion on the endpoint was struck through as being not relevant due to reasons indicated in point 9.3.1.1.

Please note that the same approach has been taken by PL as zRMS and agreed by cMS in the course of evaluation of two mesotrione formulations (Callisto and Calaris) belonging to the mesotrione data owner (Syngenta).

2. Realistic residue values

Consideration of the RUD in soil invertebrates

~~The PEC_{worm} can be calculated using the calculations for the bioaccumulation section following an application of mesotrione where:~~

~~PEC_{worm} = 21 d time-weighted average PEC_{soil} × BCF~~
~~BCF = C_{worm}/C_{soil} = (0.84 + 0.012 K_{ow}) / f_{oc} × K_{oc}~~
~~K_{ow} = Octanol-water partition coefficient~~
~~K_{oc} = Organic carbon adsorption coefficient~~
~~f_{oc} = Organic carbon content of soil (0.02 taken as a default value)~~

~~For details of the soil PEC calculation, see the supporting Registration Report Part B, environmental fate. Please note that this calculation is not done for mesotrione to investigate bioaccumulation, since mesotrione has a log P_{ow}<3 (0.11) and therefore no bioaccumulation is expected.~~

Compound	Application rate (g a.s./ha)	21d TWA PEC_{soil} (mg/kg)	K_{ow}	f_{oc}	Koc	BCF	PEC_{worm} (mg/kg)
Mesotrione	60	0.047[a]	1.29	0.02	17.39 [b]	2.46	0.116
			1.29	0.02	156.6 [c]	0.273	0.0128
	67.5	0.053[a]	1.29	0.02	17.39 [b]	2.46	0.130
			1.29	0.02	156.6 [c]	0.273	0.0145
	90	0.071[a]	1.29	0.02	17.39 [b]	2.46	0.175
			1.29	0.02	156.6 [c]	0.273	0.0194

[a] 21d TWA following application to maize (see Section 8)
[b] 90th percentile (alkaline) K_{oc} from the EFSA conclusion
[c] 10th percentile (acid) K_{oc} from the EFSA conclusion

The application rates are already included in the PEC_{worm} calculations. Therefore, the residue values for soil invertebrates do not need to be multiplied by the application rates in the risk assessment below. Also, the TWA is not applicable in the risk assessment since the 21d TWA PEC_{soil} is used to derive the PEC_{worm}.

zRMS comments:

The residues of mesotrione in earthworms were considered by the Applicant in refinement of the risk for the wood mouse with PD values determined in the study by Pelz (1989). However, refinement of the diet based on results of study by Pelz (1989) was not agreed by the zRMS (see below) and for this reason the PEC_{WORM} values calculated above were not validated and are struck through as being not necessary for the risk assessment.

Please note that the same approach has been taken by PL as zRMS and agreed by cMS in the course of evaluation of two mesotrione formulations (Callisto and Calaris) belonging to the mesotrione data owner (Syngenta).

3. Consideration of realistic residue dissipation

Mesotrione

The rapid dissipation of mesotrione has been widely reported, e.g. Lavieille *et al.* (2009[3]) reports photolytic degradation in the laboratory on cuticular wax with a DT_{50} of 100-160 minutes. The authors measured photodegradation of mesotrione on simulated leaf surfaces and showed rapid breakdown, and demonstrated that the mechanism behind rapid breakdown is photodegradation. Fantke *et al.* (2014[4]) present data to show a realistic half-life under field conditions, and according to these researchers the mean half-life for mesotrione has been estimated as 1.13 days by using a completely different method and model, and using different data, from those provided in the EU review. These published data are consistent with the results from the White (2001) study provided in the EU review, and further support the proposal that mesotrione has a short half-life in plants.
However, the White (2001) data from studies carried out in Canada were not accepted during the EU review as sufficiently representative of European conditions. Therefore an additional study has been carried out with residue trials carried out in the UK and France, and the final results are included here (North *et al*. 2016). A full study summary is presented at the end of this document.

[3] Lavieille et al. (2009) "Effect of a spreading adjuvant on mesotrione photolysis on wax films. Journal of Agriculture and Food Chemistry 57, 9624-9628 (doi:10.1021/jf901996d)"
[4] Fantke et al. (2014) "Estimating half-lives for pesticides dissipation from plants. Environmental Science and Technology 48, 8588-8602 (dx.doi.org/10.1012/es500434p)"

Table 9.3.2.2-2: Residues over time of mesotrione in maize shoots (European study; North *et al.* 2016)

Time	Mesotrione residues (mg/kg)				
	Trial No. 1	**Trial No. 2**	**Trial No. 3**	**Trial No. 4**	**Trial No. 5**
0	7.09	13.96	4.24	3.09	14.99
4h	8.48	7.75	2.98	2.74	12.63
10h	4.11	6.25	3.33	2.05	8.61
24h	3.86	3.57	1.69	0.91	4.30
34h	2.79	2.95	0.50	0.8	2.19
48h	0.92	1.37	0.41	0.36	1.07
72h	0.16	0.63	0.14	<0.01	0.31
96h	0.12	0.11	0.06	0.10	0.13
DT_{50} (d)	**0.80**	**0.51**	**0.66**	**0.62**	**0.51**
Mean DT_{50} (d)	**0.61**				

The most realistic and relevant data for Europe come from the recent trials carried out in 2015, which gave a mean DT_{50} of 14 hours. The longest DT_{50} was 19.2 hours and this will be used to represent a worst-case. The F_{twa} was then calculated in accordance with the Guidance document using the following equation:

$$f_{twa} = \frac{1 - e^{-kt}}{kt}$$ Where:

$k = \ln(2)/DT_{50}$ (rate constant)
t = Averaging time in days (21 days)

Thus the F_{twa} for the longest DT_{50} of 19.2 hours (0.80 days) = 0.055. This can be further refined considering the more realistic geomean DT_{50} of 0.61 days which gives an F_{twa} = 0.0419.
The residue decline for maize plants also acts as a surrogate for residues on other potentially contaminated plants on the field (weeds). However, weeds in the field will be subject to the herbicidal activity of the active substances and therefore are a potential diet item for only a short period after application. To further investigate this, the dissipation of mesotrione residues after application to a representative dicot (clover) was investigated (Allen, 2019). Eleven trials were carried out.

Table 9.3.2.2-3: Residues over time of mesotrione in clover (European study; Allen, 2019)

Time	Mesotrione residues (mg/kg)										
	Trial 1 (UK)	**Trial 2 (UK)**	**Trial 3 (HU)**	**Trial 4 (HU)**	**Trial 5 (FR)**	**Trial 6 (FR)**	**Trial 7 (DE)**	**Trial 8 (DE)**	**Trial 9 (PL)**	**Trial 10 (PL)**	**Trial 11 (BE)**
0	6.10	3.63	11.97	11.69	11.51	8.75	4.46	9.11	6.15	6.50	8.58
8h	6.03	4.20	11.41	8.99	8.78	9.98	5.66	2.71	4.34	4.58	8.48
24h	4.58	3.39	11.02	8.76	9.86	8.73	4.59	2.59	2.28	4.72	8.17
32h	2.69	4.09	9.02	8.80	6.72	4.77	3.98	3.29	2.06	6.37	5.65
48h	2.73	2.61	7.14	6.89	5.47	4.66	4.21	2.54	1.78	5.78	5.54
72h	1.95	2.17	6.06	5.51	2.00	4.77	0.22	2.61	3.82	1.8	3.26
96h	0.58	2.76	0.14	0.12	0.58	4.37	0.08	0.43	1.67	1.66	3.43
168h	0.27	0.19	0.11	0.07	0.09	0.70	0.09	0.05	1.23	0.27	1.70
DT_{50} (d)	**1.49**	**3.57**	**1.99**	**2.01**	**1.55**	**2.57**	**1.77**	**1.06**	**2.41**	**2.64**	**2.65**
Geomean DT_{50} (d)	**2.19 (results of trial 8 excluded from calculation due to unacceptable kinetic fit)** ~~2.05~~										

Since >ten trials are available, it is applicable to use the geometric mean DT_{50} in this case instead of the worst-case. The F_{twa} for the geomean DT_{50} of 2.19 = 0.150 ~~2.05 days = 0.14~~ for a single application.

zRMS comments:

Maize

In order to determine the DT_{50} value in maize, a residue decline study by North (2016) has been submitted by the Applicant. The study is considered acceptable and due to data from only 5 trials it is proposed by the zRMS to use the worst case DT_{50} of 0.803 days for purposes of refinement of fTWA, which is 0.055. For evaluation of the study, please refer to Appendix 2.

Weeds

In order to determine the DT_{50} value in weeds, a residue decline study by Allen (2019) performed on clover has been submitted by the Applicant. The study is considered acceptable and due to reliable DT_{50} values obtained in 10 trials it is proposed by the zRMS to use the geometric mean DT_{50} of 2.19 days for purposes of refinement of fTWA, which is 0.150. For evaluation of the study, please refer to Appendix 2.

Please note that the same approach has been taken by PL as zRMS and agreed by cMS in the course of evaluation of two mesotrione formulations (Callisto and Calaris) belonging to the mesotrione data owner (Syngenta).

4. Identification of relevant focal species

Three generic field studies have been conducted to monitor the use of maize crops by small mammals (xxxxxxxxxxxxx 2005; included in the EU review; Funkenhaus & Giessing, 2010; and Grimm 2013). Details for all studies are provided in the study summaries included towards the end of this document.
The first two maize field studies studied pre-emergence to early post-emergence growth stages (BBCH 0-14 in Austria; 0-16 in France). The third maize study looked at early growth stages post-emergence up to BBCH 16 (in Germany). In all studies no voles or shrews were trapped in-field, only off-field, indicating that the small herbivorous mammal and insectivorous mammal are not relevant generic focal species at the early growth stages considered here. The common shrew (*Sorex araneus*) is considered as the representative small insectivore consuming earthworms (Appendix A.2 of EFSA Guidance). However, reports in the literature show that shrews make relatively little use of arable land and are mostly confined to non-cropped habitat (Tew, 1994[5]). This is supported by Loman (1991[6]) who found that shrews did not occur in cropped fields in an agricultural landscape in southern Sweden, only in uncropped habitat islands.
For voles is the low attractiveness of early maize fields can be explained by the need for good vegetation cover to avoid predation (Mackin-Rogolska, 1981[7]). Further information about the presence of voles in early maize fields is available from a recent study (Fülling & Sainz-Elipe 2015). The results are summarised in the table below.

[5] Tew, T.E., Todd, I.A. and Macdonald, D.W. 1994. Field margins and small mammals. BCPC Monogr. 58:85-94. Also Todd, I.A., Tew, T.E. and Macdonald, D.W. 2000. Arable habitat use by wood mice (*Apodemus sylvaticus*). 1. Macrohabitat. Journal of Zoology **250**:299-303.

[6] Loman, J. 1991a. The small mammal fauna in an agriculture landscape in southern Sweden, with special reference to the wood mouse *Apodemus sylvaticus*. Mammalia **55**:91-96.

[7] Mackin-Rogolska, R. 1981: Spatial structure of rodent populations co-occurring in different crop fields. Pol. Ecol. Stud. 7: 213-227.

Table 9.3.2.2-4: Summary of key results for common voles

	MAIZE FIELD			ADJACENT GRASSLAND		
Summarized results	1979 trap nights* (26 trap nights with at least one capture)			1955 trap nights* (1249 trap nights with at least one capture)		
	Maize fields with common voles		2 out of 11	**Grasslands with common voles**		11 out of 11
	Trapping success	**Individuals**	27	**Trapping success**	**Individuals**	1394
		Total captures	43		**Total captures**	2606
	Trapping efficiency (mean)**	2.17		**Trapping efficiency** (mean)**	133.30	

Results per study site	MAIZE FIELD			ADJACENT GRASSLAND		
	Trapping success		**Trapping efficiency****	**Trapping success**		**Trapping efficiency****
	Individuals	**Total captures**		**Individuals**	**Total captures**	
1	0	0	0	31	41	22.91
2	0	0	0	113	177	98.33
3	0	0	0	102	200	111.73
9	0	0	0	165	273	153.37
10	24	40	22.22	283	617	358.72
16	0	0	0	181	370	206.7
17	0	0	0	125	176	97.78
18	0	0	0	165	302	168.72
21	0	0	0	52	94	55.29
22	3	3	1.67	47	96	53.33
23	0	0	0	130	260	145.25

* Traps were arranged in grids of 60 traps, with 30 traps placed on the maize field and 30 traps on the adjacent grassland habitat. All traps were activated before each trapping night but due to occasional malfunction or even the activity of other wild animals not all traps were found working the next morning. Only traps that were still working in the morning were regarded as 'active' and used for the calculation of trap nights.
**captures/100 trap nights

In total, 2606 out of the 2649 captures (i.e. 98.38%) were located in the off-crop habitat while 43 were located in maize fields (i.e. 1.62% of the total captures). Adverse conditions in the adjacent grassland at site 10 (an overpopulation explosion off-field) and 22 (mowing) are likely to explain the captures of common voles in the maize field, indicating that the animals were not attracted by the maize field *per se* but rather deterred by the adverse conditions in the neighboring habitat. The fact that in nine out of eleven maize fields no common vole was ever caught leads to the conclusion that early stage maize fields are not attractive for this species.

The relevant focal small mammal species in early growth stages of maize and cereals is clearly the small omnivorous mammal i.e. the wood mouse (*Apodemus sylvaticus)*. Indeed this was the conclusion reached in the Annex I Additional Report B.9 in Section B.9.3.3.4i) based on the xxxxxxx (2005) study and is further supported by the new study by xxxxxxxxxx (2010). In the same part of the Annex I Additional Report it is also concluded that since there were no trappings in-field in either maize or plain fields of mammals eating only earthworms (i.e. shrews), the wood mouse should be considered as the relevant focal species for risk assessment to earthworm-eating mammals.
Hares were found in maize fields in both field studies (in Austria and France) and so will also be considered here as a relevant herbivorous focal species. The method of sampling (live traps) used in the study in cereals would not detect hares, but these were noted in thermal scanning of the fields during the study, and so they are assumed to also be a relevant focal species in early spring-sown cereals.
These conclusions on relevant focal species and their relation to EFSA Tier 1 generic focal species are shown in the table below.

Table 9.3.2.2-5: Summary of focal species relevant to recommended uses in maize BBCH 12-19

Crop grouping/growth stage	Generic focal species / diet	Focal species identified in field studies
Maize BBCH 10-19	Small insectivorous mammal "shrew" ground dwelling invertebrates without interception 100% ground arthropods	No – not found in maize fields in field studies; use the omnivorous Wood mouse *Apodemus sylvaticus*
Maize BBCH 10-29	Small herbivorous mammal "vole" Grass + cereals All maize shoots + later grass	No – herbivorous focal species is the Hare *Lepus europaeus*
Maize BBCH 10-29	Small omnivorous mammal "mouse" Combination (invertebrates with interception) 25% weeds 50% weed seeds 25% ground arthropods	Yes – Wood mouse *Apodemus sylvaticus*

Therefore the relevant focal species for the supported uses are the Wood mouse and the Hare. These will be assessed in turn, below.

zRMS comments:

The studies by Grimm et al. (2013) and Funkenhaus & Giessing (2010) were already evaluated in the course of the EU review of mesotrione. On the basis of all available data in this area it was agreed that the relevant focal species for maize at BBCH 12-18 is wood mouse and brown hare.

No further consideration of this refinement option is required, as it is in line with the conclusion taken at the EU level. Taking this into account, results of studies referred to by the Applicant above were not re-evaluated by the zRMS and are thus shaded in the text above. Nevertheless, the taken conclusions are retained as being in line with the EU decision.

5. Consideration of focal species parameters

Wood mouse

i. Realistic diet for calculation of food intake rates for the wood mouse

The risk assessment for wood mouse can be refined (as done in the EU Annex I Terbuthylazine Additional Report B.9.3.3.4) using the following data available on the diet of wood mice:

Table 9.3.2.2-6: Diet composition of wood mouse in May as summarised in Gurney *et al.* (1998)[8]

Reference	% volume in stomach contents				Study site
	Plant material	Insects	Oligochaetes	Other	
Gorman & Zubaid 1993a	75	15	5		UK (woodland site)
Gorman & Zubaid 1993b	10	56	15		UK (sand-dune site)
Pelz 1989	**16**	**10**	**40**	**Cereal grain (30%) Dicot seeds (4%)**	**Arable farm site in Rhineland, Germany (n=16)**
Rogers & Gorman 1995	72 (monocots) 5 (dicots)	13	10		Set aside land, UK
Watts 1968	4	88 (nearly all examples were leaf-eating caterpillars)	0	Seed endosperm (7%)	Wytham woods, UK

The following text is quoted direct from the Additional Report B.9.3.3.4 for terbuthylazine:

[8] Mammal Bible' CONTRACT PN0910/PN0919 MILESTONE REPORT Mammals and farming: information for risk assessment. J.E. Gurney, J. Perrett, D.R. Crocker & J.A. Pascual. CSL report, 1998

"Of the five available diet compositions, the most relevant study is deemed to be Pelz (1989). This is on the basis that the study has been performed on an arable farm site, which is considered to be more relevant for the proposed use on maize than woodland, sand-dune or set aside land. The study was also performed over 7 years, obtaining 16 replicates.
For the purposes of the risk assessment, the plant material category is combined with the data for cereal grain and dicot seeds, to give a total of 50% plant material. Therefore the representative diet for wood mice in maize to be used in the risk assessment is 50% plant material, 10% insects and 40% earthworms."

For post-emergence the plant material including the crop can be foraged, and since seeds contain very different amounts of energy compared to foliar plant material, 34% of weed seeds will be kept as a separate food item. Foliar plant material will be assumed to be maize, since the herbicidal activity of the product will mean that few weeds will be available after treatment in maize fields. In addition, the standard Tier 1 mixed diet compositions will also be considered.

zRMS comments:

The diet derived by Pelz (1989) was considered by the Applicant most relevant as it was obtained in the arable area. However, from the Mammal Bible it is not known if maize fields were present at the test site and if derived diet is relevant for the intended uses of A18032E. This is particularly important as the wood mouse is an opportunistic omnivore and its diet will highly depend on the landscape composition. In order to address this issue the publication by Pelz (1989) was consulted. The study was carried out from 1980 to 1986 and in the paper the map of the study area in 1984 is given and reproduced below.

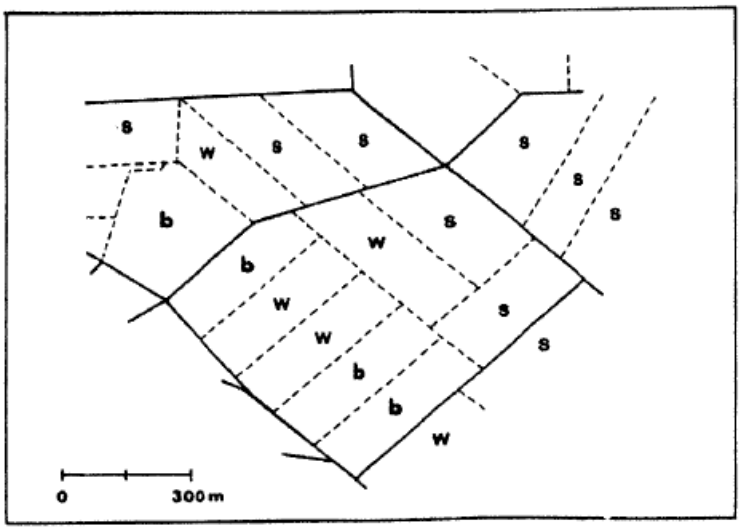


Figure 3.1 Map of the study area in the Rhineland in spring 1984. Solid lines – field paths; broken lines – field boundaries; b – winter barley; s – sugar beet; w – winter wheat.

From the above figure it is evident that the test site (at least in 1984) comprised of cereal and sugar beet fields with no maize fields present. No maps are present for remaining years, but taking into account that the study aimed at investigation of the damage by the wood mouse to sugar beet seeds and potential reduction of this damage due to the presence of cereals it may be expected that sugar beet and cereal fields represented high proportion of the area over the whole period of the study. High proportion of cereal fields explains also significant amount of cereal grain found in the mice stomachs and it is not known with what food item it would be replaced in case maize fields were present at high proportion in the test area. Taking all this into account the zRMS is of

the opinion that the study by Pelz (1989) is not relevant to refine the diet of the wood mouse exposed following application of A18032E to maize.

Overall, refinement of the risk using approach taken at the EU level, i.e. with assumption of the standard diet of the wood mouse consisting of 25% weeds, 50% weed seeds and 25% of ground arthropods as indicated in EFSA (2009) is considered acceptable by the zRMS.

Please note that the same approach has been taken by PL as zRMS and agreed by cMS in the course of evaluation of two mesotrione formulations (Callisto and Calaris) belonging to the mesotrione data owner (Syngenta).

Considering the fractions (PDi) of individual food items in a mixed diet, together with data on their respective moisture and energy content, the specific energy content of the mixed diet can be calculated as recommended by the **EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009)**. This value is used to estimate the required amount of the mixed diet to satisfy the daily energy expenditure of a wood mouse.

Hence, based on body mass data derived from EFSA Guidance, and calculations according to Appendix G of the **EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009)**, the FIR for mixed diet of woodmice according to the data presented in the text above is presented in the table below. All the terms used below are as presented in Appendix G.

$$FE_{total,fresh} = \sum_i \left[PD_{i,fresh} \times FE_i \times \left(1 - \frac{MC_i}{100}\right) \times \frac{AE_i}{100} \right]$$

$$FIR_{total,fresh} = \frac{DEE}{FE_{total,fresh}}$$

Table 9.3.2.2-7: Calculation of food intake rate for the Wood mouse considering the default EFSA mixed diet: post-emergence

Maize	April-May	Plant material [f]	Ground arthropods	Weed seeds
Fraction of food item in mixed diet [a]	PD_i fresh (%)	25%	25%	50%
Food energy of food item [i] in mixed diet [b]	FE (kJ/dry g)	17.6	22.7	21.7
Moisture content of food item [i] in mixed diet [b]	MC (%)	76.4%	68.8%	9.9
Assimilation efficiency of food item [i] in mixed diet [c]	AE (%)	47%	87%	84%
Food energy of food item in diet [d]	FE_{item},fresh (kJ/g fresh weight)	0.488	1.54	8.21
Food energy of total mixed diet [d]	FE_{total},fresh (kJ/g fresh weight)	10.2		
Daily energy expenditure [d]	DEE (kJ/day)	59		
Food intake rate of total mixed diet [d]	FIR_{total}, fresh (g fresh weight/day)	5.76		
b.w. [e]	(g)	21.7		
FIR/b.w.	(g fresh weight/b.w./day)	0.27		

[a] PD for Wood mouse Tier 1 EFSA mixed diet
[b] From Table 3 of Appendix G in EFSA (2009)
[c] From Table 4 of Appendix G in EFSA (2009)
[d] Calculated according to EFSA (2009; Appendix G)
[e] Body weight of wood mouse from the EFSA Guidance
[f] Plant material is assumed to be = maize shoots (using the default values for grasses and cereal shoots)

Table 9.3.2.2-8: Calculation of food intake rate for the Wood mouse considering mixed diet according to Pelz (1989): post-emergence

Maize	April-May	Plant material [f]	Insects	Soil invertebrates	Weed seeds
Fraction of food item in mixed diet [a]	PD_i fresh (%)	16%	10%	40%	34%
Food energy of food item [i] in mixed diet [b]	FE (kJ/dry g)	17.6	22.7	19.4	21.7
Moisture content of food item [i] in mixed diet [b]	MC (%)	76.4%	68.8%	84.3%	9.9%
Assimilation efficiency of food item [i] in mixed diet [c]	AE (%)	47%	87%	87%	84%
Food energy of food item in diet [d]	FE_{item}, fresh (kJ/g fresh weight)	0.312	0.616	1.06	5.58
Food energy of total mixed diet [d]	FE_{total}, fresh (kJ/g fresh weight)	7.57			
Daily energy expenditure [d]	DEE (kJ/day)	59			
Food intake rate of total mixed diet [d]	FIR_{total}, fresh (g fresh weight/day)	7.79			
b.w. [e]	(g)	21.7			
FIR/b.w.	(g fresh weight/b.w./day)	0.36			

[a] PD for Wood mouse estimated on data from Pelz (1989)
[b] From Table 3 of Appendix G in EFSA (2009)
[c] From Table 4 of Appendix G in EFSA (2009)
[d] Calculated according to EFSA (2009; Appendix G)
[e] Body weight of wood mouse from the EFSA Guidance
[f] Plant material is assumed to be = grasses and cereal shoots for maize

zRMS comments:

The FIR/bw for the wood mouse presented in Table 9.3.2.2-7 above was calculated by the Applicant with consideration of the bodyweight of 21.7 g and the mixed diet indicated in EFSA guidance. Calculation was performed in line with indications of Appendix G of EFSA (2009) and is agreed by the zRMS. The FIR/bw of 0.27 is relevant for the risk refinement.

FIR/bw presented in Table 9.3.2.2-8 is struck through since the diet composition based on results of the study by Pelz (1989) was not agreed by the zRMS.

Please note that the same approach has been taken by PL as zRMS and agreed by cMS in the course of evaluation of two mesotrione formulations (Callisto and Calaris) belonging to the mesotrione data owner (Syngenta).

ii. Use of early growth stage maize fields by wood mice (PT)

In the xxxxxxxxxxxx (2005) maize study, in plain fields (before drilling), the in-field trapping success of wood mice was 1.04 per 100 trapnights, *i.e.* approximately one mouse capture per trap night in the 120 in-field traps set on the 4 fields. Later when the maize was drilled the overall wood mouse populations increased but the number of in-field trappings decreased after seed bed preparation and drilling. This is reflected in terms of wood mice trapping success of 0.45 per 100 trap nights in-field, and 3.21 off-field.
In total (including in-field and off-field, and bare soil phase and all crops) 39 individual wood mice were trapped in 7040 trap nights. This number of trap nights corresponds arithmetically with trapping 23 nights on 10 fields with 30 in-field traps, or to 7 days on 10 fields with 100 traps per field.
On freshly drilled maize fields, however, only 12% of all wood mice captures were in-field. Thus, in order to radio-track the recommended number of 20 wood mice (the sample size recommended in EFSA Guidance on Bird and Mammal Risk Assessment) which are potential consumers on freshly drilled maize fields, around 180 individuals would need to be caught. Taking into account the reduced recapture rate to account for dispersal or predation between tagging and tracking, the trapping effort would even

have to be higher in order to ensure that the radio-tagged mouse would still be around on the day of intended radio-tracking.
From these calculations it is clear that radio-tracking of 20 potential consumer wood mice on freshly drilled maize fields would require unrealistic trapping efforts. Considering the trapping efficiency in-field, in order to track 20 individuals it would be necessary to set 100 traps per field in 60 fields over a 10 day period. This very high sampling effort again supports the conclusion that maize fields are not attractive habitats for wood mice and supports the low PT values and negative Jacob's Indices measured in this study.

Table 9.3.2.2-9: PT values derived from monitoring in the xxxxxxxxxxxx(2005) study

Habitat	No. of tracking sessions	No. of individuals	PT (%) mean of sessions	PT (%) 90th %ile of sessions	Jacob's Index
Plain field	4	4	17	34	-0.3
Drilled maize	2	1	0	0	-1.0
Germinated maize	3	2	2	4	-0.9
All above	9	6			-

Additional data for early post-emergence maize are available from Grimm (2013). In addition, data from the French maize study (xxxxxxxxxxxxxxx 2010) show an even lower in-field capture rate for wood mouse than in the xxxxxxxxxx (2005) study. This was 0.35 per 100 trap nights per field in-field compared to 15.4 per 100 trap nights off-field, indicating that even more fields would need to be sampled than the 60 estimated above in order to catch 20 individuals (the typical sample size recommended under EFSA Guidance), making this an unrealistic option.
The low usage of early maize fields by wood mice is supported by the data on capture locations from the three field studies which show that in-field capture rate ranged from only 2.3 – 14% of the off-field capture rate (below).

Table 9.3.2.2-10: Comparison of wood mouse capture rates in-field and off-field

Habitat	Country	In-field No. of captures per 100 trapnights	Off-field No. of captures per 100 trapnights	In-field capture rate as percentage of off-field (%)
Maize	Austria	0.45	3.21	14
Maize	France	0.35	15.4	2.3
Cereals	Germany	0.3	2.2	14

Furthermore, another study in an arable landscape in France (Ouin *et al*, 2000[9]) also found low capture rates of wood mice in maize – approximately 0.3-1 per 100 trap nights during May to July, compared to 3-6 per 100 trap nights in wheat.
The PT values from these field studies are combined in the dataset below.

[9] Ouin, A., Paillat, G., Butet, A. & Burel, F. (2000): Spatial dynamics of wood mouse (*Apodemus sylvaticus*) in an agricultural landscape under intensive use in the Mont Saint Michel Bay (France). Agriculture Ecosystems and Environment, 78, 159-165.

Table 9.3.2.2-11: PT for each wood mouse tracking session in fields relevant to uses in early post-emergence maize

Study	Crop	Wood mouse individual	PT (%)
xxxxxxxxx (2005)	Germinated maize	1	0
		2	3.8
		2	3.1
xxxxxxxxxxxxx 2013[a]	Emerging maize	3	0
		4	0
		5	3
		6	0
		7	0
		8	4
		9	7
		10	13.9
		11	3
		12	0
		13	1
		14	1
		15	0
		16	0
		17	0
		18	0
		19	3
Post-emergence only (n=20)	Emerging/ germinated maize only	90th percentile	4.3
		mean	2.1
Consumers only post-emergence (n=10) (omitting '0' PT values)	Emerging/ germinated maize only	90th percentile	7.7
		mean	4.3

[a] In this study although 18 individual tracking sessions were reported, the nine individuals included above are the ones which actively used the maize fields; therefore these data are worst-case

The PT dataset shown above comprises a total of 20 tracking sessions for 19 individual wood mice (one was tracked twice).
It is clear from the tracking data that early post-emergence maize fields are not very attractive habitats for Wood mice, as discussed above. This means that for several of the tracked individual PT values of 0 were ascertained. The PT values from both of these field studies are combined in the table above. Since all mammals were caught either in or very close to the maize crop, they will all have had the opportunity to use maize fields and so Syngenta considers that all are valid for calculating PT for use in risk assessment. However, in the past where radiotracking studies have been conducted with mammals caught in the general farmland landscape rather than in the crop in question, the only way to link these individuals to the crop in question has been to use PT values for "consumers" i.e. those mammals that visited the relevant crop. This approach biases the sample as it ignores mammals with the crop in their home-range which choose not to visit the crop. However, as a worst-case, since this approach is still required by some regulators, the PT= 0 values can be removed in order to concentrate on the worst-case, "consumers" only data.
The refined risk assessment below presents TER values considering the mean PT values and the consumer-only mean PT. The 90th percentile values are considered over-conservative since there are plentiful data, however these will also be considered below upon the request of some regulatory authorities.

Please note that during the EU review for mesotrione a PT of 13.9% was proposed, based on the worst-case value from the Grimm (2013) study. This is considered to present an extreme worst case by disregarding the additional data available. The ten data points derived from nine individuals have been produced from a very large programme of monitoring; the low numbers of individuals actually foraging in maize reflect the fact that it is not an attractive crop. Therefore the mean PT value is believed to provide a realistic estimate for risk assessment.

An additional study is available (Dittrich & Benito, 2016) which investigated PT for wood mice in pre- and early post-emergence fields in southern France. A full summary is provided in Appendix I. The

study demonstrated the presence of an abundant and stable population of Wood mice close to the study fields and the general avoidance of these fields by the animals. In a total of 4528 trap-nights, 768 captures of the focal species were made. The overall standardized trapping success for the Wood mouse was 16.1 times lower in the maize fields, with 3.2 captures per 100 trap-nights compared to 51.5 in the adjacent off-field habitat. A total of 28 potential consumers, which were trapped close to or inside the maize fields, were radio-tracked in order to measure their use of maize fields as feeding habitat. Only seven individuals entered, for a very short period of time, the study fields (a maximum of 8.1% of the time potentially foraging was spent within maize fields). Taking into account consumers for the pre-emergence period, the 90%ile PT value was 0.020 (maximum PT= 0.024). In the post-emergence period, repeated sessions of selected individuals were conducted, and the 90%ile PT value was 0.013 (maximum PT= 0.081) for consumers. These data accord well with the data presented above, but since they are less conservative and from the Southern zone, they are not included in the calculations presented below.

zRMS comments:

The studies by xxx(2005) andxxxxxxxx et al. (2013) were already evaluated in the course of the EU review of mesotrione. On the basis of all available data the maximum PT of 0.139 from study by Grimm et al. (2013) was agreed to be relevant for the risk assessment.

The Applicants' proposal to consider the mean PT due to availability of additional data is not agreed by the zRMS. The same data package was available at the EU level and consideration of the mean of 90th percentile PT was not agreed. It should be also noted majority of the tracked individuals had PT <0.1, so they cannot be considered to be crop consumers and in line with current approach for PT refinement, they would be excluded from further consideration. Nevertheless, the zRMS will not re-open the discussion on the parameters extensively discussed during the EU review and the EU agreed value will be used in the risk refinement.

Additional study to further refine the PT for the wood mouse submitted by the Applicant (Dittrich & Benito, 2016) was performed in the Southern France and is thus not relevant for evaluation performed for the Central Zone. Results of the study were not considered for risk refinement purposes.

Please note that the same approach has been taken by PL as zRMS and agreed by cMS in the course of evaluation of two mesotrione formulations (Callisto and Calaris) belonging to the mesotrione data owner (Syngenta).

iii. Risk assessment for the Wood mouse

Mesotrione

~~The risk assessment calculations presented below consider a range of possible refinements of PT (mean and 90th percentiles for all data and considering consumers only), residue decline and residues on seeds and soil invertebrates (for details please see above).~~

~~Table 9.3.2.2-12: Risk assessment for the wood mouse in early post-emergence maize (1 x 60 g a.s./ha) – mesotrione considering the EFSA default diet~~

~~Focal species~~	~~Food category, % in diet~~	~~FIR/bw~~	~~RUD_m (mg/kg food)~~	~~MAF_m × TWA~~	~~PT~~	~~DDD_m (mg/kg bw/d)~~	~~NOAEL (mg/kg bw/d)~~	~~TER_{lt}~~
~~Wood mouse~~ ~~Early post-emergence (BBCH 12-19)~~ ~~60 g/ha~~	~~Plant material, 25 %~~	~~0.27~~	~~54.2[a]~~	~~1 x 0.055[d]~~	~~0.021 (mean)~~	~~0.000254~~		
	~~Insects, 25 %~~	~~0.27~~	~~7.5[b]~~	~~1 x 0.53~~		~~0.000338~~		
	~~Seeds, 50 %~~	~~0.27~~	~~40.2[c]~~	~~1 x 0.53~~		~~0.00362~~		
	~~whole diet~~					~~0.00421~~	~~0.3~~	~~71~~
							~~1.2~~	~~290~~
	~~Plant material, 25 %~~	~~0.27~~	~~54.2[a]~~	~~1 x 0.055[d]~~	~~0.043 (90th percentile)~~	~~0.000519~~		
	~~Insects, 25 %~~	~~0.27~~	~~7.5[b]~~	~~1 x 0.53~~		~~0.000692~~		
	~~Seeds, 50 %~~	~~0.27~~	~~40.2[c]~~	~~1 x 0.53~~		~~0.00742~~		
	~~whole diet~~					~~0.00863~~	~~0.3~~	~~35~~

Wood mouse Early post-emergence (BBCH 12-19) 60 g/ha							1.2	140
Wood mouse Early post-emergence (BBCH 12-19) 60 g/ha	Plant material, 25 %	0.27	54.2[a]	1 x 0.055[d]	0.043 (mean, consumers only)	0.000519		
	Insects, 25 %	0.27	7.5[b]	1 x 0.53		0.000692		
	Seeds, 50 %	0.27	40.2[c]	1 x 0.53		0.00742		
	whole diet					0.00863	0.3	35
							1.2	140
Wood mouse Early post-emergence (BBCH 12-19) 60 g/ha	Plant material, 25 %	0.27	54.2[a]	1 x 0.055[d]	0.077 (90th percentile, consumers only)	0.000930		
	Insects, 25 %	0.27	7.5[b]	1 x 0.53		0.00124		
	Seeds, 50 %	0.27	40.2[c]	1 x 0.53		0.0133		
	whole diet					0.0155	0.3	19
							1.2	77
Wood mouse Early post-emergence (BBCH 12-19) 60 g/ha	Plant material, 25 %	0.27	54.2[a]	1 x 0.055[d]	0.139 (worst-case PT)	0.00168		
	Insects, 25 %	0.27	7.5[b]	1 x 0.53		0.00224		
	Seeds, 50 %	0.27	40.2[c]	1 x 0.53		0.0240		
	whole diet					0.0279	0.3	11
							1.2	43

TER values in **bold** are less than the trigger of 5 indicating a potential risk which is considered in further refinements

[a] RUD for grasses/cereals as a surrogate for maize. This is conservative as the Tier 1 in maize scenario has wood mice consuming weeds (non-grass herbs with mean RUD of 28.7).

[b] RUD for ground-dwelling invertebrates without interception (Appendix F of the EFSA Guidance Document)

[c] RUD for seeds; EFSA default

[d] Ftwa from longest DT_{50} measured in European trials (19.2h)

Table 9.3.2.2-13: Risk assessment for the wood mouse in early post-emergence maize (1 x 67.5 g a.s./ha) – mesotrione considering the EFSA default diet

Focal species	Food category, % in diet	FIR/bw	RUD_m (mg/kg food)	MAF_m x TWA	PT	DDD_m (mg/kg bw/d)	NOAEL (mg/kg bw/d)	TER_{lt}
Wood mouse Early post-emergence (BBCH 12-19) 67.5 g/ha	Plant material, 25 %	0.27	54.2[a]	1 x 0.055[d]	0.021 (mean)	0.000285		
	Insects, 25 %	0.27	7.5[b]	1 x 0.53		0.000380		
	Seeds, 50 %	0.27	40.2[c]	1 x 0.53		0.00408		
	whole diet					0.00475	0.3	63
							1.2	250
Wood mouse Early post-emergence (BBCH 12-19) 67.5 g/ha	Plant material, 25 %	0.27	54.2[a]	1 x 0.055[d]	0.043 (90th percentile)	0.000584		
	Insects, 25 %	0.27	7.5[b]	1 x 0.53		0.000779		
	Seeds, 50 %	0.27	40.2[c]	1 x 0.53		0.00835		
	whole diet					0.00971	0.3	31
							1.2	120
Wood mouse Early post-emergence (BBCH 12-19) 67.5 g/ha	Plant material, 25 %	0.27	54.2[a]	1 x 0.055[d]	0.043 (mean, consumers only)	0.000584		
	Insects, 25 %	0.27	7.5[b]	1 x 0.53		0.000779		
	Seeds, 50 %	0.27	40.2[c]	1 x 0.53		0.00835		
	whole diet					0.00971	0.3	31
							1.2	120
Wood mouse Early post-emergence (BBCH 12-	Plant material, 25 %	0.27	54.2[a]	1 x 0.055[d]	0.077 (90th percentile, consumers only)	0.00105		
	Insects, 25 %	0.27	7.5[b]	1 x 0.53		0.00139		
	Seeds, 50 %	0.27	40.2[c]	1 x 0.53		0.0149		
	whole diet					0.0173	0.3	17

19) 67.5 g/ha							1.2	69
Wood mouse Early post-emergence (BBCH 12-19) 67.5 g/ha	Plant material, 25 %	0.27	54.2[a]	1 x 0.055[d]	0.139 (worst-case PT)	0.00189		
	Insects, 25 %	0.27	7.5[b]	1 x 0.53		0.00252		
	Seeds, 50 %	0.27	40.2[c]	1 x 0.53		0.0270		
	whole diet					0.0314	0.3	9.6
							1.2	38

TER values in **bold** are less than the trigger of 5 indicating a potential risk which is considered in further refinements

[a] RUD for grasses/cereals as a surrogate for maize. This is conservative as the Tier 1 in maize scenario has wood mice consuming weeds (non-grass herbs with mean RUD of 28.7).

[b] RUD for ground-dwelling invertebrates without interception (Appendix F of the EFSA Guidance Document)

[c] RUD for seeds; EFSA default

[d] Ftwa from longest DT_{50} measured in European trials (19.2h)

Table 9.3.2.2-14: Risk assessment for the wood mouse in early post-emergence maize (1 x 90 g a.s./ha) – mesotrione considering the EFSA default diet

Focal species	Food category, % in diet	FIR/bw	RUD_m (mg/kg food)	MAF_m × TWA	PT	DDD_m (mg/kg bw/d)	NOAEL (mg/kg bw/d)	TER_{lt}
Wood mouse Early post-emergence (BBCH 12-19) 90 g/ha	Plant material, 25 %	0.27	54.2[a]	1 x 0.055[d]	0.021 (mean)	0.000380		
	Insects, 25 %	0.27	7.5[b]	1 x 0.53		0.000507		
	Seeds, 50 %	0.27	40.2[c]	1 x 0.53		0.00544		
	whole diet					0.00633	0.3	47
							1.2	190
Wood mouse Early post-emergence (BBCH 12-19) 90 g/ha	Plant material, 25 %	0.27	54.2[a]	1 x 0.055[d]	0.043 (90th percentile)	0.000779		
	Insects, 25 %	0.27	7.5[b]	1 x 0.53		0.00104		
	Seeds, 50 %	0.27	40.2[c]	1 x 0.53		0.0111		
	whole diet					0.0129	0.3	23
							1.2	93
Wood mouse Early post-emergence (BBCH 12-19) 90 g/ha	Plant material, 25 %	0.27	54.2[a]	1 x 0.055[d]	0.043 (mean, consumers only)	0.000779		
	Insects, 25 %	0.27	7.5[b]	1 x 0.53		0.00104		
	Seeds, 50 %	0.27	40.2[c]	1 x 0.53		0.0111		
	whole diet					0.0129	0.3	23
							1.2	93
Wood mouse Early post-emergence (BBCH 12-19) 90 g/ha	Plant material, 25 %	0.27	54.2[a]	1 x 0.055[d]	0.077 (90th percentile, consumers only)	0.00139		
	Insects, 25 %	0.27	7.5[b]	1 x 0.53		0.00186		
	Seeds, 50 %	0.27	40.2[c]	1 x 0.53		0.0199		
	whole diet					0.0232	0.3	13
							1.2	52
Wood mouse Early post-emergence (BBCH 12-19) 90 g/ha	Plant material, 25 %	0.27	54.2[a]	1 x 0.055[d]	0.139 (worst-case PT)	0.00252		
	Insects, 25 %	0.27	7.5[b]	1 x 0.53		0.00336		
	Seeds, 50 %	0.27	40.2[c]	1 x 0.53		0.0360 0.0270[e]		
	whole diet					0.0419 0.0329	0.3	7.2 9.1[e]
							1.2	29

TER values in **bold** are less than the trigger of 5 indicating a potential risk which is considered in further refinements

[a] RUD for grasses/cereals as a surrogate for maize. This is conservative as the Tier 1 in maize scenario has wood mice consuming weeds (non-grass herbs with mean RUD of 28.7).

[b] RUD for ground-dwelling invertebrates without interception (Appendix F of the EFSA Guidance Document)

[c] RUD for seeds; EFSA default

[d] Ftwa from longest DT_{50} measured in European trials (19.2h)

[e] Assuming a DF of 0.75 due to interception by the maize crop at BBCH 11-19; required for the combi-tox assessment

An acceptable risk is indicated in all cases.

Table 9.3.2.2-15: Risk assessment for the wood mouse in early post-emergence maize (1 x 60 g a.s./ha) – mesotrione considering the Pelz (1989) diet

Focal species	Food category, % in diet	FIR/bw	RUD_m (mg/kg food)	MAF_m × TWA	PT	DDD_m (mg/kg bw/d)	NOAEL (mg/kg bw/d)	TER_lt
Wood mouse Early post-emergence (BBCH 12-19) 60 g/ha	Plant material, 16 %	0.36	54.2[a]	1 x 0.055[e]	0.021 (mean)	0.000216		
	Insects, 10 %	0.36	7.5[b]	1 x 0.53		0.000180		
	Soil invertebrates, 40 %	0.36	0.116[d]	[d]		0.000351		
	Seeds, 34 %	0.36	40.2[c]	1 x 0.53		0.00329		
	whole diet					0.00404	0.3	74
							1.2	300
Wood mouse Early post-emergence (BBCH 12-19) 60 g/ha	Plant material, 16 %	0.36	54.2[a]	1 x 0.055[e]	0.043 (90th percentile)	0.000443		
	Insects, 10 %	0.36	7.5[b]	1 x 0.53		0.000369		
	Soil invertebrates, 40 %	0.36	0.116[d]	[d]		0.000718		
	Seeds, 34 %	0.36	40.2[c]	1 x 0.53		0.00673		
	whole diet					0.00826	0.3	36
							1.2	150
Wood mouse Early post-emergence (BBCH 12-19) 60 g/ha	Plant material, 16 %	0.36	54.2[a]	1 x 0.055[e]	0.043 (mean, consumers only)	0.000443		
	Insects, 10 %	0.36	7.5[b]	1 x 0.53		0.000369		
	Soil invertebrates, 40 %	0.36	0.116[d]	[d]		0.000718		
	Seeds, 34%	0.36	40.2[c]	1 x 0.53		0.00673		
	whole diet					0.00826	0.3	36
							1.2	150
Wood mouse Early post-emergence (BBCH 12-19) 60 g/ha	Plant material,16 %	0.36	54.2[a]	1 x 0.055[e]	0.077 (90th percentile, consumers only)	0.000793		
	Insects, 10 %	0.36	7.5[b]	1 x 0.53		0.000661		
	Soil invertebrates, 40 %	0.36	0.116[d]	[d]		0.00129		
	Seeds, 34 %	0.36	40.2[c]	1 x 0.53		0.0120		
	whole diet					0.0147	0.3	20
							1.2	82
Wood mouse Early post-emergence (BBCH 12-19) 60 g/ha	Plant material,16 %	0.36	54.2[a]	1 x 0.055[e]	0.139 (worst-case PT)	0.00143		
	Insects, 10 %	0.36	7.5[b]	1 x 0.53		0.00119		
	Soil invertebrates, 40 %	0.36	0.116[d]	[d]		0.00232		
	Seeds, 34 %	0.36	40.2[c]	1 x 0.53		0.0217		
	whole diet					0.0266	0.3	11
							1.2	45

TER values in **bold** are less than the trigger of 5 indicating a potential risk

[a] RUD for grasses/cereals as a surrogate for maize. This is conservative as the Tier 1 in maize scenario has wood mice consuming weeds (non-grass herbs with mean RUD of 28.7).

[b] RUD for ground-dwelling invertebrates without interception (Appendix F of the EFSA Guidance Document)

[c] RUD for seeds; EFSA default

[d] Worst-case PEC_{worm} for the 90th percentile Koc value. Note this is not multiplied by the application rate since this was taken into account in the calculation of PEC_{soil}. Also, the Ftwa is also not applicable since the 21d TWA soil PEC is used to derive the PEC_{worm}.

[e] Ftwa from longest DT_{50} measured in European trials (19.2h)

Table 9.3.2.2-16: Risk assessment for the wood mouse in early post-emergence maize (1 x 67.5 g a.s./ha) – mesotrione considering the Pelz (1989) diet

Focal species	Food category, % in diet	FIR/bw	RUD_m (mg/kg food)	$MAF_m \times TWA$	PT	DDD_m (mg/kg bw/d)	NOAEL (mg/kg bw/d)	TER_{lt}
Wood mouse Early post-emergence (BBCH 12-19) 67.5 g/ha	Plant material, 16 %	0.36	54.2[a]	1 x 0.055[e]	0.021 (mean)	0.000243		
	Insects, 10 %	0.36	7.5[b]	1 x 0.53		0.000203		
	Soil invertebrates, 40 %	0.36	0.130[d]	[d]		0.000393		
	Seeds, 34 %	0.36	40.2[c]	1 x 0.53		0.00370		
	whole diet					0.00454	0.3	66
							1.2	260
Wood mouse Early post-emergence (BBCH 12-19) 67.5 g/ha	Plant material, 16 %	0.36	54.2[a]	1 x 0.055[e]	0.043 (90th percentile)	0.000498		
	Insects, 10 %	0.36	7.5[b]	1 x 0.53		0.000415		
	Soil invertebrates, 40 %	0.36	0.130[d]	[d]		0.000805		
	Seeds, 34 %	0.36	40.2[c]	1 x 0.53		0.00757		
	whole diet					0.00929	0.3	32
							1.2	130
Wood mouse Early post-emergence (BBCH 12-19) 67.5 g/ha	Plant material, 16 %	0.36	54.2[a]	1 x 0.055[e]	0.043 (mean, consumers only)	0.000498		
	Insects, 10 %	0.36	7.5[b]	1 x 0.53		0.000415		
	Soil invertebrates, 40 %	0.36	0.130[d]	[d]		0.000805		
	Seeds, 34%	0.36	40.2[c]	1 x 0.53		0.00757		
	whole diet					0.00929	0.3	32
							1.2	130
Wood mouse Early post-emergence (BBCH 12-19) 67.5 g/ha	Plant material,16 %	0.36	54.2[a]	1 x 0.055[e]	0.077 (90th percentile, consumers only)	0.000892		
	Insects, 10 %	0.36	7.5[b]	1 x 0.53		0.000744		
	Soil invertebrates, 40 %	0.36	0.130[d]	[d]		0.00144		
	Seeds, 34 %	0.36	40.2[c]	1 x 0.53		0.0136		
	whole diet					0.0167	0.3	18
							1.2	72
Wood mouse Early post-emergence (BBCH 12-19) 67.5 g/ha	Plant material,16 %	0.36	54.2[a]	1 x 0.055[e]	0.139 (worst-case PT)	0.00161		
	Insects, 10 %	0.36	7.5[b]	1 x 0.53		0.00134		
	Soil invertebrates, 40 %	0.36	0.130[d]	[d]		0.00260		
	Seeds, 34 %	0.36	40.2[c]	1 x 0.53		0.0245		
	whole diet					0.0301	0.3	10
							1.2	40

TER values in **bold** are less than the trigger of 5 indicating a potential risk

[a] RUD for grasses/cereals as a surrogate for maize. This is conservative as the Tier 1 in maize scenario has wood mice consuming weeds (non-grass herbs with mean RUD of 28.7).

[b] RUD for ground-dwelling invertebrates without interception (Appendix F of the EFSA Guidance Document)

[c] RUD for seeds; EFSA default

[d] Worst-case PEC_{worm} for the 90th percentile Koc value. Note this is not multiplied by the application rate since this was taken into account in the calculation of PEC_{soil}. Also, the Ftwa is also not applicable since the 21d TWA soil PEC is used to derive the PEC_{worm}.

[e] Ftwa from longest DT_{50} measured in European trials (19.2h)

Table 9.3.2.2-17: Risk assessment for the wood mouse in early post-emergence maize (1 x 90 g a.s./ha) – mesotrione considering the Pelz (1989) diet

Focal species	Food category, % in diet	FIR/bw	RUD_m (mg/kg food)	$MAF_m \times TWA$	PT	DDD_m (mg/kg bw/d)	NOAEL (mg/kg bw/d)	TER_{lt}
	Plant material, 16 %	0.36	54.2[a]	1 x 0.055[e]	0.021 (mean)	0.000325		
	Insects, 10 %	0.36	7.5[b]	1 x 0.53		0.000270		
	Soil invertebrates,	0.36	0.175[d]	[d]		0.000529		

Focal species	Food category, % in diet	FIR/bw	RUD_m (mg/kg food)	MAF_m x TWA	PT	DDD_m (mg/kg bw/d)	NOAEL (mg/kg bw/d)	TER_{lt}
Wood mouse	40 %							
	Seeds, 34 %	0.36	40.2[e]	1 x 0.53		0.00493		
Early post-emergence (BBCH 12-19) 90 g/ha	whole diet					0.00605	0.3	50
							1.2	200
Wood mouse	Plant material, 16 %	0.36	54.2[a]	1 x 0.055[e]	0.043 (90th percentile)	0.000665		
	Insects, 10 %	0.36	7.5[b]	1 x 0.53		0.000554		
Early post-emergence (BBCH 12-19) 90 g/ha	Soil invertebrates, 40 %	0.36	0.175[d]	[d]		0.00108		
	Seeds, 34 %	0.36	40.2[e]	1 x 0.53		0.0101		
	whole diet					0.0124	0.3	24
							1.2	97
Wood mouse	Plant material, 16 %	0.36	54.2[a]	1 x 0.055[e]	0.043 (mean, consumers only)	0.000665		
	Insects, 10 %	0.36	7.5[b]	1 x 0.53		0.000554		
Early post-emergence (BBCH 12-19) 90 g/ha	Soil invertebrates, 40 %	0.36	0.175[d]	[d]		0.00108		
	Seeds, 34%	0.36	40.2[e]	1 x 0.53		0.0101		
	whole diet					0.0124	0.3	24
							1.2	97
Wood mouse	Plant material,16 %	0.36	54.2[a]	1 x 0.055[e]	0.077 (90th percentile, consumers only)	0.00119		
	Insects, 10 %	0.36	7.5[b]	1 x 0.53		0.000992		
Early post-emergence (BBCH 12-19) 90 g/ha	Soil invertebrates, 40 %	0.36	0.175[d]	[d]		0.00194		
	Seeds, 34 %	0.36	40.2[e]	1 x 0.53		0.0181		
	whole diet					0.0222	0.3	14
							1.2	54
Wood mouse	Plant material,16 %	0.36	54.2[a]	1 x 0.055[e]	0.139 (worst-case PT)	0.00215		
	Insects, 10 %	0.36	7.5[b]	1 x 0.53		0.00179		
Early post-emergence (BBCH 12-19) 90 g/ha	Soil invertebrates, 40 %	0.36	0.175[d]	[d]		0.00350		
	Seeds, 34 %	0.36	40.2[e]	1 x 0.53		0.0326 0.0245[f]		
	whole diet					0.0400 0.0319[f]	0.3	7.5 9.4[f]
							1.2	30

TER values in **bold** are less than the trigger of 5 indicating a potential risk

[a] RUD for grasses/cereals as a surrogate for maize. This is conservative as the Tier 1 in maize scenario has wood mice consuming weeds (non-grass herbs with mean RUD of 28.7). Furthermore the herbicidal activity of the product applied means that few if any weeds will be present in the field and therefore it is more realistic to consider maize as the relevant dietary item for long-term risk assessment.

[b] RUD for ground-dwelling invertebrates without interception (Appendix F of the EFSA Guidance Document)

[c] RUD for seeds; EFSA default

[d] Worst-case PEC_{worm} for the 90th percentile Koc value. Note this is not multiplied by the application rate since this was taken into account in the calculation of PEC_{soil}. Also, the Ftwa is also not applicable since the 21d TWA soil PEC is used to derive the PEC_{worm}.

[e] Ftwa from longest DT_{50} measured in European trials (19.2h)

[f] Assuming a DF of 0.75 due to interception by the maize crop at BBCH 11-19; required for the combi-tox assessment

TERs are above the long-term trigger value of 5 indicating acceptable long-term risk to omnivorous mammals when considering PT refinements.

~~In practice the risk to wood mice with seeds in the diet is likely to be further reduced by dehusking of seeds. Bruehl *et al* (2010[10]) have investigated dehusking behaviour in wild-caught wood mice consuming wheat, barley, maize and sunflower and showed that for all seeds at least 50% of residues were removed by dehusking by wood mice. Dehusking of maize seeds was also observed in the studies with wild wood mice by FERA (2010[11]), and also by Prescott (2004) summarised below. Such dehusking behaviour is likely to also occur with weed seeds and hence the potential long-term risk to wood mice will be further reduced.~~

zRMS comments:

Since not all refinement options proposed by the Applicant were agreed by the zRMS and correction of multiple parameters in tables above would make them not transparent, it was decided by the zRMS to struck through all calculations performed by the Applicant and re-calculate the risk assessment with consideration of the agreed input parameters. Respective evaluation is presented in table below. Only the target rate of 60 g a.s./ha (corresponding with the intended product rate at 400 g/ha) was considered. Calculations were based on unrounded values.

Refined risk assessment for wood mouse

Intended use		**Maize, 1 × 0.4 kg product/ha**						
Active substance/product		mesotrione						
Application rate (g a.s./ha)		1 × 60						
Reprod. toxicity (mg/kg bw/d)		0.3						
TER criterion		5						
Crop scenario Growth stage	**Generic focal species**	**PD/diet type**	**FIR/bw**	**RUD_m**	**MAF_m × TWA**	**PT**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
BBCH 10 -29	Wood mouse	0.25 (maize)	0.27	54.2	1.0 × 0.055	0.139	0.002	
		0.5 (seeds)		40.2	1.0 × 0.53		0.024	
		0.25 (arthropods)		3.5 [1)]	1.0 × 0.53		0.001	
						Sum of DDD_m	0.027	11.2

[1)] according to Appendix A of EFSA (2009) RUD values for arthropods with interception are relevant for scenario maize at BBCH 10-29

The risk assessment based on refined parameters agreed by the zRMS demonstrated acceptable risk to the wood mouse following application of A18032E according to the intended use pattern (maize at BBCH 12-18, at 1 x 0.4 kg product/ha).

The Applicant is kindly reminded that in case the risk envelope approach is not considered, only calculations performed for the target rate(s) should be presented in the report, since calculations for rates not indicated in GAP table will not be validated and make the report less transparent.

[10] ~~Bruehl et al. (2010) Exposure reduction of seed treatments through dehusking behaviour of the wood mouse (*Apodemus sylvaticus*). Environ Sci Pollut Res. DOI 10.1007/s11356-010-0351-x.~~

[11] ~~Anonymous (2010) Dehusking of seed by small mammals – default values for use in risk assessment. Available at: http://randd.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&ProjectID=16264&FromSearch=Y&Publisher=1&SearchText=ps2349&SortString=ProjectCode&SortOrder=Asc&Paging=10#Description~~

Hare

This section considers the attractiveness and use of post-emergence maize fields to Brown hares in Europe. It considers information from a variety of sources including the open literature plus industry generic mammalian radio-tracking and observational field studies. Relevant data and information from these sources are summarised below and combined to generate an overall statement on the use of post-emergence maize field by hares for foraging.

i. Review paper on hare use of habitat in Europe (Smith et al, 2005)

The use of crops by Brown hares in Europe was reviewed by Smith et al. (2005[12]). This paper summarised the results of 77 research papers from twelve European countries. The findings for maize have been extracted and summarised below. Comparative findings from this review paper for wheat/cereals are also summarised below. This will help to show whether PT data for hares in cereals can be considered as representative of usage of maize crops.

Table 9.3.2.2-18: Preference for maize crops by hares (extracted and summarised from Smith et al, 2005)

Country	Preference for crop	Method	Reference
Germany	Neutral	Spotlight counts	Pegel (1986)
	-	Spotlight counts	Späth (1989)
	Neutral	Spotlight counts	Petrak (1990)
	-	*Not known*	Schäfers (1986
	+	Hunting bag	Nyenhuis (1999)
Hungary	-	*Not known*	Bertoti (1975)
Italy	-	Clearance netting	Meriggi & Alieri (1989)
	Neutral	Spotlight counts, belt assessment	Prigioni & Pelizza (1992)
UK	Neutral	Relative abundance as perceived by farmers	Vaughan et al (2003)

Table 9.3.2.2-19: Preference for wheat and cereals crops by hares (extracted and summarised from Smith et al, 2005)

Country	Preference for crop	Method	Reference
Austria	Neutral	Spotlight counts	Hackländer et al (2001)
France	+	Clearance counts	Pepin (1985,1987)
	+	Radiotracking	Marboutin & Aebischer (1996)
Germany	+	Hunting bag	Schröpfer & Nyenhuis (1999)
	Neutral	Spotlight counts	Pegel (1986)
	-	Spotlight counts	Späth (1989)
	Neutral	Spotlight counts	Petrak (1990)
	+	Spotlight counts	Ahrens et al (1995)
	-*	*Not known*	Schäfers (1986)
	+	Hunting bag	Nyenhuis (1999)
	+	Spotlight counts	Kilias & Ackermann (2001)
Hungary	+	Hunting bags	Szederjei (1959)
Italy	Neutral	Clearance netting	Meriggi & Alieri (1989)
	+	Spotlight counts, belt assessment	Prigioni & Pelizza (1992)
Sweden	+	Spotlight counts	Frylestam (1979, 1980)
	+	Spotlight counts	Frylestam (1992)
UK	+	Relative abundance as perceived by farmers	Vaughan et al (2003)

*indicates summer only

Table 9.3.2.2-20: Summary of hare crop preference for wheat/cereals and maize (extracted and summarised from Smith et al, 2005)

	+	Neutral	-
Wheat/cereals	11	3	2
Maize	1	4	4

[12] Smith RK, Vaughan Jennings N & Harris S. 2005a. A quantitative analysis of the abundance and demography of European hares *Lepus europaeus* in relation to habitat type, intensity of agriculture and climate. Mammal Review 35 (1) pp 1-24

~~It is clear from the summary above, the majority of studies showed a positive association of wheat/cereals with hare abundance whilst maize shows either a neutral or negative association with abundance. This also reflects the conclusions as set out in Smith et al (2005).~~

zRMS comments:

The paper by Smith et al. (2005) was not provided by the Applicant for review and for this reason validation of presented above results was not possible.

Nevertheless, it is not fully clear how findings of Smith et al. (2005) would overcome results of the field monitoring studies on the basis of which the brown hare was selected as the relevant focal species representative for herbivorous feeding guild.

The information from the study by Smith et al. (2005) was struck through as being not validated and not relevant for the risk assessment.

ii. Use of early growth stage maize fields by hares (PT)

The potential risk to hares can be investigated by considering the proportion of diet obtained from the treated area (PT).

Generic field monitoring of hares in a mixed landscape in Germany (Voigt & Zaccaroni, 2013)

An industry study was conducted in which 16 hares were radio-tracked in an agricultural landscape during the spring in Germany (Voigt & Zaccaroni 2013) to specifically investigate hares in maize. An OECD study summary is provided in Appendix I.
The landscape was composed mainly of agricultural crops, which occupied around 83% of the total study area of 1,125 ha. The landscape composition changed throughout the study period, according to the growth of the crops. Over the study period, a maximum of 43.6% of the fields were cereals, up to 25.6% was sugar beet and up to 16.5% was maize.

Table 9.3.2.2-21: $PT_{foraging}$ inside post-emergent maize

	$PT_{foraging}$	Week	Date
Hare # 3	2.1	20	16 May
Hare # 6	-	-	-
Hare # 7	-	-	-
Hare # 11	-	-	-
Hare # 13	-	-	-
Hare # 15	-	-	-
Hare # 16	-	-	-
Hare # 18	-	-	-
Hare # 23	-	-	-
Hare # 25	-	-	-
Hare # 28	-	-	-
Hare # 29	-	-	-
Hare # 30	3.8	18	4 May
Hare # 30	20.8	20	14 May

The highest individual PT was 20.8% at BBCH 10-19 and this could be considered as a worst-case PT for hare use of maize.

In an alternative approach to study the preference of the hare for different crop types, data from total surface of the study area were estimated, and the availability of the crop types was measured to calculate the Jacobs preference index D (Jacobs, 1974[13]). These calculations are presented in a separate addendum (Voigt & Zaccaroni 2015) to the original report as detailed in the OECD summary in Appendix I. The Jacobs index results are presented below.

[13]Jacobs J (1974) Quantitative measurement of food selection Oecologia, 14 (4), 413-417.

Table 9.3.2.2-22: Monthly Jacobs index (D) related to the BBCH-stages of each crop type.

Dates (from-to)		**19/03-15/04**	**16/04-13/05**	**14/05-10/06**	**11/06-02/07**
Month (Weeks)		**3 (12-15)**	**4 (16-19)**	**5 (20-23)**	**6 (24-27)**
Cereals	BBCH	3-27	9-33	31-69	55-87
	D	0,56	0,71	0,73	-0,78
Sugar beet	BBCH	Up to 9	5-16	14-38	19 and above
	D	-0,31	-0,43	-0,43	0,86
Maize	BBCH	--	0-12	13-18	15-33
	D	--	-0,91	-0,94	-1,00
Oilseed rape	BBCH	19-55	10-67	51-80	67-80
	D	0,02	-1,00	-1,00	-1,00
Others		-0,34	-0,10	-0,22	-0,68
Bare soil		-0,56	-1,00	-1,00	--
Off-crop		-0,34	-0,49	-0,46	-0,08
Number of hares		**12**	**6**	**5**	**2**

The Jacobs Indices show a strong negative value, indicating avoidance, for maize (D = -0.91 to -1.0) and a positive preference for cereals up to early June (D = +0.56 up to +0.73). These findings are in close agreement with the review of Smith *et al.* (2005) summarised above.

zRMS comments:

The study by Voigt & Zaccaroni (2015) was evaluated by the zRMS and is considered unreliable. Taking this into account, its results were not used in the risk refinement. For details, please refer to Appendix 2.

Please note that the same approach has been taken by PL as zRMS and agreed by cMS in the course of evaluation of two mesotrione formulations (Callisto and Calaris) belonging to the mesotrione data owner (Syngenta).

Generic field monitoring of hares in a mixed arable landscape in Germany and Hungary (Grimm & Katzschner, 2019)

A further monitoring study has been carried out to investigate the use of maize fields by Brown hares in five sites in Germany and Hungary specifically chosen to be representative of high density maize-growing regions in central Europe (Grimm & Katzchner 2019). An OECD study summary is provided in Appendix I. Hares were individually radio-tracked over 24 hours. For each telemetry session the proportion of active time of an individual hare in maize (PT) was calculated as the proportion of time the hare spend 'potentially foraging' in that crop, thus the 'time potentially foraging' is the sum of the time periods covered by behavioural categories when foraging could not be excluded. A mean PT value (plus standard deviation of the mean and 90%ile values) was calculated based on all single PT values. Radio-tracking sessions of 21 individual hares at five study sites were performed during the early crop development of maize from late April until early June 2018 and covered the maize BBCH growth stages <20. Twenty-three 24h telemetry sessions were carried out (17 in Germany, six in Hungary), since two individuals were radio tracked twice. One session had to be excluded from analysis, as this session was a no 'consumer session' according to EFSA (2009), i.e. the animal was never located being active (i.e. showed behaviour categorized as potentially foraging) in a maize field during the session or at least had maize in the 24h home range.
The landscape was a mixed rural landscape, the majority covered in agricultural crops, which occupied around 60% of the total study area, with the remaining being 13% forest/hedges; 22% meadow, and 5 % 'other'. 36.2% of the habitat was made up of maize, which is considered a high proportion and therefore worst-case for European arable landscapes.
The calculated individual daily PT values ranged from 0.02 to 0.94. Calculated PT values did not differ substantially between different study sites; mean values were slightly higher in Germany. Also PT values between sexes did not differ substantially even though mean values were slightly higher in male hares (0.43) than in female hares (0.28). Regarding BBCH growth stages, no clear trend was detectable, although hares tended to spend slightly more active time inside maize fields at BBCH growth stages 15 or higher. In tracking sessions in which maize fields in later stages (i.e. BBCH ≥15) were part of the

home range, the mean PT value was 0.39, whereas the mean PT value of sessions including only fields in early stages (i.e. BBCH <15) within the home range was 0.35.

Table 9.3.2.2-23: Calculated PT values of hares in maize fields in early BBCH growth stages (BBCH growth stage <20) in Central Europe

Country	Session ID	PT	Date (dd.mm.yyyy)
Germany	398_GER_01	0.56	18.05.2018
	398_GER_02	0.40	19.05.2018
	398_GER_03	0.17	20.05.2018
	398_GER_04	~~0.08~~	23.05.2018
	398_GER_05	0.50	22.05.2018
	398_GER_06	~~0.09~~	23.05.2018
	398_GER_07	~~0.08~~	22.05.2018
	398_GER_08	~~0.02~~	24.05.2018
	398_GER_09	0.44	25.05.2018
	398_GER_10	0.41	26.05.2018
	398_GER_12	0.18	27.05.2018
	398_GER_13	0.89	28.05.2018
	398_GER_14	0.37	30.05.2018
	398_GER_15	0.26	01.06.2018
	398_GER_16	0.94	04.06.2018
	398_GER_17	0.63	05.06.2018
Hungary	398_HU_01	0.42	29.04.2018
	398_HU_02	0.38	30.04.2018
	398_HU_03	0.56	02.05.2018
	398_HU_04	0.28	13.05.2018
	398_HU_05	0.21	14.05.2018
	398_HU_06	~~0.02~~	15.05.2018
Mean		**0.447** ~~0.36~~	-
90th percentile		**0.734** ~~0.62~~	-

zRMS comments:

The study by Grimm & Katzschner (2019) on monitoring brown hares in two Central Zone countries (Germany and Hungary) was evaluated and agreed by the zRMS. PT values for 21 individuals were >, but for 5 were <0.1. In line with current Polish national requirements in area of environmental risk assessment, individuals with PT values <0.1 are not considered as crop consumers. Nevertheless, when these 5 individuals are excluded, reliable PT values are available for 17 individuals and since the number is >10, the 90th percentile PT value may be used for purposes of the risk refinement. After exclusion of the PT values <0.1 the 90th percentile PT of 0.734 could be calculated. For details of the study evaluation, please refer to Appendix 2.

Radio-tracking of hares in Germany (Späth, 1989)

Radio-tracking data was collected by Späth (1989)[14] in southern Germany. The hares were radiotracked and time spent potentially foraging was determined for several crops, including maize. These data can therefore provide appropriate information for a realistic estimate of PT for the European hare, in early growth stages in maize, i.e. post-emergence. Since herbicide treatment is restricted BBCH<19, this accords to a time period from about mid-May to mid-July in southern Germany, and therefore only the telemetry results from May to July will be taken into account here. The home range sizes of 9 individual hares were determined via radio-tracking and land use / cropping pattern within these areas was assigned. The table below summarizes the percentage of maize fields within the home ranges of radio-tracked hares (N = 9).

[14] Späth V (1989) Untersuchungen zur Populationsökologie des Feldhasen (*Lepus europaeus* Pallas) in der Oberrheinebene. PhD thesis, University of Freiburg, Freiburg im Breisgau

Table 9.3.2.2-24: Percentage of maize in the home range of the hare in southern Germany

Animal No	Time period	Percentage maize in home range [%]
1	May-July	51
2		2
3		6
4		6
5		4
6		24
7		12
8		35
9		7
Mean (n=9)		**16.3**
90th percentile		**38**

In order to use these data on percentage of maize in the home range as PT data i.e. to indicate the proportion of time spent foraging in maize, we need to factor in the relative attractiveness of maize to hares. We know from evidence already presented above that maize is not an attractive crop for hares and in general we can expect this crop to be used for foraging either less than expected based on occurrence in landscape (-ve Jacobs Index) or, as a most conservative assumption, in proportion to its occurrence in the landscape (Jacobs Index of 0 or neutral association in point 1 above). Therefore, taking the most conservative assumption, that maize will be used for foraging in proportion to its percentage area in the home range of hares, then it would be reasonable to consider a 90th percentile PT of 0.38 as being conservative for hares use of maize.
Please note that the Späth (1989) study has been previously reviewed and accepted in the Central Zone (by Ctgb in the Netherlands). They state '*The hares were radiotracked and time spent potentially foraging was determined for several crops, including maize. However, the 90-percentile PT should be used, especially since the number of hare are not very high. The 90-percentile in maize is 0.38.*'

zRMS comments:

The study by Späth (1989) was already evaluated by PL as the zRMS in the course of evaluation of two mesotrione formulations (Callisto and Calaris) belonging to the mesotrione data owner (Syngenta) and considered not reliable since the PT was calculated on the basis of percentage maize in the home range of brown hare, which is not relevant measure for derivation of PT values. Taking this into account, results of the study by Späth (1989) are not reliable to be used for purposes of the risk refinement for mesotrione in A18032E.

These studies can be put together in order to derive an estimate of the mean and 90th percentile PT values for post-emergent maize:

Table 9.3.2.2-25: Derivation of PT in post-emergence maize for the hare

Author	Animal No / session ID	Time period	PT [%]
~~Späth (1989)~~	~~1~~	~~May-July~~	~~51~~
	~~2~~	~~May-July~~	~~2~~
	~~3~~	~~May-July~~	~~6~~
	~~4~~	~~May-July~~	~~6~~
	~~5~~	~~May-July~~	~~4~~
	~~6~~	~~May-July~~	~~24~~
	~~7~~	~~May-July~~	~~12~~
	~~8~~	~~May-July~~	~~35~~
	~~9~~	~~May-July~~	~~7~~
~~Voigt & Zaccaroni (2013)~~	~~3~~	~~9-26th May~~	~~2.1~~
	~~30~~	~~1-22nd May~~	~~3.8~~
	~~30~~	~~14th May-7th June~~	~~20.8~~
Grimm & Katzschner (2019)	398_GER_01	18.05.2018	56
	398_GER_02	19.05.2018	40

	398_GER_03	20.05.2018	17
	398_GER_04	23.05.2018	8
	398_GER_05	22.05.2018	50
	398_GER_06	23.05.2018	9
	398_GER_07	22.05.2018	8
	398_GER_08	24.05.2018	2
	398_GER_09	25.05.2018	44
	398_GER_10	26.05.2018	41
	398_GER_12	27.05.2018	18
	398_GER_13	28.05.2018	89
	398_GER_14	30.05.2018	37
	398_GER_15	01.06.2018	26
	398_GER_16	04.06.2018	94
	398_GER_17	05.06.2018	63
	398_HU_01	29.04.2018	42
	398_HU_02	30.04.2018	38
	398_HU_03	02.05.2018	56
	398_HU_04	13.05.2018	28
	398_HU_05	14.05.2018	21
	398_HU_06	15.05.2018	2
~~Consumers only~~	~~N=34 tracking sessions~~ ~~N= 31 individuals~~	~~mean~~	~~28.6~~
		~~90th percentile~~	~~56.0~~

zRMS comments:

From studies listed in Table 9.3.2.2-25, only study by Grimm & Katzschner (2019) was considered reliable by the zRMS. The 90th percentile of 0.734 based on this study results is considered relevant for purposes of refinement of the risk for mesotrione in A18032E.

Please note that the same approach has been taken by PL as zRMS and agreed by cMS in the course of evaluation of two mesotrione formulations (Callisto and Calaris) belonging to the mesotrione data owner (Syngenta). However, evaluation for these two formulations was carried out before it was decided that at the Polish national level that individuals with PT <0.1 are not considered to be actual crop consumers and should be rejected from calculations.

iii. Hare diet

The default risk assessment assumes that a hare spends all of its time feeding in treated maize fields and feeds exclusively on maize shoots. This is a highly conservative assumption as is justified below.
Zörner (1989[15]) summarises data from a study by Onderscheka *et al.* (1981) looking at hare diet from analysis of botanical composition of stomach contents. This study looked at stomach contents of 366 hares in 8 regions of Austria as shown in the table below. This study of hare diet showed that across 366 individuals, the average percentage stomach content of maize ranged from 0 – 14.2%.

[15] Zörner, H. (1989): *Lepus europaeus* (Pallas), 286-321. In: Stubbe, M. Buch der Hege Band 1 Haarwild. Deutscher Landwirtschaftsverlag, Berlin.

Table 9.3.2.2-26: Botanic composition of the stomach contents of brown hares (annual average in %) in different areas of Austria

Number of stomachs	**16**	**33**	**32**	**16**	**57**	**70**	**84**	**58**
Territory	**Wiener Neustadt**	**Theresienfeld**	**Weikersdorf**	**Sollenau**	**Zurndorf-Süd**	**Zurndorf-Nord**	**Schrems**	**Meires**
Cultivated plants								
Barley	7.3	11.8	7.6	13.1	6.1	5.2	2.7	10.4
Wheat	2.4	-	1.1	0.0	16.2	15.5	0.0	8.4
Oats	1.9	2.0	3.1	1.4	0.2	0.0	3.2	8.9
Rye	17.1	21.5	19.0	18.3	0.6	3.3	23.7	11.4
Maize	11.6	14.2	11.5	13.6	4.8	9.2	0.0	0.0
Beet	4.9	-	9.1	5.7	-	3.7	0.0	0.0
Alfalfa	5.1	3.7	6.1	2.8	1.0	0.2	-	-
Canola	-	0.0	0.0	0.0	2.3	-	1.0	0.0
Soybeans	-	-	-	-	1.2	0.3	-	-
Red clover	-	-	-	-	-	-	12.5	16.6
Potatoes	-	-	-	-	-	-	0.1	0.1
Total	50.3	53.2	57.5	54.9	32.4	37.4	43.2	55.8
Non-cultivated plants								
Grasses	11.8	13.8	10.0	9.9	21.6	26.0	39.7	29.1
Faboidea	11.3	6.0	9.4	7.8	1.5	0.3	2.0	4.3
Asteraceae	2.7	4.5	1.8	5.6	4.0	3.2	5.4	4.2
Brassicaceae	2.3	4.2	1.8	0.4	1.2	0.0	0.0	0.2
Plantain	2.4	2.1	3.7	5.0	2.2	1.7	0.7	0.0
Other occurring sporadically	7.4	8.7	6.3	5.3	16.9	13.3	4.5	3.1
Total	37.9	39.3	33.0	34.0	47.4	44.5	52.3	40.9
Supplemental food								
Cabbage					1.2	2.6		
Carrots					5.1	1.5		
Indeteminable	10.5	5.5	5.1	8.1	13.6	12.5	3.8	2.5
Animal hair	1.3	2.0	4.4	3.0	0.3	1.5	0.7	0.8

Another study of hare diet was conducted by Reichlin *et al.* (2006[16]) in Austria. The study area comprised 2,820 ha dominated by arable land with the main crops being wheat, sugar beet, sunflower, maize and potato. Food use by hares was investigated by analysis of stomach contents in February, May, August and November 2003. In May, the time most relevant to mesotrione use, the plants most commonly found in hares stomachs (N=28) were: wheat (50%), barley (13%), soya (11%), clover (8%), sugar beet (5%) and poppy (3%) with others being recorded at <3%. Maize was found, but at <0.1%. In August (n=32), the plants most commonly found in hares stomachs were: lucerne (39%), barley (25%), sugar beet (14%) and *Artemisia vulgaris* (4%) with maize present at 2.3% and other foods being recorded at <3%.

A third study of hare diet has been reported by Chapuis (1990[17]). This study was based in France on a 2000 ha study area consisting predominantly of winter wheat (40-50%), maize (30%), peas (10%) and oilseed rape (up to 10%). Diet was determined by analysis of faeces samples collected in the study area. The maximum consumption of maize recorded was 40% in June 1983 and 30% in August 1984.

An estimate of the brown hare diet has also been published in the Northern Zone Guidance for bird and mammal risk assessment[18]. This is based on published data from studies carried out in Sweden (Frylestam 1980a), England (Tapper and Barnes 1986), France (Chapuis 1990) and Denmark (Olesen & Asferg 2006; Hansen 1990); please refer to the Northern Zone Guidance for full references. It is

[16] Reichlin T, Klansek E, Hackländer K (2006) Diet selection by hares (*Lepus europaeus*) in arable land and its implications for habitat management. Eur J Wildl Res 52 109-118. DOI 10.1007/s10344-005-0013-3

[17] Chapuis JL (1990) Comarison of the diets of two sympatric lagomorphs, *Lepus europeaus* (Pallas) and *Oryctolagus cuniculus* (L) in agroecosystems of the Ile-de-France. Z. Saugetierkunde 55 176-185

[18] Northern Zone 2015. PESTICIDE RISK ASSESSMENT FOR BIRDS AND MAMMALS. Selection of relevant species and development of standard scenarios for higher tier risk assessment in the Northern Zone in accordance with Regulation EC 1107/2009. Version 1.6, March 2018.

therefore considered that this dietary estimate is adequate for considering the potential mixed diet for hares in treated maize fields, however it should be noted that the herbicidal activity of the product applied means that any weeds will not form part of a long-term diet, since full weed control is expected to occur rapidly (please refer to the section on biological efficacy).

Table 9.3.2.2-27: Estimated diet composition of brown hares feeding in different crops from the Northern Zone Guidance. Spring: April-May; Summer: June-September

Crop	Growth stage	Season	PD (fresh weight)		
			Monocotyledons (cereals, grasses)	Dicotyledons (leafy crops, non-grass weeds)	Bush berry plants (buds, leaves)
Maize	BBCH 10-19	Spring	0.84	0.16	-
	BBCH 10-39	Summer	0.72	0.28	-

Since A18032E is only proposed for maize in BBCH 11-19 which occurs mainly in May in Central Europe, the diet for 'spring' is considered most applicable and only those calculations will be presented in the risk assessments below.

zRMS comments:

The brown hare diet was refined by the Applicant in Alvarez (2019) on the basis of results of several publications, however neither was submitted in support of this evaluation and for this reason their reliability and relevance for intended uses of A18032E in maize could not be validated by the zRMS.

In addition to that it was pointed out by the Applicant that the brown hare diet relevant for maize is indicated in the Northern Zone Guidance Document. The derived PD values presented in Table 9.3.2.2-27 were based on published data from studies carried out in Sweden (Frylestam 1980a), England (Tapper and Barnes 1986), France (Chapuis 1990) and Denmark (Olesen & Asferg 2006; Hansen 1990).

In opinion of the zRMS, the PD values derived for spring may be also used for purposes of the evaluation performed in the Central Zone, as they were based on results of the studies performed in Member States of all zones, including Central Zone. Furthermore, all publications were already assessed, validated and agreed by the ecotoxicology experts of the Northern Zone and there is no reason to challenge derived conclusions.

Moreover, in opinion of the zRMS it is highly unlikely that the brown hare would feed exclusively on maize shoots, which is confirmed by data obtained by Zörner (1989), presented in Table 9.3.2.2-26. Zörner (1989) summarised data from a study by Onderscheka et al. (1981) that was investigating hare diet from analysis of botanical composition of stomach contents. This study looked at stomach contents of 366 hares in 8 regions of Austria. From the table 9.3.2.2-24 it is evident that brown hare do feed on dicotyledonous plants, although monocots represent the major part of their diet. It may be also seen that maize is definitely not the preferred food of the brown hare.
Please note that the publication was not submitted by the Applicant so could not be fully validated by the zRMS, however results summarised above are presented as supportive information that the brown hare feed on dicotyledonous plants and that the diet composition as proposed by the Northern Zone Guidance Document is relevant.

Taking all this into account the zRMS is of the opinion that PD values of 0.84 and 0.16 for monocots and dicots, respectively, as proposed by the Northern Zone Guidance Document are sufficiently supported by the available data and may be used also in evaluation performed for Poland.

Please note that the same approach has been taken by PL as zRMS and agreed by cMS in the course of evaluation of two mesotrione formulations (Callisto and Calaris) belonging to the mesotrione data owner (Syngenta).

iv. Hare bodyweight

The body weight for a brown hare is 3.8 kg according to Appendix A of the EFSA Bird and Mammal Guidance Document (EFSA 2009) and this is accepted by most MS in the Central Zone. The Northern Zone Guidance gives a mean body weight of brown hares in Sweden of 4.2 kg. However, these values are generic and not season-specific, and mainly derived from hare hunt data which tends to occur in winter, when hares are expected to be lighter due to lower food availability. In the PT study for brown hares by Grimm & Katzschner (2019), individual hares were weighed as part of the study. Eleven males and 9 females were investigated; 3 hares were not weighed in order to minimize stress to those individuals. The remaining results were as follows:

	Bodyweight (kg)	**Sex**
	3.8	male
	3.5	male
	4.3	female
	4.6	female
	4.1	male
	4.8	female
	4.6	male
	3.8	male
	3.9	female
	3.7	male
	4.6	female
	3.6	female
	4.6	female
	3.4	male
	4.6	male
	3.6	female
	4.3	female
	4.5	male
	4.1	male
	4.2	male
Average	4.13	

Thus females were marginally larger than males (4.3 kg versus 4.03 kg), and both were heavier than the default value of 3.8 kg used in the EFSA guidance (source not referenced). The measured values for bodyweight from the PT monitoring study are considered to be more reliable than the defaults considered above, as they are representative for both the crop and time of year of the proposed uses. The bodyweight of 4.13 kg is therefore considered suitable to refine the risk assessments presented below, but the default of 3.8 kg will be initially addressed for conservatism in the assessments below.

zRMS comments:

Although in EFSA (2009) it is not indicated on the basis of what data the bodyweight of brown hare of 3800 g was determined, in opinion of the zRMS the study with data for 20 individuals originating from one test site are not sufficient to rule out indications of the guidance document. It should be also noted that in the Mammal Bible brown hare mean bodyweight of 3230 and 3430 g is reported for male and female, respectively. This is considerable less than bodyweight obtained in the study by Grimm & Katzschner (2019). Taking this into account, the FIR/bw should be calculated for the commonly agreed bodyweight of 3800 g.

v. Realistic diet for calculation of food intake rates for the hare

The bodyweight for the hare reported in Appendix A of the EFSA Guidance document is 3800 g; the FIR/bw can then be calculated using the equation provided in Appendix G of the Guidance document using:

$$FIR = \left(\frac{DEE}{FE * \left(1 - \frac{MC}{100}\right) * \left(\frac{AE}{100}\right)} \right) \quad \text{[g fresh weight/d]}$$

In which

$$\log DEE = \log a + b \times \log bw$$

Table 9.3.2.2-28: Calculation of food intake rate for the hare considering a single item diet in post-emergence maize

Food item	Hare bodyweight	DEE	FIR	FIR/bw
Maize shoots (grasses/ cereals)	3800g	2363	1210.7	0.32 [a]
~~Non-grass herbs~~	~~3800g~~	~~2363~~	~~1468.1~~	~~0.39~~ [b]
~~Maize shoots (grasses/ cereals)~~	~~4130g~~	~~2508.4~~	~~1284.94~~	~~0.31~~
~~Non-grass herbs~~	~~4130g~~	~~2508.4~~	~~1558.2~~	~~0.38~~

[a] This is the same as the FIR/bw of 0.32 given for the herbivorous brown hare *Lepus europaeus* 3800 g bodyweight, feeding on 100% grass in the Grassland scenario in Appendix A of the EFSA Guidance document.
[b] This is the same as the FIR/bw of 0.39 given for the herbivorous brown hare *Lepus europaeus* 3800 g bodyweight, feeding on 100% plant matter (non-grass herbs) in the Vineyard scenario in Appendix A of the EFSA Guidance document.

zRMS comments:

As already indicated above, the zRMS is of the opinion that the available data are not sufficient to overrule the generic brown hare bodyweight of 3800 g and FIR/bw should be calculated with the commonly agreed parameter. It is further noted that the diet consisting exclusively of non-grass herbs was neither discussed nor agreed in point related to the brown hare diet. The available data clearly indicate that monocotyledons are the main component of the brown hare diet with only some addition of dicotyledons. Taking this into account, diet consisting exclusively of dicotyledons is highly unlikely and should not be considered in the risk refinement.

Not agreed data are struck through in Table 9.3.2.2-28.

FIR/bw for mixed diet:

Considering the fractions (PDi) of individual food items in a mixed diet, together with data on their respective moisture and energy content, the specific energy content of a mixed diet can be calculated as recommended by the EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009). This value is used to estimate the required amount of the mixed diet to satisfy the daily energy expenditure of a hare. Based on each appropriate bodyweight and calculations according to Appendix G of the EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009), the FIR for mixed diet of hares is presented in the table below. All the terms used below are as presented in Appendix G.

$$FE_{total,fresh} = \sum_i \left[PD_{i,fresh} \times FE_i \times \left(1 - \frac{MC_i}{100}\right) \times \frac{AE_i}{100} \right]$$

$$FIR_{total,fresh} = \frac{DEE}{FE_{total,fresh}}$$

Table 9.3.2.2-29: Calculation of food intake rate for the 3800g hare considering a mixed diet in post-emergence maize

Maize		April-May	
		Maize shoots [f]	Non-grass herbs
Fraction of food item in mixed diet [a]	PD_i fresh (%)	84%	16%
Food energy of food item [i] in mixed diet [b]	FE (kJ/dry g)	17.6	17.8
Moisture content of food item [i] in mixed diet [b]	MC (%)	76.4	88.1
Assimilation efficiency of food item [i] in mixed diet [c]	AE (%)	47	76
Food energy of food item in diet [d]	FE_{item},fresh (kJ/g fresh weight)	1.640	0.258
Food energy of total mixed diet [d]	FE_{total},fresh (kJ/g fresh weight)	1.897	
Daily energy expenditure [d]	DEE (kJ/day)	2363.4	
Food intake rate of total mixed diet [d]	FIR_{total}, fresh (g fresh weight/day)	1245.61	
b.w. [e]	(g)	3800	
FIR/b.w.	(g fresh weight/b.w./day)	0.328	

[a] PD for the hare according to published papers, as discussed above
[b] From Table 3 of Appendix G in EFSA (2009)
[c] From Table 4 of Appendix G in EFSA (2009)
[d] Calculated according to EFSA (2009; Appendix G)
[e] Body weight of brown hare from either the EFSA Guidance (3800g)
[f] Monocot plant material is assumed to be = maize shoots (using the default values for grasses and cereal shoots)

~~**Table 9.3.2.2-30: Calculation of food intake rate for the 4130g hare considering a mixed diet in post-emergence maize**~~

~~Maize~~		~~April-May~~	
		~~Maize shoots~~ [f]	~~Non-grass herbs~~
~~Fraction of food item in mixed diet~~ [a]	~~PD_i fresh (%)~~	~~84%~~	~~16%~~
~~Food energy of food item [i] in mixed diet~~ [b]	~~FE (kJ/dry g)~~	~~17.6~~	~~17.8~~
~~Moisture content of food item [i] in mixed diet~~ [b]	~~MC (%)~~	~~76.4~~	~~88.1~~
~~Assimilation efficiency of food item [i] in mixed diet~~ [c]	~~AE (%)~~	~~47~~	~~76~~
~~Food energy of food item in diet~~ [d]	~~FE_{item},fresh (kJ/g fresh weight)~~	~~1.659~~	~~0.258~~
~~Food energy of total mixed diet~~ [d]	~~FE_{total},fresh (kJ/g fresh weight)~~	~~1.917~~	
~~Daily energy expenditure~~ [d]	~~DEE (kJ/day)~~	~~2508.4~~	
~~Food intake rate of total mixed diet~~ [d]	~~FIR_{total}, fresh (g fresh weight/day)~~	~~1308.57~~	
~~b.w.~~ [e]	~~(g)~~	~~4130~~	
~~FIR/b.w.~~	~~(g fresh weight/b.w./day)~~	~~0.317~~	

~~[a] PD for the hare according to published papers, as discussed above~~
~~[b] From Table 3 of Appendix G in EFSA (2009)~~
~~[c] From Table 4 of Appendix G in EFSA (2009)~~
~~[d] Calculated according to EFSA (2009; Appendix G)~~
~~[e] Body weight of brown hare from either the EFSA Guidance (3800g)~~
~~[f] Monocot plant material is assumed to be = maize shoots (using the default values for grasses and cereal shoots)~~

zRMS comments:

As already indicated above, the zRMS is of the opinion that the available data are not sufficient to overrule the generic brown hare bodyweight of 3800 g and FIR/bw should be calculated with the commonly agreed parameter. Taking this into account, calculations presented in Table 9.3.2.2-30 above are struck through.

Calculations for bodyweight of 3800 g presented in Table 9.3.2.2-29 are agreed by the zRMS.

vi. Deposition factor

An interception factor can be applied for exposure from non-grass herbs food material, as this food item will be weeds on the ground surface which are partially shaded by the crop at growth stage 10-19 in accordance with FOCUS gw version 2.2 (May 2014). Therefore a deposition factor (DF) of 0.75 can be reasonably applied for non-grass herbs under maize at BBCH 10-19.

zRMS comments:

Consideration of the deposition factor proposed by the Applicant is not agreed by the zRMS since according to EFSA (2009) \the deposition factor is relevant for maize at principal growth stages ≥3.

vii. Risk assessment for the hare

~~The hare will be initially assumed to feed on maize shoots or cereal shoots in a similar way to the "large herbivorous lagomorphs" Tier 1 scenario in cereals. T~~
~~The risk assessment calculations presented below consider a range of possible refinements of PT and residue dissipation.~~

~~**Mesotrione**~~

~~**Table 9.3.2.2-31: Risk assessment for the 3800g hare in early post-emergence maize – mesotrione (1 x 60 g a.s./ha)**~~

Focal species	Food category, % in diet	FIR/bw	RUD_m (mg/kg food)	MAF_m x TWA	Deposition factor	PT	DDD_m (mg/kg bw/d)	NOAEL (mg/kg bw/d)	TER_lt
Hare post-emergence BBCH 12-18 1 x 60 g/ha	Monocots, 100%	0.319	54.2[a]	1 x 0.055[b]	1	28.55% (mean all data)	0.0163	0.3	18
								1.2	73
						56% (90th percentile; all data)	0.0320	0.3	9.4
								1.2	38
	Non-grass herbs 100%	0.39	28.7[c]	1 x 0.14[d]	0.75	28.55% (mean all data)	0.0202	0.3	15
								1.2	74
						56% (90th percentile; all data)	0.0395	0.3	7.6
								1.2	30

TER values in **bold** are less than the trigger of 5 indicating a potential risk
[a] Default RUD for grasses/cereals
[b] Ftwa from longest DT_{50} measured in European trials (19.2h)
[c] Default RUD for non-grass herbs as given in Appendix A of the Guidance Document
[d] Ftwa considering a geomean DT_{50} = 2.05 days for non-grass herbs

Table 9.3.2.2-32: Risk assessment for the 3800g hare in early post-emergence maize - mesotrione (1 x 60 g a.s./ha) considering a mixed diet

Focal species	Food category, % in diet	FIR/bw	RUD_m (mg/kg food)	MAF_m × TWA	DF	PT	DDD_m (mg/kg bw/d)	DDD_{sum} (mg/kg bw/d)	NOAEL (mg/kg bw/d)	TER_{lt}
Hare post-emergence BBCH 12-18 1 x 60 g/ha	Maize, 84%	0.328	54.2[a]	1 x 0.055[b]	1	28.55% (mean all data)	0.0141	0.0168	0.3	18
	Non-grass weeds 16%		28.7[c]	1 x 0.14[d]	0.75		0.00271		1.2	72
	Maize, 84%	0.328	54.2[a]	1 x 0.055[b]	1	56% (90th percentile; all data)	0.0276	0.0329	0.3	9.1
	Non-grass weeds 16%		28.7[c]	1 x 0.14[d]	0.75		0.00531		1.2	36

TER values in **bold** are less than the trigger of 5 indicating a potential risk
[a] Default RUD for grasses/cereals
[b] Ftwa from longest DT_{50} measured in European trials (19.2h)
[c] Default RUD for non-grass herbs as given in Appendix A of the Guidance Document
[d] Ftwa considering a geomean DT_{50} = 2.05 days for non-grass herbs

Table 9.3.2.2-33: Risk assessment for the 3800g hare in early post-emergence maize - mesotrione (1 x 67.5 g a.s./ha)

Focal species	Food category, % in diet	FIR/bw	RUD_m (mg/kg food)	MAF_m × TWA	Deposition factor	PT	DDD_m (mg/kg bw/d)	NOAEL (mg/kg bw/d)	TER_{lt}
Hare post-emergence BBCH 12-18 1 x 67.5 g/ha	Monocots, 100%	0.319	54.2[a]	1 x 0.055[b]	1	28.55% (mean all data)	0.0183	0.3	16
								1.2	65
						56% (90th percentile; all data)	0.0359	0.3	8.3
								1.2	33
	Non-grass herbs 100%	0.39	28.7[c]	1 x 0.14[d]	0.75	28.55% (mean all data)	0.0227	0.3	13
								1.2	52
						56% (90th percentile; all data)	0.0444	0.3	6.8
								1.2	27

TER values in **bold** are less than the trigger of 5 indicating a potential risk
[a] Default RUD for grasses/cereals
[b] Ftwa from longest DT_{50} measured in European trials (19.2h)
[c] Default RUD for non-grass herbs as given in Appendix A of the Guidance Document
[d] Ftwa considering a geomean DT_{50} = 2.05 days for non-grass herbs

Table 9.3.2.2-34: Risk assessment for the 3800g hare in early post-emergence maize - mesotrione (1 x 67.5 g a.s./ha) considering a mixed diet

Focal species	Food category, % in diet	FIR/bw	RUD_m (mg/kg food)	MAF_m × TWA	DF	PT	DDD_m (mg/kg bw/d)	DDD_{sum} (mg/kg bw/d)	NOAEL (mg/kg bw/d)	TER_{lt}
Hare post-emergence BBCH 12-18 1 x 67.5 g/ha	Maize, 84%	0.328	54.2[a]	1 x 0.055[b]	1	28.55% (mean all data)	0.0158	0.0189	0.3	16
	Non-grass weeds 16%		28.7[c]	1 x 0.14[d]	0.75		0.00305		1.2	63
	Maize, 84%	0.328	54.2[a]	1 x 0.055[b]	1	56% (90th percentile; all data)	0.0310	0.0370	0.3	8.1
	Non-grass weeds 16%		28.7[c]	1 x 0.14[d]	0.75		0.00598		1.2	32

TER values in **bold** are less than the trigger of 5 indicating a potential risk
[a] Default RUD for grasses/cereals
[b] Ftwa from longest DT_{50} measured in European trials (19.2h)
[c] Default RUD for non-grass herbs as given in Appendix A of the Guidance Document
[d] Ftwa considering a geomean DT_{50} = 2.05 days for non-grass herbs

Table 9.3.2.2-35: Risk assessment for the 3800g hare in early post-emergence maize - mesotrione (1 x 90 g a.s./ha)

Focal species	Food category, % in diet	FIR/bw	RUD_m (mg/kg food)	MAF_m × TWA	Deposition factor	PT	DDD_m (mg/kg bw/d)	NOAEL (mg/kg bw/d)	TER_{lt}
Hare post-emergence BBCH 12-18 1 x 90 g/ha	Monocots, 100%	0.319	54.2[a]	1 x 0.055[b]	1	28.55% (mean all data)	0.0244	0.3	12
								1.2	49
						56% (90th percentile; all data)	0.0479	0.3	6.3
								1.2	25
	Non-grass herbs 100%	0.39	28.7[c]	1 x 0.14[d]	0.75	28.55% (mean all data)	0.0303	0.3	9.9
								1.2	39
						56% (90th percentile; all data)	0.0592	0.3	5.1
								1.2	20

TER values in **bold** are less than the trigger of 5 indicating a potential risk
[a] Default RUD for grasses/cereals
[b] Ftwa from longest DT_{50} measured in European trials (19.2h)
[c] Default RUD for non-grass herbs as given in Appendix A of the Guidance Document
[d] Ftwa considering a geomean DT_{50} = 2.05 days for non-grass herbs

~~**Table 9.3.2.2-36: Risk assessment for the 3800g hare in early post-emergence maize - mesotrione (1 x 90 g a.s./ha) considering a mixed diet**~~

~~Focal species~~	~~**Food category, % in diet**~~	~~**FIR/bw**~~	~~**RUD_m (mg/kg food)**~~	~~**MAF_m × TWA**~~	~~**DF**~~	~~**PT**~~	~~**DDD_m (mg/kg bw/d)**~~	~~**DDD_{sum} (mg/kg bw/d)**~~	~~**NOAEL (mg/kg bw/d)**~~	~~**TER_{lt}**~~
~~Hare post-emergence BBCH 12-18 1 x 90 g/ha~~	~~Maize, 84%~~	~~0.328~~	~~54.2[a]~~	~~1 x 0.055[b]~~	~~1~~	~~28.55% (mean all data)~~	~~0.0211~~	~~0.0252~~	~~0.3~~	~~12~~
	~~Non-grass weeds 16%~~		~~28.7[c]~~	~~1 x 0.14[d]~~	~~0.75~~		~~0.00406~~		~~1.2~~	~~48~~
	~~Maize, 84%~~	~~0.328~~	~~54.2[a]~~	~~1 x 0.055[b]~~	~~1~~	~~56% (90th percentile; all data)~~	~~0.0414~~	~~0.0494~~	~~0.3~~	~~6.1~~
	~~Non-grass weeds 16%~~		~~28.7[c]~~	~~1 x 0.14[d]~~	~~0.75~~		~~0.00797~~		~~1.2~~	~~24~~

~~TER values in **bold** are less than the trigger of 5 indicating a potential risk~~
~~[a] Default RUD for grasses/cereals~~
~~[b] Ftwa from longest DT_{50} measured in European trials (19.2h)~~
~~[c] Default RUD for non-grass herbs as given in Appendix A of the Guidance Document~~
~~[d] Ftwa considering a geomean DT_{50} = 2.05 days for non-grass herbs~~

~~An acceptable risk is indicated in all cases, and furthermore this estimate is unrealistically worst-case as indicated by the weight of evidence assessment presented below.~~

Table 9.3.2.2-37: Weight of evidence and uncertainty in the hare refined risk assessment for mesotrione

Refinement	Source of uncertainty	Discussion and Conclusion regarding uncertainty	Effect on conservativeness
NOEL derived from 2-generation study with the rat	Extrapolation from rat to the focal species lagomorph	The rat is known to be particularly sensitive to mesotrione; tests on rabbits, also lagomorphs, indicate lower sensitivity. Hares are more likely to be similar to rabbits than to rats	+
NOEL	Use of lowest NOEL in the risk assessment	The NOEL is based on effects in the 2nd generation of continual exposure, but use of mesotrione is limited to a very short time period and it dissipates quickly	+++
~~NOEAEL~~	~~Use of F1 NOEAEL~~	~~The NOEAEL is based on results from the F1 generation of continuous exposure. This is still conservative as exposure is longer than expected from mesotrione's dissipation patterns and short application window~~	~~++~~
Refined DT_{50} for maize	Early maize was used to estimate the DT_{50} Longest DT_{50} is used	Maize at an appropriate representative BBCH was used to measure the DT_{50} The longest DT_{50} from 5 trials was used instead of a geomean	+/- ++
Mixed diet including weeds	Oversprayed weeds were included in diet	Oversprayed weeds have been included in the hare diet. This is unlikely to occur over chronic time periods due to the senescence caused by the herbicidal activity. Instead exposure through feeding on maize will occur; this has higher residues, but faster dissipation, so overall this is considered neither over- nor under-conservative.	+/-

Refinement	Source of uncertainty	Discussion and Conclusion regarding uncertainty	Effect on conservativeness
Refined DT_{50} for monocot weeds	Early maize was used to estimate the DT_{50} for monocot weeds	Monocot weeds may be part of the diet of hares, and the DT_{50} from maize has been used to estimate the dissipation in them. However the activity of the herbicide means that sprayed monocot weeds will senesce quickly within the field, and therefore the monocot portion of the diet is more likely to be maize itself in-field. Overall this is considered neither over- nor under-conservative.	+/-
Refined DT_{50} for dicots	Geomean DT_{50} was used	The use of the geomean DT_{50} is considered appropriate as 10 trials were available. This is considered appropriately realistic.	+/-
90th percentile daily PT	It has been assumed that each hare is exposed to the 90th percentile PT every day of the exposure period	The PT value used is a daily PT based on 24h of monitoring, thus it is assumed that the 90th percentile PT is spent every day of the entire potential exposure period. Taken augmentatively, use of a daily 90th percentile PT will result in an overall 99th percentile PT over a 21day period. Over a 21-day averaging period hares will use different parts of their home range as indicated by variations in the PT of individual hares monitored twice (e.g. Grimm & Katzschner 2019), and so the overall PT for long-term exposure is expected to be lower. ~~The mean PT is expected to provide a more realistic estimate of exposure. Experience with modelling a 90th percentile 21 day PT has shown that this tended to be approximately the same as the mean daily PT (Ludwigs *et al.*, 2017[19])~~	+++
Bodyweight	Default bw = 3800g	The assessment has used the default bw of 3800g. Data for hares from the PT study (Grimm & Katzschner 2019) indicate that hares at the appropriate time of year and crop for the proposed uses will be heavier (mean = 4130g)	+
Final Conclusion	The uncertainty analysis above indicates a high likelihood that the true "worst-case" risk is lower than indicated by the current risk assessment. For 6 of the 12 factors considered above, there is believed to be no likelihood of true worst-case risk being higher and for these factors the true risk is considered to be 2 to 10 times lower in each case. This is driven by the use of a conservative 90th percentile daily PT (2 times higher than the mean) and selection of an unrealistically conservative reproduction endpoint (NOEL is 4 x lower than the NOEAEL), so multiplicatively these two factors have an impact of making the assessment 8 times more conservative. It is clear that a relaxation of worst-case nature of the risk assessment in one or two of these six areas would be enough to indicate an acceptable risk assessment for hares for all uses.		

zRMS comments:

Since not all refinement options proposed by the Applicant were agreed by the zRMS and correction of multiple parameters in tables above would make them not transparent, it was decided by the zRMS to struck through all calculations performed by the Applicant and re-calculate the risk assessment with consideration of the agreed input parameters. Respective evaluation is presented in table below. Only the target rate of 60 g a.s./ha

[19] ~~Ludwigs, J.D et al (2017). Appropriate exposure estimates for wildlife risk assessments of crop protection products based on continuous radio telemetry: a case study with woodpigeons. Environmental Toxicology and Chemistry, Vol. 36, No. 5, pp. 1270–1277.~~

(corresponding with the intended product rate at 400 g/ha) was considered. Calculations were based on unrounded values.

Refined risk assessment for brown hare

Intended use		**Maize, 1 × 0.4 kg product/ha**						
Active substance/product		mesotrione						
Application rate (g a.s./ha)		1 × 60						
Reprod. toxicity (mg/kg bw/d)		0.3						
TER criterion		5						
Crop scenario Growth stage	**Focal species**	**PD/diet type**	**FIR/bw**	**RUD_m**	**MAF_m × TWA**	**PT**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
BBCH 10 -29	Brown hare	0.84 (maize)	0.328	54.2	1.0 × 0.055	0.734	0.036	
		0.16 (dicot weeds)		28.7	1.0 × 0.150		0.010	
						Sum of DDD_m	0.046	6.5
BBCH 10 -29	Brown hare	1.0 (maize)	0.32	54.2	1.0 × 0.055	0.734	0.042	7.1

The risk assessment based on refined parameters agreed by the zRMS demonstrated acceptable risk to the brown hare from both types of the diet following application of A18032E according to the intended use pattern (maize at BBCH 12-18, at 1 x 0.4 kg product/ha).

The uncertainty analysis provided in Table 9.3.2.2-37 above is in general agreed by the zRMS with exception of some parameters that were struck through as being not agreed in the course of the evaluation.

The Applicant is kindly reminded that in case the risk envelope approach is not considered, only calculations performed for the target rate(s) should be presented in the report, since calculations for rates not indicated in GAP table will not be validated and make the report less transparent.

Overall conclusion

Taking all the above arguments into account (refined ecotoxicologically-relevant endpoints, focal species, residue dissipation, PT) it is considered that the recommended uses will pose an acceptable long-term risk to mammals.

zRMS comments:

The risk assessment based on refined parameters agreed by the zRMS demonstrated acceptable risk to both focal species (wood mouse and brown) exposed to mesotrione applied as A18032E.

viii. Refined TER values for combi-tox assessments for the relevant focal species

~~For assessment of chronic effects, according to the EFSA guidance, if a given formulation contains several active substances all known to cause similar effects via a similar biochemical mechanism (e.g. aromatase inhibition) and if this type of effects is actually driving the risk assessment, it is recommended to perform an assessment for combined effects on a case by case basis.~~

~~For A18032E the active ingredients, mesotrione (HPPD inhibitor) has a different mode of action in plants than the active ingredient nicosulfuron (ALS/AHAS inhibitor) which are both different from the active ingredient dicamba (synthetic auxin), and their toxicity profiles in mammals are very different as demonstrated in Section 6. The mammalian toxicity of mesotrione is well understood, with the principal feature of the toxicology being low NOAEL values in the rat (particularly male), due to the induction of tyrosinemia which leads to reversible effects including reduced bodyweight, increased liver and kidney weights and ocular lesions as corneal opacity. The NOAEL is based on effects on litter size and pup survival. The NOAEL for nicosulfuron showed no significant adverse effects on reproductive performance at the top dose level (EFSA Conclusion 2007). For dicamba the NOAEL is based on~~

decreased body weight gain in adults and does' aborting effects in the rabbit teratology study. Whilst a common mode of action is highly unlikely for these different active ingredients, since the effects seen include reduced bodyweight for mesotrione and dicamba, an assessment for combined effects shall be carried out in order to be conservative. Consequently an assessment for combined effects will be conducted and is based on a concentration addition approach. However, please note that the toxicity is clearly driven by the mesotrione content.

In case of concentration addition each substance contributes to the total toxicity of a mixture in proportion to its concentration using the following equation:

$TER_{combi} = trigger / ((trigger_{<mesotrione>}/TER_{<mesotrione>}) + (trigger_{<active substance 2>}/TER_{<active substance 2>}))$ etc.

An acceptable risk is expected when $TER_{combi} > trigger$.

In this formula, 'triggers' are the EU triggers.

Table 9.3.2.2-38: Higher tier assessment of the long-term/reproductive risk for mammals due to the maximum use of A18032E in maize: combination risk assessment

Intended use	Maize					
Application rate (g/ha)	187.5 g/ha dicamba 90 g/ha mesotrione 60 g/ha nicosulfuron					
Trigger$_{combi}$	5					
TER criterion	5					
GAP Crop scenario	**Focal species**	**Refinement**	**TER**$_{dicamba}$	**TER** $_{mesotrione}$	**TER** $_{nicosulfuron}$	**TER**$_{combi}$
Post-emergence BBCH 12-19	Wood mouse *Apodemus sylvaticus*	PT = 0.077 Mixed diet (Pelz)	21 (screening level)	14[a] 54[b]	1700 (screening level)	8.4[a] 15[b]
Post-emergence BBCH 12-19	Wood mouse *Apodemus sylvaticus*	PT = 0.139 Mixed diet (Pelz)	21(screening level)	7.5[a] or 9.4[c] 30[b]	1700 (screening level)	5.5 or 6.5[a] 12[b]
Post-emergence BBCH 12-19	Hare *Lepus europaeus*	PT = 0.2855, mixed diet	21(screening level)	12[a] 48[b]	1700 (screening level)	7.6[a] 14[b]
		PT = 0.56, mixed diet	21(screening level)	6.1[a] 24[b]	1700 (screening level)	**4.7**[a] 11[b]

PT: proportion of diet obtained from treated area; TER: toxicity-to-exposure ratio. TER values shown in bold fall below the relevant trigger.
[a] Considering the conservative endpoint for mesotrione of 0.3 mg/kg bw/d
[b] Considering the ecologically relevant endpoint for mesotrione of 1.2 mg/kg bw/d

An acceptable risk is indicated in all cases, except for the combi-tox for the brown hare considering the worst-case NOEL in combination with the worst-case PT value. However, this is extremely worst-case, as an acceptable risk is expected from the individual actives and additive effects are not expected due to the different modes of action. Furthermore, this is based on the screening level TER values for dicamba and nicosulfuron, and these can be refined for the focal species as follows:

Table 9.3.2.2-39: Risk assessment for the 3800g hare in early post-emergence maize - dicamba (1 x 187.5 g a.s./ha) and nicosulfuron (1 x 60g a.s./ha) considering a mixed diet

Focal species	Food category, % in diet	FIR/bw	RUD_m (mg/kg food)	$MAF_m \times TWA$	DF	PT	DDD_m (mg/kg bw/d)	DDD_{sum} (mg/kg bw/d)	NOEL (mg/kg bw/d)	TER_{lt}
Hare post-emergence BBCH 12-18 1 x 187.5 g dicamba/ha	Maize, 84%	0.328	54.2[a]	1 x 0.53[c]	1	56% (90th percentile; all data)	0.831	0.894	150	168
	Non-grass weeds 16%		28.7[c]	1 x 0.53[c]	1		0.0629			
Hare post-emergence BBCH 12-18 1 x 60 g nicosulfuron/ha	Maize, 84%	0.328	54.2[a]	1 x 0.53[c]	1	56% (90th percentile; all data)	0.266	0.286	3861	13500
	Non-grass weeds 16%		28.7[c]	1 x 0.53[c]	1		0.0201			

TER values in **bold** are less than the trigger of 5 indicating a potential risk
[a] Default RUD for grasses/cereals
[b] Default Ftwa
[c] Default RUD for non-grass herbs as given in Appendix A of the Guidance Document

Table 9.3.2.2-40: Higher tier assessment of the long-term/reproductive risk for mammals due to the maximum use of A18032E in maize: combination risk assessment

Intended use	Maize					
Application rate (g/ha)	187.5 g/ha dicamba 90 g/ha mesotrione 60 g/ha nicosulfuron					
$Trigger_{combi}$	5					
TER criterion	5					
GAP Crop scenario	Focal species	Refinement	$TER_{dicamba}$	$TER_{mesotrione}$	$TER_{nicosulfuron}$	TER_{combi}
Post-emergence BBCH 12-19	Hare *Lepus europaeus*	PT = 0.56, mixed diet	168 (refined)	6.1[a]	13500 (refined)	5.9[a]

PT: proportion of diet obtained from treated area; TER: toxicity-to-exposure ratio. TER values shown in bold fall below the relevant trigger.
[a] Considering the conservative endpoint for mesotrione of 0.3 mg/kg bw/d

An acceptable risk is indicated by combi-TER values considering the refined TER values.

Utilising the above data, risk assessment parameters and rationale in the context of this application, the below risk assessments are therefore presented:

Mesotrione

Table 9.3.2.2-41: Higher-tier assessment of the long-term/reproductive risk for mammals due to the use of mesotrione in A18032E in maize early post-emergence – refined parameters (*) are further described and justified in the text above

Intended use		Maize, post-emergence BBCH 12-19						
Active substance **Application rate (g/ha)**		Mesotrione 1 × 60						
Reprod. toxicity (mg/kg bw/d) **TER criterion**		0.3 (Worst-case NOEL) 1.2 (refined ecologically relevant NOAEL) 5						
Focal species	**Food category, % in diet**	**FIR/bw**	**RUD_m × DF (mg/kg food)**	**MAF_m × TWA**	**PT**	**DDD_m (mg/kg bw/d)**	**NO(A)EL (mg/kg bw/d)**	**TER_{lt}**
Wood mouse *Apodemus sylvaticus**	Plant material, 25%	0.27*	54.2 × 1	1 × 0.055*	0.021 0.139*	0.000254 0.00168		
	Insects, 25%		7.5 × 1	1 × 0.53		0.000338 0.00224		
	Seeds, 50%		40.2 × 1	1 × 0.53		0.00362 0.0240		
	whole diet					0.00421 0.0279	0.3 1.2	11-71 43-290
Hare *Lepus europaeus**	Maize, 84%*	0.328*	54.2 x 1	1 x 0.055*	0.286 0.56*	0.0141 0.0276		
	Non-grass weeds 16%		28.7 x 0.75*	1 x 0.14*		0.0027 0.0053		
	Whole diet					0.0168 0.0329	0.3 1.2	9.1-18 36-72

FIR/bw: Food intake rate per body weight; RUD: residue unit dose; DF: deposition factor (considering possible interception by the crop); MAF: multiple application factor; TWA: time-weighted average factor; PT: proportion of diet obtained from treated area; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Table 9.3.2.2-42: Higher-tier assessment of the long-term/reproductive risk for mammals due to the use of mesotrione in A18032E in maize early post-emergence – refined parameters (*) are further described and justified in the text above

Intended use		Maize, post-emergence BBCH 12-19						
Active substance **Application rate (g/ha)**		Mesotrione 1 × 67.5						
Reprod. toxicity (mg/kg bw/d) **TER criterion**		0.3 (Worst-case NOEL) 1.2 (refined ecologically relevant NOAEL) 5						
Focal species	**Food category, % in diet**	**FIR/bw**	**RUD_m × DF (mg/kg food)**	**MAF_m × TWA**	**PT**	**DDD_m (mg/kg bw/d)**	**NO(A)EL (mg/kg bw/d)**	**TER_{lt}**
Wood mouse *Apodemus sylvaticus**	Plant material, 25%	0.27*	54.2 × 1	1 × 0.055*	0.021 0.139*	0.000285 0.00189		
	Insects, 25%		7.5 × 1	1 × 0.53		0.000380 0.00252		
	Seeds, 50%		40.2 × 1	1 × 0.53		0.00408 0.0270		
	whole diet					0.00475 0.0314	0.3 1.2	9.6-63 38-250
Hare *Lepus europaeus**	Maize, 84%*	0.328*	54.2 x 1	1 x 0.055*	0.286 0.56*	0.0158 0.0310		
	Non-grass weeds 16%		28.7 x 0.75*	1 x 0.14*		0.002305 0.00598		
	Whole diet					0.0189 0.0370	0.3 1.2	8.1-16 32-63

FIR/bw: Food intake rate per body weight; RUD: residue unit dose; DF: deposition factor (considering possible interception by the crop); MAF: multiple application factor; TWA: time-weighted average factor; PT: proportion of diet obtained from treated area; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Table 9.3.2.2-43: Higher-tier assessment of the long-term/reproductive risk for mammals due to the use of mesotrione in A18032E in maize early post-emergence – refined parameters (*) are further described and justified in the text above

Intended use		Maize, post-emergence BBCH 12-19						
Active substance **Application rate (g/ha)**		Mesotrione 1 × 90						
Reprod. toxicity (mg/kg bw/d) **TER criterion**		0.3 (Worst-case NOEL) 1.2 (refined ecologically relevant NOAEL) 5						
Focal species	**Food category, % in diet**	**FIR/bw**	**RUD_m × DF (mg/kg food)**	**MAF_m × TWA**	**PT**	**DDD_m (mg/kg bw/d)**	**NO(A)EL (mg/kg bw/d)**	**TER_lt**
Wood mouse *Apodemus sylvaticus**	Plant material, 25%	0.27*	54.2 × 1	1 × 0.055*	0.021 0.139*	0.000380 0.00252		
	Insects, 25%		7.5 × 1	1 × 0.53		0.000507 0.00336		
	Seeds, 50%		40.2 × 1	1 × 0.53		0.00544 0.0360		
	whole diet					0.00633 0.0419	0.3 1.2	7.2-47 29-190
Hare *Lepus europaeus**	Maize, 84%*	0.329*	54.2 x 1	1 x 0.055*	0.286 0.56*	0.0211 0.0414		
	Non-grass weeds 16%		28.7 x 0.75*	1 x 0.14*		0.00406 0.00797		
	Whole diet					0.0252 0.0494	0.3 1.2	6.1-12 24-48

FIR/bw: Food intake rate per body weight; RUD: residue unit dose; DF: deposition factor (considering possible interception by the crop); MAF: multiple application factor; TWA: time-weighted average factor; PT: proportion of diet obtained from treated area; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

A18032E - dicamba/mesotrione/nicosulfuron mixture - Chronic risk

The EFSA Guidance Document (2009) states that if one active substance can be identified where the two quotients "tox per fraction (a.s.)" and "tox per fraction (mix)" deviate by ≤10%, this indicates that this active substance contributes to more than 90% to mixture toxicity. The other component(s) of the mixture then only have a marginal impact on the predicted risk. The tox per fractions were calculated as given in Appendix B of the EFSA Guidance Document and the results are given in the table below.

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

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

Whilst this approach is recommended for consideration of acute toxicity, it can be used to also provide an estimate for chronic toxicity, although this is not mentioned in the Guidance – in fact the guidance states that chronic mixture toxicity should only be considered when it is clear that the different active substances cause similar effects by a similar biochemical mechanism – which is not the case for mesotrione, nicosulfuron and dicamba, as discussed above. However, for conservatism, the tox per fraction approach is considered below:

Table 9.3.2.2-44: Calculation of tox per fraction quotients

Active substance	Chronic NOEC (mg a.s./kg bw)	X (a.s.)	NOEC/ X (a.s.) (mg a.s./kg bw)	NOEC (mix)	NOEC (mix) /∑X (a.s.)	% Deviation tox per fraction (a.s.) to NOEC (mix) [a]
Dicamba	150	0.556	270	1.12	1.12	The deviation is = 270-1.12 = 268.88 Then % deviation = 24000%
Mesotrione	0.3	0.267	1.125		1.12	The deviation is = 1.125-1.12 = 0.00467 Then % deviation = 0.42%
Nicosulfuron	3861	0.178	21718		1.12	The deviation is = 21718-1.12 = 21717 Then % deviation = 1938443%

[a] Please note that these "tox per fraction" quotients themselves have no biological meaning; they are only to be used for comparison

The "tox per fraction" and "tox per fraction (mix)" deviate by ≤ 10 % for mesotrione. This means that a chronic risk assessment for the mixture does not need to be performed as the toxicity is clearly driven by the mesotrione content.

However, for completeness an assessment for combined effects will be conducted and is based on a concentration addition approach. In case of concentration addition each substance contributes to the total toxicity of a mixture in proportion to its concentration using the following equation:

$TER_{combi} = trigger / ((trigger_{<mesotrione>}/TER_{<mesotrione>}) + (trigger_{<active\ substance\ 2>}/TER_{<active\ substance\ 2>}))$ etc.

An acceptable risk is expected when $TER_{combi} > trigger$.

Table 9.3.2.2-45: Higher tier assessment of the long-term/reproductive risk for mammals due to the maximum use of A18032E in maize: combination risk assessment as above

Intended use	Maize					
Application rate (g/ha)	187.5 g/ha dicamba 90 g/ha mesotrione 60 g/ha nicosulfuron					
$Trigger_{combi}$	5					
TER criterion	5					
GAP Crop scenario	Focal species	Refinement	$TER_{dicamba}$	TER mesotrione	TER nicosulfuron	TER_{combi}
Post-emergence BBCH 12-19	Wood mouse *Apodemus sylvaticus*	PT = 0.077 Mixed – diet (Pelz)	21 (screening level)	14[a] 54[b]	1700 (screening level)	8.4[a] 15[b]
Post-emergence BBCH 12-19	Wood mouse *Apodemus sylvaticus*	PT = 0.139 Mixed – diet (Pelz)	21(screening level)	7.5[a] or 9.4[c] 30[b]	1700 (screening level)	5.5 or 6.5[a] 12[b]
Post-emergence BBCH 12-19	Hare *Lepus europaeus*	PT = 0.2855, mixed – diet (Alvarez)	21(screening level)	12[a] 48[b]	1700 (screening level)	7.6[a] 14[b]
		PT = 0.56, mixed – diet (Alvarez)	168 (refined) 21(screening level)	6.1[a] 24[b]	13500 (refined) 1700 (screening level)	5.9[a] 11[b]

PT: proportion of diet obtained from treated area; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

[a] Considering the conservative endpoint for mesotrione of 0.3 mg/kg bw/d

[b] Considering the ecologically relevant endpoint for mesotrione of 1.2 mg/kg bw/d

~~Considering the maximum application rate of 0.6 kg A18032E/ha all TER_{combi} values exceed the trigger of 5 indicating acceptable risk when considering PT refinement.~~

zRMS comments:

The combined chronic risk assessment provided by the Applicant above was not validated by the zRMS since it is difficult to follow: some calculations are based on the exaggerated rate corresponding to 0.6 kg product/ha, some are based on the target rate of A18032E in Poland, range of TER values is presented for particular compounds and the mixture and at some point results of the screening step of the evaluation for dicamba and nicosulfuron are considered. Furthermore, part of calculations was based on refined parameters that were not agreed by the zRMS. Taking this into account, the combined chronic risk has been performed by the zRMS with consideration of the agreed refined parameters and only for the target rate. Please note that all calculations were based on unrounded values.

Since in the course of the risk refinement it was concluded that two focal species are relevant for maize at BBCH 12-18 (wood mouse and brown hare), the combined risk assessment should be focused on these two species. Taking this into account, the respective TER values for dicamba and nicosulfuron were calculated below with consideration of the non-substance specific refined parameters (i.e. PD, PT, FIR/bw). Results are presented below.

Dicamba

Intended use		**Maize, 1 × 0.4 kg product/ha**						
Active substance/product		dicamba						
Application rate (g a.s./ha)		1 × 125						
Reprod. toxicity (mg/kg bw/d)		150						
TER criterion		5						
Crop scenario Growth stage	**Generic focal species**	**PD/diet type**	**FIR/bw**	**RUD_m**	**MAF_m × TWA**	**PT**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
BBCH 10 -29	Wood mouse	0.25 (maize)	0.27	54.2	1.0 × 0.53	0.139	0.034	
		0.5 (seeds)		40.2	1.0 × 0.53		0.050	
		0.25 (arthropods)		3.5 [1)]	1.0 × 0.53		0.002	
						Sum of DDD_m	0.086	1747
BBCH 10 -29	Brown hare	0.84 (maize)	0.328	54.2	1.0 × 0.53	0.734	0.726	
		0.16 (dicot weeds)		28.7	1.0 × 0.53		0.073	
						Sum of DDD_m	0.799	188
BBCH 10 -29	Brown hare	1.0 (maize)	0.32	54.2	1.0 × 0.53	0.734	0.843	178

[1)] according to Appendix A of EFSA (2009) RUD values for arthropods with interception are relevant for scenario maize at BBCH 10-29

Nicosulfuron

Intended use		**Maize, 1 × 0.4 kg product/ha**						
Active substance/product		nicosulfuron						
Application rate (g a.s./ha)		1 × 40						
Reprod. toxicity (mg/kg bw/d)		3861						
TER criterion		5						
Crop scenario Growth stage	**Generic focal species**	**PD/diet type**	**FIR/bw**	**RUD_m**	**MAF_m × TWA**	**PT**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
BBCH 10 -29	Wood mouse	0.25 (maize)	0.27	54.2	1.0 × 0.53	0.139	0.011	
		0.5 (seeds)		40.2	1.0 × 0.53		0.016	
		0.25 (arthropods)		3.5 [1)]	1.0 × 0.53		0.0007	
						Sum of DDD_m	0.027	140557
BBCH 10 -29	Brown hare	0.84 (maize)	0.328	54.2	1.0 × 0.53	0.734	0.232	
		0.16 (dicot weeds)		28.7	1.0 × 0.53		0.023	
						Sum of DDD_m	0.256	15093

BBCH 10 -29	Brown hare	1.0 (maize)	0.32	54.2	1.0 × 0.53	0.734	0.270	14306

[1)] according to Appendix A of EFSA (2009) RUD values for arthropods with interception are relevant for scenario maize at BBCH 10-29

The combined chronic risk assessment based on TER values for individual substances calculated for both focal species is presented below.

Generic focal species	Compound						Σ1/TER	Σ1/TER^{-1}	Trigger
	Dicamba		Mesotrione		Nicosulfuron				
	TER	1/TER	TER	1/TER	TER	1/TER			
Wood mouse	1747	0.0006	11.1	0.090	140557	0.0000007	0.091	11.0	5
Brown hare (mixed diet)	188	0.005	6.5	0.154	15093	0.00007	0.159	6.3	
Brown hare (single diet)	178	0.006	7.1	0.141	14306	0.00007	0.147	6.8	

Values in **bold** indicate unacceptable risk

All refined TERmix values for both focal species are above the trigger of 5 demonstrating acceptable risk to mammals exposed to the mixture of dicamba, mesotrione and nicosulfuron applied as A18032E to maize at BBCH 12-18 as 0.4 kg product/ha.

9.3.2.3 Drinking water exposure

When necessary, the assessment of the risk for mammals due to uptake of contaminated drinking water is conducted for a small omnivorous mammal with a body weight of 21.7 g (*Apodemus sylvaticus*) and a drinking water uptake rate of 0.24 L/kg bw/d (*cf.* Appendix K of EFSA/2009/1438).

Puddle scenario

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).

Dicamba

With a K(f)oc of 12.4, dicamba belongs to the group of less sorptive substances. Here, the maximum use rate of 1 x 187.5 g a.s./ha has been used to cover the risk to mammals from all intended uses (see Table 9.1-3).

Effective application rate (g/ha)* =	187.5		
Acute toxicity (mg/kg bw) =	1581	quotient =	0.12
Reprod. toxicity (mg/kg bw/d) =	150	quotient =	1.3

* Effective application rate = Maximum application rate x MAF of 1

The resulting ratios fall below the trigger of 50 indicating that further assessment of the acute and long-term risk to mammals from drinking water from puddles is not required for dicamba.

Mesotrione

With a K(f)oc of 50 (geomean; the worst-case is 14 in the final EFSA endpoints), mesotrione belongs to the group of less sorptive substances. Here, the maximum use rate of 1 x 90 g a.s./ha has been used to cover the risk to mammals from all intended uses (see Table 9.1-3).

Effective application rate (g/ha)* =	90		
Acute toxicity (mg/kg bw) =	>5000	quotient =	<0.018
Reprod. toxicity (mg/kg bw/d) =	0.3 (NOEL) ~~1.2 (NOAEL)~~	quotient =	**300** ~~75~~

* Effective application rate = Maximum application rate x MAF of 1

The resulting ratio for acute risk falls below the trigger of 50 indicating that further assessment of the acute risk to mammals from drinking water from puddles is not required for mesotrione.

For the chronic risk since the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) exceeds the critical value of 50 for reproductive risk, a quantitative risk assessment (calculation of TER values) is necessary.

Here, the maximum use rate of 1 x 90 g a.s./ha is used to cover the risk to mammals from all intended uses (see Table 9.1-3).

The predicted environmental concentration in puddles is calculated as follows in accordance with the EFSA Guidance Document:

$$PEC_{puddle} = \frac{AR/10}{1000\ (w + Koc \times s)}$$

where:
AR = application rate (g/ha); divisor of 10 to achieve rate in mg/m^2
w = 0.02 (pore water term; volume)
s = 0.0015 (soil term: volume, density, organic carbon content)

Table 9.3-11: Assessment of the risk for mammals due to exposure to mesotrione via contaminated drinking water in puddles

Intended use	Maize					
Active substance	Mesotrione					
Application rate (g/ha)	1 × 90					
Reprod. toxicity (mg/kg bw/d)	0.3 (NOEL) ~~1.2 (NOAEL)~~					
TER criterion	5					
Soil-relevant applic. rate (g/ha)	**Koc (L/kg)**	**PEC_{puddle} (mg/L)**	**DW uptake (L/kg bw/d)**	**Daily dose (mg/kg bw/d)**	**NO(A)EL (mg/kg bw/d)**	**TER_{lt}**
90	50 (geomean)	0.0947	0.24	0.0227	0.3 ~~1.2~~	13 ~~53~~
90	14 (worst-case in the EFSA conclusion) [a]	0.220	0.24	0.0527	0.3 ~~1.2~~	5.7 ~~23~~

PEC_{puddle}: concentration in puddles; DW: drinking water; TER: toxicity to exposure ratio.
[a] The final DAR states: "As the adsorption is pH dependent a worst-case value should be used for risk assessment rather than a mean"

Nicosulfuron

With a K(f)oc of 20.7, nicosulfuron belongs to the group of less sorptive substances. Here, the maximum use rate of 1 x 60 g a.s./ha has been used to cover the risk to mammals from all intended uses (see Table 9.1-3).

Effective application rate (g/ha)*	=	60		
Acute toxicity (mg/kg bw)	=	>5000	quotient =	<0.012
Reprod. toxicity (mg/kg bw/d)	=	3861	quotient =	0.016

* Effective application rate = Maximum application rate x MAF of 1

The resulting ratios fall below the trigger of 50 indicating that further assessment of the acute and long-term risk to mammals from drinking water from puddles is not required for nicosulfuron.

zRMS comments:

The drinking water risk assessment for particular active substances provided in tables above is agreed by the zRMS. Acceptable acute and chronic risk could be concluded at the screening step for dicamba and nicosulfuron. For mesotrione acceptable acute risk could be concluded, while quantitative risk assessment was necessary to address the long-term risk.

It is noted that all calculations were performed for exaggerated application rate corresponding to 600 g product/ha, covering the rate of 400 g product/ha intended in Poland.

No calculations were provided by the Applicant for the pertinent soil metabolites of all three active compounds. However, the risk would be acceptable for dicamba and nicosulfuron since the maximum ratio for metabolites based on the worst case assumptions (10 times toxicity of the parent and parent exposure) would be <50 (worst case trigger assumed, covering also risk from less sorptive metabolites) for the acute and long-term risk. The same is relevant for acute risk from mesotrione metabolites, while additional calculations might be necessary to address the long-term risk. Nevertheless, in the course of the EU renewal of mesotrione it was concluded that its metabolites have similar or lower toxicity and for this specific evaluation of the drinking water risk was deemed not necessary and is considered to be covered by the evaluation performed for the active compound.

Overall, acceptable risk from the drinking water may be concluded for all active compounds ant their pertinent soil metabolites.

9.3.2.4 Effects of secondary poisoning

The log P_{OW} value for dicamba is 0.55 – 1.9 (at pH 5.0 – 8.9) and for its metabolite NOA414746 the log P_{OW} value is -0.84 (pH 6.8). The log P_{OW} values of mesotrione and its main metabolites MNBA, AMBA and SYN546974 amount to 0.11, -1.3, 0.32 and 1.62 respectively and thus do not exceed the trigger value of 3. Nicosulfuron has a log P_{OW} value of 0.6 and its major aquatic metabolites ASDM, AUSN and HMUD have log P_{OW} values of < 1.0.

Therefore, risk assessment for effects due to secondary poisoning is not required for dicamba, mesotrione, nicosulfuron and their relevant metabolites.

zRMS comments:

Provided above information is agreed by the zRMS. The evaluation of the risk of secondary poisoning is triggered due to log Pow <3 for all active compounds and their relevant metabolites.

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 acute and long-term risks of A18032E to mammals were assessed from toxicity exposure ratios between toxicity endpoints, estimated from studies with A18032E, dicamba, mesotrione and nicosulfuron, and maximum residues occurring on food items following applications according to the proposed use pattern. The combined toxicity and risk assessment was also performed.

The risk to mammals from exposure via drinking water has also been assessed. Risk of secondary poisoning has not been assessed, as dicamba, mesotrione, nicosulfuron and their relevant metabolites have log P_{OW} <3.0.

The TER values, calculated for recommended scenarios, all exceed the trigger values of 10 for acute risk, indicating that the acute risk to mammals is acceptable following use of A18032E according to the proposed use pattern. Acceptable combined acute risk could be concluded.

The long-term TER values for dicamba and nicosulfuron, calculated for recommended scenarios, exceed the trigger value of 5, indicating acceptable risk. However, the long-term TER values for mesotrione fall below the trigger of 5 and the combined long-term risk was also unacceptable at Tier 1.

Acceptable long-term risk to ~~small omnivorous and small herbivorous~~ mammals from mesotrione could be demonstrated in a refined risk assessment by identifying the brown hare and wood mouse as relevant focal species for the intended use pattern, refining the residue decline of mesotrione ~~residues~~ in potential food items, and considering the realistic amount of time spent foraging in early maize fields (PT). Considered refinement options were also sufficient to resolve the combined long-term risk to the relevant focal species. ~~A more realistic NOAEL has also been used in the refined assessments, and justification for use of this has been provided.~~

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

No relevant data on amphibians and reptiles is available for dicamba, mesotrione or nicosulfuron, consequently no further assessment of potential effects on reptiles and amphibians will be presented in this document.

zRMS comments:

As currently there are no agreed rules or criteria for evaluation of the risk to other terrestrial vertebrates like reptiles and amphibians, this issue should be addressed once respective guidance is available and EU agreed endpoints concluded.

9.5 Effects on aquatic organisms (KCP 10.2)

9.5.1 Toxicity data

Studies on the toxicity to aquatic organisms have been carried out with dicamba, mesotrione, nicosulfuron, and their 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).

Effects of A18032E on aquatic organisms were not evaluated as part of the EU assessment of dicamba, mesotrione and nicosulfuron. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process. There are some deviations for which justifications are provided below.

Table 9.5-1: Endpoints and effect values relevant for the risk assessment for aquatic organisms – dicamba and relevant metabolites

Species	Substance	Exposure system	Results	Reference
Cyprinus carpio	Dicamba	96 h, s	LC_{50} > 100 mg a.s./L $_{nom}$	EFSA Conclusion 2011 Volz, 2004a SAN837/6142
Oncorhynchus mykiss	Banvel 480 SL ~~Dicamba~~	96 h, s	LC_{50} > 41.0 mg a.s./L $_{nom}$ [a]	EFSA Conclusion 2011 Baetscher, 2005 SAN837/6503
Oncorhynchus mykiss	NOA414746 (DCSA)	96 h, ss	LC_{50} > 100 mg/L $_{im}$	EFSA Conclusion 2011 Douglas et al., 1993a NOA414746/0003
Oncorhynchus mykiss	Dicamba	21 d, ss	NOEC = 180 mg a.s./L $_{nom}$	EFSA Conclusion 2011 Scheerbaum, 1990 SAN837/5331
Daphnia magna	Dicamba (as 480 SL formulation)	48 h, s	EC_{50} > 41 mg a.s./L $_{nom}$	EFSA Conclusion 2011 Bätscher, 2005b SAN837/6502
Daphnia magna	NOA414746 (DCSA)	48 h, s	EC_{50} = 89 mg/L $_{mm}$	EFSA Conclusion 2011 Douglas et al., 1993b NOA414746/0005
Daphnia magna	Dicamba	21 d, ss	NOEC = 97 mg a.s./L $_{mm}$	EFSA Conclusion 2011 Douglas, 1993 SAN837/5332
Selenastrum capricornutum (currently: *Pseudokirchneriella subcapitata*)	Dicamba	72 h, s	E_rC_{50} > 3.7 mg a.s./L $_{mm}$ E_bC_{50} > 3.7 mg a.s./L $_{mm}$	DAR, Vol 3, 2007, revised September 2010 and October 2010 Hoberg, 1993c SAN837/5230
Skeletonema costatum	Dicamba	72 h, s	E_rC_{50} > 4.1 mg a.s./L $_{mm}$ E_bC_{50} = 1.8 mg a.s./L $_{mm}$	EFSA Conclusion 2011 Hoberg 1993a SAN837/5224
Navicula pelliculosa	Dicamba	72 h, s	E_rC_{50} > 3.8 mg a.s./L $_{mm}$ E_bC_{50} > 3.8 mg a.s./L $_{mm}$	EFSA Conclusion 2011 Hoberg 1993b SAN837/5229
Anabaena flos-aque	Dicamba	72 h, s	E_rC_{50} > 32 mg a.s./L $_{nom}$ E_bC_{50} > 32 mg a.s./L $_{nom}$	EFSA Conclusion 2011 Smyth et al., 1998 SAN837/0411
Pseudokirchneriella subcapitata	NOA414746 (DCSA)	72 h, s	E_rC_{50} = 138 mg/L $_{im}$ E_bC_{50} = 118 mg/L $_{im}$	EFSA Conclusion 2011 Douglas et al., 1993c NOA414746/0004

Species	Substance	Exposure system	Results	Reference
Myriophyllum spicatum	Dicamba	26 d, s	E_rC_{50} > 0.45 mg a.s./L nom E_bC_{50} > 0.45 mg a.s./L nom	EFSA Conclusion 2011 Volz, 2003 SAN837/6133
Lemna gibba	Dicamba	7 d, s	E_rC_{50} n.a. E_bC_{50} > 3.25 mg a.s./L mm	EFSA Conclusion 2011 Hoberg 1993d SAN837/5223
Lemna gibba	NOA414746 (DCSA)	7 d, s	E_rC_{50} > 73 mg/L mm E_bC_{50} = 11.9 mg/L mm	EFSA Conclusion 2011 Grade, 2002 NOA414746/0013
Higher-tier studies (micro- or mesocosm studies)				
Not required.				

s: static; ss: semi-static; nom: based on nominal concentrations; mm: based on mean measured concentrations; im: based on initial measured concentrations

zRMS comments:

Endpoints presented in Table 9.5-1 are in line with the EU agreed endpoints reported in EFSA Journal 2011;9(1):1965. Some corrections were introduced by the zRMS so presented data are fully agreed with information presented in respective EU documents.

Table 9.5-2: Endpoints and effect values relevant for the risk assessment for aquatic organisms – mesotrione and potentially relevant metabolites

Species	Substance	Exposure system	Results	Reference
Oncorhynchus mykiss	Mesotrione	96 h, s	LC_{50} >120 mg a.s./L nom s	EFSA Conclusion 2016 Kelso et al., 1994 ZA1296/0557
Oncorhynchus mykiss	MNBA	96 h, s	LC_{50} >120 mg a.s./L nom s	EFSA Conclusion 2016 Smyth et al., 1997a ZA1296/0529
Oncorhynchus mykiss	AMBA	96 h, s	LC_{50} = 150 mg a.s./L nom s	EFSA Conclusion 2016 Magor and Gore, 1998a R44276/0017
Pimephales promelas	Mesotrione	36 d chronic, f	NOEC = 12.5 mg a.s./L nom ~~mm~~	EFSA Conclusion 2016 Shillabeer and Kent, 1997 ZA1296/0560
Daphnia magna	Mesotrione	48 h, s	EC_{50} >622 mg a.s./L mm	EFSA Conclusion 2016 Gentle and Hamer, 1995 ZA1296/0561
Daphnia magna	MNBA	48 h, s	EC_{50} = 130 mg a.s./L nom	EFSA Conclusion 2016 Kent and Shillabeer, 1997 ZA1296/0531
Daphnia magna	AMBA	48 h, s	EC_{50} = 160 mg a.s./L nom	EFSA Conclusion 2016 Magor and Gore, 1998b R44276/0018
Daphnia magna	Mesotrione	21 d, ss	NOEC = 180 mg a.s./L mm	EFSA Conclusion 2016 Morris et al., 1996 ZA1296/0564
Pseudokirchneriella subcapitata	Mesotrione	120 h, s	E_rC_{50} = 13 mg a.s./L nom ~~mm~~ E_bC_{50} = 3.5 mg a.s./L nom ~~mm~~	EFSA Conclusion 2016 Shillabeer et al., 1997 ZA1296/0186
Pseudokirchneriella subcapitata	MNBA	72 h, s	E_rC_{50} = 42 mg a.s./L nom ~~mm~~ E_bC_{50} = 38 mg a.s./L nom ~~mm~~	EFSA Conclusion 2016 Smyth et al., 1997b ZA1296/0533

Species	Substance	Exposure system	Results	Reference
Pseudokirchneriella subcapitata	AMBA	72 h, s	E_rC_{50} = 14 mg a.s./L nom ~~mm~~ E_bC_{50} = 9.4 mg a.s./L nom ~~mm~~	EFSA Conclusion 2016 Smyth et al., 1998 R44276/0019
Lemna gibba	Mesotrione	14 d, ss	E_bC_{50} frond no.= 0.022 mg a.s./L nom ~~mm~~ E_bC_{50} dry weight = 0.0077 mg a.s./L nom ~~mm~~	EFSA Conclusion 2016 Smyth et al., 1997c ZA1296/0182
Lemna gibba	Mesotrione	7 d, ss	E_rC_{50} frond no or biomass = 0.0241 mg a.s./L nom E_bC_{50} yield = 0.0045 mg a.s./L nom ~~E_rC_{50} frond no or biomass = 0.028 mg a.s./L nom~~ ~~E_bC_{50} yield = 0.0052 mg a.s./L nom~~	Hengsberger and Wydra, 2015 ZA1296_10438
Myriophyllum spicatum	Mesotrione	14d, ss	E_rC_{50} total shoot length = 0.0287 mg a.s./L nom E_yC_{50} yield = 0.00255 mg a.s./L nom ~~E_rC_{50} total shoot length = 0.0339 mg a.s./L nom~~ ~~E_yC_{50} yield = 0.00301 mg a.s./L nom~~	Gonsior 2017 ZA1296_10504
Aquatic macrophytes	Mesotrione	Geometric mean	E_rC_{50} = 0.0263 mg a.s./L E_yC_{50} = 0.00339 mg a.s./L ~~E_rC_{50} = 0.031 mg a.s./L~~ ~~E_yC_{50} = 0.0040 mg a.s./L~~	See section 9.5.1.1
Lemna gibba	MNBA	7 d, ss	E_rC_{50} >97 mg a.s./L T E_yC_{50} >97 mg a.s./L T	EFSA Conclusion 2016 Liedtke, 2013a CA3511_10001
Lemna gibba	AMBA	7 d, ss	E_rC_{50} >90 mg a.s./L T E_yC_{50} >90 mg a.s./L T	EFSA Conclusion 2016 Liedtke, 2013b R044276_10001.
Lemna gibba	SYN546974	7 d, ss	E_rC_{50} >95 mg a.s./L mm ~~nom~~ E_yC_{50} = 93 mg a.s./L mm ~~nom~~	EFSA Conclusion 2016 Liedtke, 2013c SYN546974_10001
Higher-tier studies (micro- or mesocosm studies)				
None				

s: static; ss: semi-static; f: flow-through; nom: based on nominal concentrations; mm: based on mean measured concentrations; im: based on initial measured concentrations T:based on time-weighted mean measured concentrations

zRMS comments:

Endpoints presented in Table 9.5-2 are in line with the EU agreed endpoints reported in EFSA Journal 2016;14(3):4419.

In support of this submission the applicant provided two additional studies on toxicity of mesotrione to *Lemna gibba* (Hengsberger & Wydra, 2015) and *Myriophyllum spicatum* (Gonsior, 2017).
In general, in the course of the EU review it was concluded that sufficient data are available for *Lemna gibba* and no data gap in this area was identified in EFSA Journal 2016;14(3):4419. Nevertheless, as endpoints were not based on growth rates, the zRMS decided to evaluate the new study provided by the Applicant in order to perform the risk assessment in line with EFSA aquatic guidance (2013).
As in EFSA conclusion a data gap regarding testing of additional aquatic macrophyte species was identified, the study performed with *M. spicatum* was evaluated by the zRMS as it was necessary to finalise the risk assessment.

Both studies were agreed by the zRMS, however the derived endpoints were corrected for purity of the test item which resulted also with correction of the geometric mean E_rC_{50} for aquatic macrophytes. Respective corrections were thus made in Table 9.5-2. For details of evaluation of the studies, please refer to Appendix 2.

Table 9.5-3: Endpoints and effect values relevant for the risk assessment for aquatic organisms – nicosulfuron and relevant metabolites

Species	Substance	Exposure system	Results	Reference
Oncorhynchus mykiss	Nicosulfuron	96 h, s	LC_{50} = 65.7 mg a.s./L $_{nom}$	EFSA Conclusion 2007 Jenkins, 1991a 91/ISK169/182/0012
Lepomis macrochirus	ASDM	96 h, ss	LC_{50} > 100 mg/L $_{nom}$	EFSA Conclusion 2007 Buchanan and Knight, 1997a 15168
Brachydanio rerio	AUSN	96 h, s	LC_{50} > 100 mg/L $_{nom}$	EFSA Conclusion 2007 Wüthrich, 1996b 601031
Oncorhynchus mykiss	MU-466	96 h, s	LC_{50} > 100 mg/L $_{nom}$	EFSA Conclusion 2007 Wüthrich, 1996a 613080
Oncorhynchus mykiss	HMUD	96 h, s	LC_{50} > 100 mg/L $_{nom}$	EFSA Conclusion 2007 Grützner, 1996a 613912
Oncorhynchus mykiss	ADMP	96 h, s	LC_{50} > 100 mg/L $_{nom}$	EFSA Conclusion 2007 Hertl, 1997a 658034
Oncorhynchus mykiss	Nicosulfuron	28 d, f	NOEC = 10 mg a.s./L $_{nom}$	EFSA Conclusion 2007 Bogers, 1994a, 117473
Daphnia magna	Nicosulfuron	48 h, s	EC_{50} = 90 mg a.s./L $_{nom}$	EFSA Conclusion 2007 Jenkins, 1991c 91/ISK171
Daphnia magna	ASDM	48 h, s	EC_{50} > 954 mg/L $_{mm}$	EFSA Conclusion 2007 Jenkins, 1993b 93/ISK203/0628
Daphnia magna	AUSN	48 h, s	EC_{50} > 100 mg/L $_{nom}$	EFSA Conclusion 2007 Wüthrich, 1995a 601053
Daphnia magna	MU-466	48 h, s	EC_{50} > 100 mg/L $_{nom}$	EFSA Conclusion 2007 Wüthrich, 1996d 613078
Daphnia magna	HMUD	48 h, s	EC_{50} > 100 mg/L $_{nom}$	EFSA Conclusion 2007 Grützner, 1996c 613890
Daphnia magna	UCSN	48 h, s	EC_{50} > 100 mg/L $_{nom}$	EFSA Conclusion 2007 Grützner, 1996d 601042
Daphnia magna	ADMP	48 h, s	EC_{50} > 100 mg/L $_{nom}$	EFSA Conclusion 2007 Hertl, 1997b 658012
Daphnia magna	Nicosulfuron	21 d, ss	NOEC = 5.2 mg a.s./L $_{nom}$	EFSA Conclusion 2007 Bogers, 1994b 117484
Anabaena flos-aquae	Nicosulfuron	72 h, s	E_rC_{50} = 8.4 mg a.s./L $_{nom}$ E_bC_{50} = 7.8 mg a.s./L $_{nom}$	EFSA Conclusion 2007 and DAR (2005) Memmert, 1998a 692278
Pseudokirchneriella subcapitata	ASDM	72 h, s	E_rC_{50} > 336 mg/L $_{mm}$ E_bC_{50} > 54 mg/L $_{mm}$	EFSA Conclusion 2007 Jenkins, 1993c 93/ISK206/0750
Scenedesmus subspicatus	AUSN	72 h, s	E_rC_{50} > 100 mg/L $_{nom}$ E_bC_{50} > 100 mg/L $_{nom}$	EFSA Conclusion 2007 Wüthrich, 1996f 601108

Species	Substance	Exposure system	Results	Reference
Scenedesmus subspicatus	MU-466	72 h, s	E_rC_{50} > 100 mg/L $_{nom}$ E_bC_{50} = ≥ 84.4 mg/L $_{nom}$	EFSA Conclusion 2007 Grützner, 1996g 613056
Scenedesmus subspicatus	HMUD	72 h, s	E_rC_{50} > 100 mg/L $_{nom}$ E_bC_{50} > 100 mg/L $_{nom}$	EFSA Conclusion 2007 Grützner, 1996f 613901
Scenedesmus subspicatus	UCSN	72 h, s	E_rC_{50} > 100 mg/L $_{nom}$ E_bC_{50} > 100 mg/L $_{nom}$	EFSA Conclusion 2007 Grützner, 1996e 601097
Scenedesmus subspicatus	ADMP	72 h, s	E_rC_{50} > 100 mg/L $_{nom}$ E_bC_{50} > 100 mg/L $_{nom}$	EFSA Conclusion 2007 Hertl, 1997c 657990
Lemna gibba	Nicosulfuron	7 d, ss	E_rC_{50} = 0.0027 mg a.s./L $_{mm}$ EC_{50} = 0.0017 mg a.s./L $_{mm}$ (frond count) EC_{50} = 0.034 mg a.s./L $_{mm}$ (dry weight)	EFSA Conclusion 2007 and DAR (2005) Memmert, 1998c 693854
Lemna gibba	ASDM	7 d, ss	E_rC_{50} > 100 mg/L $_{nom}$ E_bC_{50} > 100 mg/L $_{nom}$ EC_{50} > 100 mg/L $_{nom}$ (frond count)	EFSA Conclusion 2007 Memmert, 1998d 693876
Lemna gibba	AUSN	7 d, ss	E_rC_{50} > 100 mg/L $_{nom}$ E_bC_{50} > 100 mg/L $_{nom}$ EC_{50} > 100 mg/L $_{nom}$ (frond count)	EFSA Conclusion 2007 Memmert, 1998f 693898
Lemna gibba	HMUD	7 d, ss	E_rC_{50} > 1 mg/L $_{nom}$ E_bC_{50} > 1 mg/L $_{nom}$ EC_{50} > 1 mg/L $_{nom}$ (frond count)	EFSA Conclusion 2007 Kitajima, 2004 ET0104
Lemna gibba	UCSN	7 d, ss	E_rC_{50} > 100 mg/L $_{nom}$ E_bC_{50} > 100 mg/L $_{nom}$ EC_{50} > 100 mg/L $_{nom}$ (frond count)	EFSA Conclusion 2007 Memmert, 1998e 693911
Myriophyllum aquaticum	*Nicosulfuron*	*7 d, s*	*E_bC_{50} = 3.071 mg a.s./L $_{mm}$* *E_rC_{50} >3.523 (length increase)* [1)]	*Wenzel, 2010* *185 NIS*
Higher-tier studies (micro- or mesocosm studies)				
Not required.				

[1)] Endpoints from study by Wenzel (2010) on toxicity of nicosulfuron to *M. aquaticum* are not relevant for pusporse of the risk assessment due to too short study duration (7 days instead of 14 d required by the current test guideline OECD TG 239). Nevertheless, endpoints from the study are retained above since they may be used as supportive information in identification of the most sensitive aquatic macrophyte species.

s: static; ss: semi-static; f: flow-through; nom: based on nominal concentrations; mm: based on mean measured concentrations

zRMS comments:

Endpoints presented in Table 9.5-3 are in line with the EU agreed endpoints reported in EFSA Scientific Report (2007) 120 with exception of results of the study on toxicity of nicosulfuron to *Myriophyllum aquaticum* (Wenzel, 2010), which was submitted in support of evaluation of A18032E.

In general, generation of new active substance data at the zonal/national level should be avoided, nevertheless no toxicity data for the second macrophyte species were available from the nicosulfuron EU review and it was not possible to confirm that *Lemna gibba* is actually most sensitive aquatic macrophyte species. Taking this into account, the study by Wenzel (2010) was evaluated by the zRMS. The study would be acceptable in terms of the test design and test conditions, however, too short test duration (7 days) makes the derived endpoints not relevant for purposes of the risk assessment performed in line with EFSA (2013). Nevertheless, obtained results may be used as supportive information to confirm that *Lemna gibba* is the aquatic macrophyte species more sensitive to nicosulfuron than *M. aquaticum*.

Table 9.5-4: Endpoints and effect values relevant for the risk assessment for aquatic organisms – A18032E

Species	Substance	Exposure system	Results	Reference
Oncorhynchus mykiss	A18032E	96 h, s	LC_{50} = 3.44 mg/L $_{nom}$ (1.97 mg sum of a.s./L)	Weich, 2012 A18032E_10001
Daphnia magna	A18032E	48 h, s	EC_{50} = 1.22 mg/L $_{nom}$ (0.69 mg sum of a.s./L)	Weber, 2012 A18032E_10008
Pseudokirchneriella subcapitata	A18032E	72 h, s	E_rC_{50} = 0.0728 mg/L $_{nom}$ (0.041 mg sum of a.s./L) E_yC_{50} = 0.0389 mg/L $_{nom}$ E_bC_{50} = 0.0374 mg/L $_{nom}$	Falk, 2012 A18032E_10002
Lemna gibba	A18032E	7 d, ss	E_rC_{50} = 18.1 µg/L_{nom} (10.31 µg sum of a.s./L) E_yC_{50} = 6.43 µg/L_{nom} EC_{50} = 12.7 µg/L_{nom} (dry weight)	Weber, 2012 A18032E_10009

s: static; ss: semi-static; nom: based on nominal concentrations

zRMS comments:

Studies on toxicity of A18032E to aquatic organisms were evaluated and agreed by the zRMS. For details of evaluation, please refer to Appendix 2.

It is noted that in the studies the measured concentrations were verified for mesotrione only. However, information available in area of environmental fate and behaviour of particular active compounds indicates that with mean water DT_{50} of 5.6 days determined in the water/sediment studies, mesotrione is the least stable active substance (mean water DT_{50} of 41 and 65 days was determined for dicamba and nicosulfuron, respectively, in water/sediment systems). Dicamba was stable in EU agreed hydrolysis studies, while nicosulfuron was stable at pH 7 and 9. At pH 5 hydrolytic degradation was observed with DT_{50} determined to be 15 days.
In order to further support conclusion on stability of dicamba and nicosulfuron, summaries of the aquatic toxicity studies of these two compounds were consulted in monographs. Dicamba was stable in all acute and chronic aquatic toxicity studies. Nicosulfuron was stable in all acute studies as well as chronic toxicity with algae and majority studies with *Lemna gibba* and single static *Myriophyllum aquaticum* study summarised in this report (Wenzel, 2010), where nicosulfuron was stable over 7 days of exposure. Concentrations of nicosulfuron dropped below 80% in two *Lemna* studies available in the course of EU review (for active compound and formulated product). However, following explanation has been provided by the RMS:

Data on the hydrolytic stability of the active substance, indicate that although stable at pH 7, at the test system pH of 5 significant hydrolysis of the active substance may be expected - this being likely to account for the recorded drop in a.s. concentration 2-3 days after medium renewal.

In the study on toxicity of A18032E to *Lemna gibba* (Weber, 2012) pH of the fresh and aged test solutions was >7 over the whole study period and it may be thus concluded that nicosulfuron was stable over the study period.

Overall, performed chemical analyses confirmed that mesotrione was stable in all aquatic toxicity studies with A18032E, while dicamba and nicosulfuron may be concluded to be stable based on the data available in area of the fate and behaviour. Taking this into account, endpoints reported in Table 9.5-4 based on nominal concentrations are confirmed to be correct. Endpoints expressed in terms of the sum of active compounds were added by the zRMS.

Study on toxicity of the formulated product to second aquatic macrophyte species are deemed not necessary since based on the toxicity data available for particular active compounds it may be concluded that:

- None of the primary producers is especially sensitive to dicamba. All E_rC_{50} values for all relevant species were greater than values ranging from >0.45 to >32.0 mg a.s./L.
- Aquatic macrophytes are more sensitive to mesotrione comparing to algae. However, sensitivity of both tested species with E_rC_{50} of 0.0241 and 0.0287 mg a.s./L for *L. gibba* and *M. spicatum*, respectively, is comparable with none being more sensitive.
- Algae are not especially sensitive to nicosulfuron (E_rC_{50} of 8.4 mg a.s./L). Of two aquatic macrophyte species With E_rC_{50} of 0.0027 mg a.s./L *Lemna gibba* is clearly more sensitive comparing to

Myriophyllum aquaticum with E_rC_{50} of >3.523 mg a.s./L (please note, however, that this latter endpoint is quoted for informative purposes only since it was derived from the study of 7 days duration instead of 14 days).

Overall, since none of active substances is toxic to algae and for two active compounds aquatic macrophytes were of comparable sensitivity and for one compound Lemna gibba was clearly more sensitive, study on toxicity of the formulated product to *Lemna gibba* is considered sufficient.

9.5.1.1 Justification for new endpoints

According to the recommendations in 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), focus on growth rate endpoints for algae and aquatic plants are recommended for European risk assessment. The advantage of using the growth rate endpoints is that growth rate is less dependent on study duration and is relevant to 'recovery potential'.

Based on the recommendations from the Aquatic Guidance, Syngenta propose that the worst-case E_rC_{50} values for A18032E, dicamba, mesotrione, nicosulfuron and their metabolites are used in the algal and aquatic plant risk assessments. This approach is widely accepted for algae. However, it is recognised that some regulatory authorities may have reservations about the use of E_rC_{50} values for macrophyte risk assessment when these are less conservative than $E_{b\text{ or }y}C_{50}$ values, and therefore the $E_{b\text{ or }y}C_{50}$ values will also be assessed.

Lemna endpoint for mesotrione

The laboratory study for *Lemna* was repeated due to issues in the original study submitted in which concentrations were not maintained within 20% of nominal throughout the exposure period, and endpoints were not reported in terms of growth rate. The new 7d study (Hengsberger & Wydra 2015) fulfils all the current acceptability criteria, and concentrations were maintained within 20% of nominal throughout the study. The biomass endpoints of the Hengsberger & Wydra study (2015, $E_bC_{50\text{ dry weight}}$ = 0.0052 mg a.s./L) and the previous study of Smyth et al. (1997c, E_bC_{50} = 0.0077 mg a.s./L) are very similar, and the new endpoints will be used in the risk assessment, in preference, as they are considered more reliable.

Myriophyllum endpoint for mesotrione

In the EU review a data gap was identified for a dicot aquatic macrophyte, and therefore a new test has been carried out with *Myriophyllum spicatum*. The results for *Lemna* and *Myriophyllum* are remarkable similar as shown in the table above, indicating that there is no indication of selectivity to dicot or monocot aquatic macrophytes.

Use of geomean values for macrophyte risk assessment

In accordance with the Aquatic Guidance document ('Tier 2a'), the geometric mean of endpoints can be used when there are data from more than 1 species, and when these differ by less than a factor of 10. Since suitable data for mesotrione are available from studies with *Myriophyllum* and *Lemna*, the geometric mean E_rC_{50} and E_yC_{50} can be used.

However for nicosulfuron the endpoints from the studies with *Myriophyllum* and *Lemna* are more than a factor of 10 apart, and therefore the geometric mean has not been used.

zRMS comments:

In general, in the course of the EU review it was concluded that sufficient data are available for *Lemna gibba* and no data gap in this area was identified in EFSA Journal 2016;14(3):4419. Nevertheless, as endpoints were not based on growth rates, the zRMS decided to evaluate the new study by Hengsberger & Wydra (2015) provided by the Applicant in order to perform the risk assessment in line with EFSA aquatic guidance (2013). The study was considered acceptable. For evaluation, please refer to Appendix 2, KCP 10.2.1/01.
As in EFSA conclusion a data gap regarding testing of additional aquatic macrophyte species was identified, the study by Gonsior (2017) performed with *M. spicatum* was evaluated by the zRMS as it was necessary to finalise the risk assessment.

Calculation of the geometric mean E_rC_{50} and E_yC_{50} values for aquatic macrophytes is agreed by the zRMS, as data for more than one species are available and endpoints do not differ by more than a factor of 10. Actually, it can be concluded that endpoints for both species are in similar range.

It is noted that *Lemna gibba* and *Myriophyllum spicatum* belong to different taxonomic groups and according to EFSA (2013) separate risk assessment should be performed for each group. However, in a footnote c) to Table 26 in point 8.3.3 of EFSA (2013) it is indicated that the geometric mean may be calculated for various taxonomic groups of primary producers, provided that it is demonstrated that certain taxonomic groups may be combined.
For *Lemna gibba* and *Myriophyllum spicatum* E_rC_{50} values of 0.0241 and 0.0287 mg a.s./L (corrected for purity of the test item) were derived from the newly submitted studies, respectively, and they do not differ by more than a factor of 10. Actually it may be concluded that difference between both endpoints is due to inter-laboratory variation and not due to higher sensitivity of *Lemna gibba* to mesotrione. In opinion of the zRMS this confirms that endpoints for these two taxonomic groups may be combined to calculate the geomean E_rC_{50}.

Formulation studies

New studies are available for A18032E which are required to fulfil the data requirements for plant protection products in accordance with Regulation (EC) No 1107/2009. The endpoints are summarised in Table 9.5-4.

The results from the toxicity tests using the formulation indicate that the formulation may be more toxic than the content of individual active ingredients would suggest. In order to compare the measured toxicity data for the formulation with the predicted toxicity data, taking into account additive toxicity of the different active ingredient contents, the Finney formula[20] has been applied as follows for each species:

$$\frac{100}{CE_{50}} = \sum\left[\frac{x}{CE_{50}i}\right]$$

Where:
x, the percentage of the active substance i (in weight) in the formulation
CE_{50}i, the toxicity of the active substances i for the aquatic organisms

This formula assumes an additivity of the toxicities of each active substance. A18032E contains 312.5 g dicamba/kg, 150 g mesotrione/kg and 100 g nicosulfuron/kg. Therefore the contents of the active substances are 31.25%, 15% and 10% for dicamba, mesotrione and nicosulfuron, respectively (taking into account a 1 kg product).

[20] Finney, D. J. (1952). Probit Analysis, 2nd Ed. Cambridge Univ. Press, London and New York.

~~**Table 9.5-5: Comparison between observed acute toxicity of the formulation and active substances and calculated toxicity (Finney formula) for the formulation A18032E**~~

~~Organism~~	~~Test endpoint~~	~~Active substance~~	~~Endpoint used (mg/L)~~	~~Calculated value based on additive toxicity of mesotrione, nicosulfuron and dicamba (mg formulation/L)~~	~~Observed value for formulation (mg formulation/L)~~
~~*Oncorhynchus mykiss*~~	~~96h LC_{50}~~	~~Dicamba~~	~~>100~~	~~170~~	~~3.44~~
		~~Mesotrione~~	~~>120~~		
		~~Nicosulfuron~~	~~65.7~~		
~~*Daphnia magna*~~	~~48h EC_{50}~~	~~Dicamba~~	~~>41~~	~~111~~	~~1.22~~
		~~Mesotrione~~	~~622~~		
		~~Nicosulfuron~~	~~90~~		
~~*Pseudokirchneriella subcapitata* [a]~~	~~120h E_bC_{50}~~	~~Dicamba~~	~~>3.7~~	~~7.14~~	~~0.0374~~
	~~72h E_bC_{50}~~	~~Mesotrione~~	~~3.5~~		
	~~72h E_bC_{50}~~	~~Nicosulfuron~~ [b]	~~7.8~~		
~~*Lemna gibba* [c]~~	~~14d EC_{50}~~	~~Dicamba~~	~~>3.25~~	~~0.020~~	~~0.0127~~
	~~7d EC_{50}~~	~~Mesotrione~~	~~0.0077~~		
	~~7d EC_{50}~~	~~Nicosulfuron~~	~~0.0034~~		

~~[a] Formerly *Selenastrum capricornatum*~~
~~[b] Endpoint derived from study with *Anabaena flos-aquae*~~
~~[c] Endpoints presented here for *Lemna* all based on dry weight in order to be comparable~~

zRMS comments:

Calculations presented above are not agreed by the zRMS since they were based on not correct assumptions:

- the estimated mixture endpoints were based on percentage concentration of individual active compounds, while in line with EFSA (2013) fraction of particular compounds should be taken into account with their sum being 1,
- E_bC_{50} values were considered in estimation of the endpoints for algae and *L. gibba* while E_rC_{50} values are relevant in line with EFSA (2013),
- the estimated endpoints expressed in terms of sum of active substances were compared with measured formulation toxicity expressed in terms of the product, while the formulation endpoints should be also expressed in terms of the active substance,
- MDR values were not calculated.

Respective calculations based on unrounded values were thus performed by the zRMS and their results are presented below.

Species	Fraction of a.s. in formulation			LC_{50}/EC_{50} [µg a.s./L]			EC_x [µg a.s./L]		MDR [c]
	Dicamba	Meso	Nico	Dicamba	Meso	Nico	PPP [a]	Mix-ca [b]	
Fish	0.56	0.27	0.18	100000	120000	65700	1970	94431	**47.9**
D. magna				41000	622000	90000	690	62140	**90.1**
Algae				3700	13000	8400	41	5167	**126**
Lemna gibba				3250	24.1	2.7	10.31	12.8	1.2

Meso: mesotrione Nico: nicosulfuron
[a] measured mixture toxicity, based on sum of active substances, see Table 9.5-4
[b] calculated mixture toxicity, ECxmix-CA
[c] MDR = $EC_{xmix\text{-}CA}/EC_{xPPP}$

MDR values for fish, *Daphnia magna* and algae are greater than 5 demonstrating that formulation is more toxic than the individual active substances. MDR of 1.2 (i.e. between 0.2 and 5) indicates that the measured and estimated toxicity of the mixture are in good agreement.

From this table it is clear that the toxicity of the formulation A18032E to the most sensitive species, the aquatic macrophyte *Lemna gibba*, is a reflection of the toxicity of the individual active substances dicamba, mesotrione and nicosulfuron and their relative loading in the formulated product. This is as expected for the herbicidal active substances.

However; the toxicity of the formulation to fish and invertebrate species is not a reflection of the additive toxicity of dicamba, mesotrione and nicosulfuron as the measured toxicity is lower than the predicted toxicity.

The formulated product A18032E contains multiple constituents alongside the active substances; the most toxic of the major constituents of this formulation were identified as dispersing agents named Morwet® D-425™ at a concentration in the formulation A18032E of 80 g/kg, equivalent to 8%, and Dispergator B Gran at a concentration in the formulation of 40 g/kg, equivalent to 4%.

Morwet® D-425™ has a three major ingredients, sodium sulphate, formaldehyde and naphthalene. The toxicities for these ingredients are as follows:

Sodium sulphate: EC_{50} fish = 7960 mg/L, *Daphnia* sp. = unavailable [21]
Formaldehyde: EC_{50} fish = 1.41 mg/L, *Daphnia magna* = 14 mg/L 21
Naphthalene: EC_{50} fish = 1.6 mg/L, *Daphnia pulex* = 1.0 mg/L 21

Dispergator B Gran contains a mixture of isomers of dibutylnaphthalene, sulphonic and acid sodium-salt. Thus, as a worst-case the toxicity endpoint for naphtalene can also be applied to Dispergator B Gran.
If the lowest endpoint for *Daphnia* sp. and *Oncorhynchus mykiss* for any of the three ingredients is taken and assumed as a worst-case to represent the toxicity of 100% of the Morwet® D-425™ content (80 g/kg in A18032E; 8% of the formulation) and the endpoints for naphtalene are taken to represent the toxicity of 100% of the Dispergator B Gran content (40 g/kg in A18032E; 4% of the formulation), the predicted mixture toxicity can be re-calculated and compared to the measured formulation toxicity.

Table 9.5-6: Comparison between observed acute toxicity of the formulation and predicted additive toxicity (Finney formula) for the formulation A18032E

Organism	Test endpoint	Active substance	Endpoint used (mg/L)	Calculated value based on additive toxicity of dicamba, mesotrione, nicosulfuron and co formulants Morwet D-425 & Dispergator B Gran (mg formulation/L)	Observed value for formulation (mg formulation/L)
Oncorhynchus mykiss	96h LC_{50}	Dicamba	>100	11.4	3.44
		Mesotrione	>120		
		Nicosulfuron	65.7		
		Morwet D-425 (as formaldehyde)	1.41		
		Dispergator B Gran (as napthalene)	1.6		
Daphnia magna	48h EC_{50}	Dicamba	>41	7.8	1.22
		Mesotrione	622		
		Nicosulfuron	90		
		Morwet D-425 (as naphthalene)	1.0		
		Dispergator B Gran (as napthalene)	1.0		

[21] AkzoNobel Material Safety Data Sheet. MSDS# 15-01856 Morwet® D-425, 25 May 2010.

It is clear that when the toxicity of Morwet® D-425™ and Dispergator B Gran is accounted for (as presented in the table above), the toxicity of A18032E is likely to be a reflection of the toxicity of the individual substances dicamba, mesotrione and nicosulfuron and the co-formulants Morwet® D-425™ and Dispergator B Gran when considering their relative loadings in the formulated product.

zRMS comments:

The zRMS agrees with the Applicant statement that synergistic effects of the mixture of all three compounds are not the only explanation of the increased toxicity of the formulation to fish and *Daphnia magna*, especially all three compounds are practically non-toxic to these groups of species. However, calculations presented in Table 9.5-6 are not agreed for the same reasons as indicated in the commenting box above for calculations of the combined toxicity based on active substance data. In order to derive reliable estimated and measured endpoints, both co-formulants must be included in formulation endpoints expressed in terms of the sum of compounds contributing to the toxicity of the formulation. Furthermore, estimated endpoints should be calculated with consideration of the fractions of particular compounds in formulation rather than their concentration. Respective calculations are presented below.

Organism	Active substance	Fraction in mixture	LC_{50}/EC_{50} [µg a.s./L]	EC_x [µg a.s./L]		MDR
				PPP [a]	Mix-ca [b]	
Fish	Dicamba	0.46	>100000	2390	7615	3.2
	Mesotrione	0.22	>120000			
	Nicosulfuron	0.15	65700			
	Morwet D-425 (as formaldehyde)	0.12	1410			
	Dispergator B Gran (as napthalene)	0.06	1600			
D. magna	Dicamba	0.46	>41000	840	5175	**6.2**
	Mesotrione	0.22	622000			
	Nicosulfuron	0.15	90000			
	Morwet D-425 (as naphthalene)	0.12	1000			
	Dispergator B Gran (as napthalene)	0.06	1000			

When toxic co-formulants are taken into account, MDR for fish indicates that the measured and estimated mixture toxicity are in good agreement and that toxicity of the formulation may be explained by the presence of co-formulants.
MDR calculated for *Daphnia magna* is above the maximum MDR of 5, but it is clearly lower than calculated based on the data only for active substances, which shows that co-formulants significantly contribute to the overall mixture toxicity.

No calculations could be performed for algae, but it cannot be excluded that similar pattern will be observed.

Although performed calculations indicate that synergistic effects of the active substances in A18032E are unlikely, the combined risk assessment is still necessary. However, due to presence of the toxic co-formulants calculation based on the endpoints expressed in terms of active substances compared with $PEC_{SW,MIX}$ calculated as a sum of PEC_{SW} values for individual compounds may be not relevant, since due to lack of respective efate data the toxic co-formulants will not be accounted for in the surface water exposure. Taking this into account the zRMS is of the opinion that in this particular case the risk assessment for the formulated product should be based on measured formulation endpoints expressed in terms of the product and PEC_{SW} values for the formulation derived using Spray Drift Calculator.
Nevertheless, risk assessment based on $PEC_{SW,MIX}$ for individual compounds compared with measured formulation endpoints expressed in terms of the sum of active substances will be also performed for completeness.

Assessment for the nicosulfuron metabolite MU-466 for all organism groups
MU-466 is not currently part of the SW residue definition (EFSA 2007) as it was only detected in lysimeter studies and hence it is not normally included in SW modelling.

"***Surface water***
Definitions for risk assessment (water): nicosulfuron, HMUD, AUSN, UCSN, ASDM, ADMP (all metabolites except HMUD only via soil)"
No unacceptable risk is expected for this non-active metabolite.

Assessment for the nicosulfuron metabolite DUDN for all organism groups
In the EFSA Scientific Report (2007) 120, 1-91 it is stated " The metabolite DUDN was formed in amount of > 10% in a hydrolysis study at a pH of 5 but was not found at a pH of 7 and higher. DUDN was not found in the water sediment study and it is not expected that surface waters with such a low pH occur frequently in agricultural landscapes." As major metabolites of nicosulfuron have low toxicity to the most sensitive aquatic organisms therefore due the low probability of exposure, DUDN is considered unlikely to represent a risk to aquatic organisms in surface water.

zRMS comments:

Information on nicosulfuron metabolites MU-466 and DUDN provided above is agreed by the zRMS. In line with current EU agreements, no specific risk assessment is required for these compounds.

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).

In the context of a risk envelope approach, risk assessments are only conducted for 0.4 and 0.6 kg A18032E/ha. The application rate of 0.45 kg A18032E/ha is covered by 0.6 kg A18032E/ha.

PEC_{SW} values for the active substances dicamba and mesotrione were calculated with higher application rates than given in GAP for this product. The application rates of 132 and 264 g dicamba/ha and the application rates of 75 and 100 g mesotrione/ha cover the maximum use rates given in the GAP of A18032E (125 and 187.5 g dicamba/ha, 60 and 90 g mesotrione/ha).

For mesotrione and its metabolites, PEC_{SW} values were calculated for acidic soils (pH 5.1), neutral soils (pH 6.5) and alkaline soils (pH 7 to 9). In a risk envelope approach the maximum PEC_{SW} values arising from any of the three soil types were used in the risk assessment.

In the tables below, for A18032E, dicamba, mesotrione, nicosulfuron and relevant metabolites, the regulatory acceptable concentrations (RACs) were derived by taking into account relevant endpoint values and the default safety factors in accordance with the EFSA Aquatic Guidance.

Table 9.5-7: Derivation of RAC values used in the Tier 1 risk assessment – dicamba and relevant metabolites

Species	Substance	Exposure system	Results (µg/L)	Assessment Safety factor	RAC (µg/L)
Cyprinus carpio	Dicamba	96 h, s	LC_{50} >100 000	100	>1000
Oncorhynchus mykiss	NOA414746 (DCSA)	96 h, ss	LC_{50} >100 000	100	>1000
Oncorhynchus mykiss	Dicamba	21 d, ss	NOEC = 180 000	10	18000
Daphnia magna	Dicamba (as 480 SL formulation)	48 h, s	EC_{50} >41 000	100	>410
Daphnia magna	NOA414746 (DCSA)	48 h, s	EC_{50} = 89 000	100	890
Daphnia magna	Dicamba	21 d, ss	NOEC = 97 000	10	9700
Selenastrum capricornutum (currently: *Pseudokirchneriella subcapitata*)	Dicamba	72 h, s	E_rC_{50} >3 700	10	>370
Pseudokirchneriella subcapitata	NOA414746 (DCSA)	72 h, s	E_rC_{50} = 138 000	10	13800
Lemna gibba	Dicamba	7 d, s	E_bC_{50} >3 250	10	>325
Lemna gibba	NOA414746 (DCSA)	7 d, s	E_rC_{50} >73 000 ~~E_bC_{50} >11900[a]~~	10	>7300 ~~>1190~~
Myriophyllum spicatum	Dicamba	26 d, s	E_rC_{50} or E_bC_{50} >450[a]	10	>45

d: days; h: hours; s: static; ss: semi-static; RAC: Regulatory acceptable concentration

[a] Based on the recommendations from the EFSA Aquatic Guidance, Syngenta propose that the worst case E_rC_{50} values are used in the algal and aquatic plant risk assessments. ~~This approach is widely accepted for algae. However, it is recognised that some regulatory authorities may have reservations about the use of E_rC_{50} values for macrophyte risk assessment when these are less conservative than $E_{b\ or\ y}C_{50}$ values, and therefore the $E_{b\ or\ y}C_{50}$ values will also be assessed~~

Table 9.5-8: Derivation of RAC values used in the Tier 1 risk assessment – mesotrione and relevant metabolites

Species	Substance	Exposure system	Results (µg/L)	Assessment Safety factor	RAC (µg/L)
Oncorhynchus mykiss	Mesotrione	96h, s	LC_{50} >120 000	100	>1200
Oncorhynchus mykiss	MNBA	96h, s	LC_{50} >120 000	100	>1200
Oncorhynchus mykiss	AMBA	96h, s	LC_{50} = 150 000	100	1500
Pimephales promelas	Mesotrione	36d chronic, f	NOEC = 12500	10	1250
Daphnia magna	Mesotrione	48h, s	EC_{50} >622 000	100	>6220
Daphnia magna	MNBA	48h, s	EC_{50} = 130 000	100	1300
Daphnia magna	AMBA	48h, s	EC_{50} = 160 000	100	1600
Daphnia magna	Mesotrione	21d, ss	NOEC = 180 000	10	18000
Pseudokirchneriella subcapitata	Mesotrione	120h, s	E_rC_{50} = 13 000	10	1300
Pseudokirchneriella subcapitata	MNBA	72h, s	E_rC_{50} = 42 000	10	4200
Pseudokirchneriella subcapitata	AMBA	72h, s	E_rC_{50} = 14 000	10	1400
Lemna gibba	Mesotrione	7d, ss	E_rC_{50} = 24.1 ~~E_rC_{50} = 28~~ ~~E_bC_{50} = 5.2[a]~~	10	2.41 ~~2.8~~ ~~0.52~~

Species	Substance	Exposure system	Results (µg/L)	Assessment Safety factor	RAC (µg/L)
Myriophyllum spicatum	Mesotrione	14d, ss	E_rC_{50} = 28.7	10	2.87
Macrophytes	Mesotrione	Geometric mean	E_rC_{50} = 26.3 ~~E_rC_{50} = 31~~ ~~E_yC_{50} = 4.0[a]~~	10	2.63 ~~3.1~~ ~~0.40~~
Lemna gibba	MNBA	7d, ss	E_rC_{50} or E_yC_{50} >97 000[a]	10	>9700
Lemna gibba	AMBA	7d, ss	E_rC_{50} or E_yC_{50} >90 000[a]	10	>9000
Lemna gibba	SYN546974	7d, ss	E_rC_{50} >95 000 ~~E_yC_{50} = 93 000[a]~~	10	>9500 ~~9300~~

d: days; h: hours; s: static; ss: semi-static; f: flow-through; RAC: Regulatory acceptable concentration

[a] Based on the recommendations from the EFSA Aquatic Guidance, Syngenta propose that the worst case E_rC_{50} values are used in the algal and aquatic plant risk assessments. ~~This approach is widely accepted for algae. However, it is recognised that some regulatory authorities may have reservations about the use of E_rC_{50} values for macrophyte risk assessment when these are less conservative than $E_{b \text{ or } y}C_{50}$ values, and therefore the $E_{b \text{ or } y}C_{50}$ values will also be assessed~~

Table 9.5-9: Derivation of RAC values used in the Tier 1 risk assessment – nicosulfuron and relevant metabolites

Species	Substance	Exposure system	Results (µg/L)	Assessment Safety factor	RAC (µg/L)
Oncorhynchus mykiss	Nicosulfuron	96 h, s	LC_{50} = 65 700	100	657
Lepomis macrochirus	ASDM	96 h, ss	LC_{50} > 100 000	100	>1000
Brachydanio rerio	AUSN	96 h, s	LC_{50} > 100 000	100	>1000
Oncorhynchus mykiss	HMUD	96 h, s	LC_{50} > 100 000	100	>1000
~~*Brachydanio rerio*~~	~~UCSN~~	~~96 h, s~~	~~LC_{50} > 100 000~~	~~100~~	~~>1000~~
Oncorhynchus mykiss	ADMP	96 h, s	LC_{50} > 100 000	100	>1000
Oncorhynchus mykiss	Nicosulfuron	28 d, f	NOEC = 10 000	10	1000
Daphnia magna	Nicosulfuron	48 h, s	EC_{50} = 90 000	100	900
Daphnia magna	ASDM	48 h, s	EC_{50} > 954 000	100	>9540
Daphnia magna	AUSN	48 h, s	EC_{50} > 100 000	100	>1000
Daphnia magna	HMUD	48 h, s	EC_{50} > 100 000	100	>1000
Daphnia magna	UCSN	48 h, s	EC_{50} > 100 000	100	>1000
Daphnia magna	ADMP	48 h, s	EC_{50} > 100 000	100	>1000
Daphnia magna	Nicosulfuron	21 d, ss	NOEC = 5 200	10	520
Anabaena flos-aquae	Nicosulfuron	72 h, s	E_rC_{50} = 8 400	10	840
Pseudokirchneriella subcapitata	ASDM	72 h, s	E_rC_{50} > 336 000	10	>33600
Scenedesmus subspicatus	AUSN	72 h, s	E_rC_{50} > 100 000	10	>10000
Scenedesmus subspicatus	HMUD	72 h, s	E_rC_{50} > 100 000	10	>10000
Scenedesmus subspicatus	UCSN	72 h, s	E_rC_{50} > 100 000	10	>10000
Scenedesmus subspicatus	ADMP	72 h, s	E_rC_{50} > 100 000	10	>10000
Lemna gibba	Nicosulfuron	7 d, ss	E_rC_{50} = 2.7 ~~E_bC_{50} = 1.7[a]~~	10	0.27 ~~0.17~~
Lemna gibba	ASDM	7 d, ss	E_rC_{50} or E_bC_{50} > 100 000[a]	10	>10000
Lemna gibba	AUSN	7 d, ss	E_rC_{50} or E_bC_{50} > 100 000[a]	10	>10000
Lemna gibba	HMUD	7 d, ss	E_rC_{50} or E_bC_{50} > 1 000[a]	10	>100

Species	Substance	Exposure system	Results (µg/L)	Assessment Safety factor	RAC (µg/L)
Lemna gibba	UCSN	7 d, ss	E_rC_{50} or E_bC_{50} > 100 000[a]	10	>10000
~~*Myriophyllum aquaticum*~~	~~Nicosulfuron~~	~~7 d, s~~	~~E_rC_{50} > 3523~~ ~~E_bC_{50} = 3071[a]~~	~~10~~	~~>352.3~~ ~~307.1~~

d: days; h: hours; s: static; ss: semi-static; f: flow-through; RAC: Regulatory acceptable concentration

[a] Based on the recommendations from the EFSA Aquatic Guidance, Syngenta propose that the worst case E_rC_{50} values are used in the algal and aquatic plant risk assessments. ~~This approach is widely accepted for algae. However, it is recognised that some regulatory authorities may have reservations about the use of E_rC_{50} values for macrophyte risk assessment when these are less conservative than $E_{b \text{ or } y}C_{50}$ values, and therefore the $E_{b \text{ or } y}C_{50}$ values will also be assessed~~

Table 9.5-10: Derivation of RAC values used in the Tier 1 risk assessment – A18032E

Species	Substance	Exposure system	Results (µg/L)	Assessment Safety factor	RAC (µg/L)
Oncorhynchus mykiss	A18032E	96 h, s	LC_{50} = 3 440 (1970 for Σ of a.s.)	100	34.4 (19.7 for Σ of a.s.)
Daphnia magna	A18032E	48 h, s	EC_{50} = 1 220 (690 for Σ of a.s.)	100	12.2 (6.9 for Σ of a.s.)
Pseudokirchneriella subcapitata	A18032E	72 h, s	E_rC_{50} = 72.8 (41 for Σ of a.s.)	10	7.28 (4.1 for Σ of a.s.)
Lemna gibba	A18032E	7 d, ss	E_rC_{50} = 18.1 (10.31 for Σ of a.s.) ~~E_yC_{50} = 6.43[a]~~	10	1.81 (1.03 for Σ of a.s.) ~~0.643~~

d: days; h: hours; s: static; ss: semi-static; RAC: Regulatory acceptable concentration

[a] Based on the recommendations from the EFSA Aquatic Guidance, Syngenta propose that the worst case E_rC_{50} values are used in the algal and aquatic plant risk assessments. ~~This approach is widely accepted for algae. However, it is recognised that some regulatory authorities may have reservations about the use of E_rC_{50} values for macrophyte risk assessment when these are less conservative than $E_{b \text{ or } y}C_{50}$ values, and therefore the $E_{b \text{ or } y}C_{50}$ values will also be assessed~~

zRMS comments:

Derivation of RAC values presented in Tables 9.5-7 to 9.5-10 above is in general agreed by the zRMS with some minor corrections resulting from evaluation of the additional studies submitted in support of evaluation of A18032E. For discussion on agreed endpoints and justification for consideration of new endpoints for some species and compounds, please refer to zRMS comments in point 9.5.1 above.

It is noted that no fish endpoint is reported for metabolite UCSN and for this reason RAC for this compound is struck through in Table 9.5-9. Furthermore, study on toxicity of nicosulfuron to *Myriophyllum aquaticum* (Wenzel, 2010) was agreed by the zRMS as supportive information to confirm higher sensitivity of *Lemna gibba* to nicosulfuron, but due to too short exposure duration endpoints from the study were considered to be not relevant for the risk assessment purposes. For this reason RAC derived for M. aquaticum has been struck through in Table 9.5-9.

In addition to RAC values based on formulation endpoints, also RAC values based on the sum of individual active compounds were added to Table 9.5-10.

For primary producers RAC values based E_bC_{50} / E_yC_{50} are struck through in tables above, since in line with EFSA (2013) only E_rC_{50} values are relevant for the risk assessment.

In the following tables, the ratios between predicted environmental concentrations in surface water bodies (PEC_{SW}, PEC_{SED}) and regulatory acceptable concentrations (RAC) for aquatic organisms are given per intended use for each FOCUS scenario, each organism group, and each substance (active substances, relevant metabolites, and formulation A18032E).

Dicamba

Table 9.5-11: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for dicamba for each organism group based on maximum FOCUS PEC_{SW} calculations for the use of A18032E in maize (1 x 264 g a.s./ha, post-emergence; risk envelope covering the proposed uses at 125 g a.s./ha)

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Aquatic macrophyte - *Lemna* (based on E_bC_{50})	Aquatic macrophyte - *Myriophyllum* (based on E_rC_{50} and E_bC_{50})
RAC (µg/L)		>1000	18000	>410	9700	>370	>325	>45
FOCUS Scenario	**PEC_{gl-max} (µg/L)**							
Step 1								
	44.7 ~~89.3~~	<0.045 ~~<0.089~~	0.02 ~~0.0050~~	<0.109 ~~<0.22~~	0.005 ~~0.0092~~	<0.121 ~~<0.24~~	<0.138 ~~<0.27~~	0.993 ~~<2.0~~
~~**Step 2**~~								
~~N-Europe~~	~~8.77~~	~~*~~	~~*~~	~~*~~	~~*~~	~~*~~	~~*~~	~~<0.19~~
~~S-Europe~~	~~15.3~~	~~*~~	~~*~~	~~*~~	~~*~~	~~*~~	~~*~~	~~<0.34~~

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
* No further assessmentis required as an acceptable risk has been demonstrated in a more conservative FOCUS scenario

Dicamba metabolite NOA414746

Table 9.5-12: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the dicamba metabolite NOA414746 (DCSA) for each organism group based on maximum FOCUS PEC_{SW} calculations for the use of A18032E in maize (1 x 264 g a.s./ha, post-emergence)

Group		Fish acute	Inverteb. acute	Algae	Aquatic macrophyte (based on E_rC_{50})	~~Aquatic macrophyte (based on E_bC_{50})~~
RAC (µg/L)		>1000	890	13800	>7300	~~>1190~~
FOCUS Scenario	**PEC_{gl-max} (µg/L)**					
Step 1						
	17.5 ~~35.0~~	<0.018 ~~<0.035~~	0.020 ~~0.039~~	0.001 ~~0.0025~~	<0.002 ~~<0.0048~~	0.018 ~~<0.029~~

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

zRMS comments:

It is noted that calculations were based on PEC_{SW} values derived for exaggerated application rate of 264 g a.s./ha, however PEC_{SW} calculations for this application rate were not presented in area of Section 8, where PEC_{SW} values for dicamba and DCSA were based on rate of 132 g dicamba/ha (covering application rate of dicamba in A18032E at 125 g/ha). Taking this into account Tables 9.5-11 and 9.5-12 were amended with surface water exposure as agreed in Section 8.

Acceptable risk to aquatic organisms from exposure to dicamba and metabolite DCSA may be concluded following application of A18032E to maize (BBCH 12-14) at 0.4 kg/ha (corresponding to 125 g dicamba/ha) with no need for risk mitigation measures.

The Applicant is kindly reminded that exposure estimates considered in the risk assessment presented in area of Section 9 must correspond with PEC values presented in area of Section 8.

Calculations for primary producers based on RAC values derived using E_bC_{50} / E_yC_{50} are struck through in tables above, since in line with EFSA (2013) only E_rC_{50} values are relevant for the risk assessment.

Mesotrione

Table 9.5-13: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for mesotrione for each organism group based on maximum FOCUS PEC_{SW} calculations for the use of A18032E in maize (1 x 75 g a.s./ha, post-emergence; risk envelope covering the proposed uses at 60 g a.s./ha)

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Aquatic macrophyte (geomean E_rC_{50})	~~Aquatic macrophyte (geomean E_yC_{50})~~
RAC (µg/L)		>1200	1250	>6220	18000	1300	2.63 ~~3.1~~	~~0.40~~
FOCUS Scenario	**PEC_{gl-max} (µg/L)**							
Step 1								
	25.1	<0.021	0.020	<0.0040	0.0014	0.019	**9.5** ~~**8.1**~~	~~**63**~~
Step 2								
N-Europe	3.28	*	*	*	*	*	**1.2** ~~**1.1**~~	~~**8.2**~~
S-Europe	6.17	*	*	*	*	*	**2.3** ~~**2.0**~~	~~**15**~~
Step 3								
D3/Ditch	0.394	*	*	*	*	*	0.150 ~~0.13~~	~~0.99~~
D4/Pond	0.042	*	*	*	*	*	0.016 ~~0.014~~	~~0.11~~
D4/Stream	0.339	*	*	*	*	*	0.129 ~~0.11~~	~~0.85~~
D5/Pond	0.023	*	*	*	*	*	0.009 ~~0.0074~~	~~0.058~~
D5/Stream	0.344	*	*	*	*	*	0.131 ~~0.11~~	~~0.86~~
D6/Ditch	0.396	*	*	*	*	*	0.151 ~~0.13~~	~~0.99~~
R1/Pond	0.057	*	*	*	*	*	0.022 ~~0.018~~	~~0.14~~
R1/Stream	1.20	*	*	*	*	*	0.456 ~~0.39~~	~~**3.0**~~
R2/Stream	1.61	*	*	*	*	*	0.612 ~~0.52~~	~~**4.0**~~
R3/Stream	2.95	*	*	*	*	*	**1.1** ~~0.95~~	~~**7.4**~~
R4/Stream	3.12	*	*	*	*	*	**1.2** ~~**1.0**~~	~~**7.8**~~

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
* No further assessmentis required as an acceptable risk has been demonstrated in a more conservative FOCUS scenario

For macrophytes, calculated PEC/RAC ratios did not indicate an acceptable risk to aquatic macrophytes in FOCUS Steps 1 and 2, and in the R4/Stream scenario in Step 3 considering the E_rC_{50}, or R1-4/Stream scenarios considering the E_yC_{50}. Therefore, further refinement is required. The risk to macrophytes will be refined by considering mitigation at Step 4, and higher tier realistic pulsed dose exposure studies.

Table 9.5-14: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for mesotrione for each organism group based on maximum FOCUS PEC_{SW} calculations for the use of A18032E in maize (1 x 100 g a.s./ha, post-emergence; risk envelope covering the proposed use at 60 g a.s./ha)

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Aquatic macrophyte (geomean E_rC_{50})	Aquatic macrophyte (geomean E_yC_{50})
RAC (µg/L)		>1200	1250	>6220	18000	1300	3.1	0.40
FOCUS Scenario	$PEC_{gl\ max}$ (µg/L)							
Step 1								
-	33.5	<0.028	0.027	<0.0054	0.0019	0.026	**11**	**84**
Step 2								
N-Europe	4.38	*	*	*	*	*	**1.4**	**11**
S-Europe	8.22	*	*	*	*	*	**2.7**	**21**
Step 3								
D3/Ditch	0.525	*	*	*	*	*	0.17	**1.3**
D4/Pond	0.056	*	*	*	*	*	0.018	0.14
D4/Stream	0.451	*	*	*	*	*	0.15	**1.1**
D5/Pond	0.031	*	*	*	*	*	0.010	0.078
D5/Stream	0.459	*	*	*	*	*	0.15	**1.1**
D6/Ditch	0.527	*	*	*	*	*	0.17	**1.3**
R1/Pond	0.076	*	*	*	*	*	0.025	0.19
R1/Stream	1.60	*	*	*	*	*	0.52	**4.0**
R2/Stream	2.16	*	*	*	*	*	0.70	**5.4**
R3/Stream	3.94	*	*	*	*	*	**1.3**	**9.9**
R4/Stream	4.16	*	*	*	*	*	**1.3**	**10**

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
* No further assessmentis required as an acceptable risk has been demonstrated in a more conservative FOCUS scenario

For macrophytes, calculated PEC/RAC ratios did not indicate an acceptable risk to aquatic macrophytes in FOCUS Steps 1 and 2, and in the R3/Stream and R4/Stream scenarios in Step 3 considering the E_rC_{50}, or all ditch and stream scenarios considering the E_yC_{50}. Therefore, further refinement is required. The risk to macrophytes will be refined by considering mitigation at Step 4, and higher tier realistic pulsed dose exposure studies.

Refinement of risk for macrophytes considering FOCUS Step 4

Table 9.5-15: Aquatic macrophytes: refined higher-tier risk assessment for acceptability of risk (PEC/RAC < 1) for mesotrione based on mitigation at FOCUS Step 4 following application of mesotrione to maize at 1 x 75 g a.s./ha, post-emergence (risk envelope covering the proposed uses at 60 g a.s./ha)

Group						Aquatic macrophyte E_rC_{50}	~~Aquatic macrophyte E_yC_{50}~~
RAC (µg/L)						Tier 1 geomean RAC based on E_rC_{50} = 2.63 ~~3.1~~	~~Tier 1 geomean RAC based on E_yC_{50} = 0.40~~
Application regime	**FOCUS Scenario**	**Vegetative filter strip (m)** [a]	**No spray buffer (m)**	**Nozzle reduction (%)**	**PEC (µg/L)**	**PEC/RAC**	~~**PEC/RAC**~~
1 x 75 g a.s./ha, post-emergence	**Step 4**						
	R1/stream (acidic scenario)	-	-	50	1.20	*	~~**3.0**~~
		-	5	-	1.20	*	~~**3.0**~~
		10 (L&M)	10	-	0.544	*	~~**1.4**~~
		20 (L&M)	20	-	0.284	*	~~0.71~~
		5 (VFSmod)	5	-	0.113	*	~~0.28~~
	R2/stream (neutral scenario)	-	-	50	1.61	*	~~**4.0**~~
		-	5	-	1.61	*	~~**4.0**~~
		10 (L&M)	10	-	0.708	*	~~**1.8**~~
		20 (L&M)	20	-	0.367	*	~~0.92~~
		5 (VFSmod)	5	-	0.154	*	~~0.39~~
	R3/stream (neutral scenario)	-	-	50	2.95	**1.1**	~~**7.4**~~
		-	5	-	2.95	**1.1**	~~**7.4**~~
		10 (L&M)	10	-	1.33	0.506	~~**3.3**~~
		20 (L&M)	20	-	0.697	0.265	~~**1.7**~~
		5 (VFSmod)	5	-	0.161	0.061	~~0.40~~
	R4/stream (neutral scenario)	-	-	50	3.12	**1.2** ~~1.0~~	~~**7.8**~~
		-	5	-	3.12	**1.2** ~~1.0~~	~~**7.8**~~
		10 (L&M)	10	-	1.42	0.540 ~~0.46~~	~~**3.6**~~
		20 (L&M)	20	-	0.742	0.282 ~~0.24~~	~~**1.9**~~
		5 (VFSmod)	5	-	0.114	0.043 ~~0.037~~	~~0.29~~

[a] L&M = mitigation according to FOCUS Landscape and Mitigation V1 (2007); reduction for 10 / 20 m buffer is 60 / 80 % in runoff flux and volume and 85 / 95 % in sediment flux and mass
VFSmod = simulated using VFSMod tool included in SWAN v 4.0.1

* No further assessment is required as an acceptable risk has been demonstrated in a more conservative FOCUS scenario

PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

The comparison of the refined PEC and RAC values indicates acceptable risk to aquatic macrophytes following 1 application of 75 g mesotrione/ha to maize post-emergence as follows:

- R scenarios:
  - Considering the E_rC_{50}:
    - R3 and R4: when a 10 m vegetated buffer zone according to FOCUS Landscape and Mitigation V1 (2007) buffer is considered, or a 5 m VFSmod buffer is used
  - ~~Considering the E_yC_{50}:~~
    - ~~R1, R2: when a 20 m vegetated buffer zone according to FOCUS Landscape and Mitigation V1 (2007) is considered, or a 5 m VFSmod buffer is used~~
    - ~~R3, R4: when a 5 m VFSmod buffer is used.~~

Table 9.5-16: Aquatic macrophytes: refined higher-tier risk assessment for acceptability of risk (PEC/RAC < 1) for mesotrione based on mitigation at FOCUS Step 4 following application of mesotrione to maize at 1 x 100 g a.s./ha, post-emergence (risk envelope covering the proposed uses at 60 g a.s./ha)

Group						Aquatic macrophyte E_rC_{50}	Aquatic macrophyte E_yC_{50}
RAC (µg/L)						Tier 1 geomean RAC based on E_rC_{50} = 3.1	Tier 1 geomean RAC based on E_yC_{50} = 0.40
Application regime	FOCUS Scenario	Vegetative filter strip (m) [a]	No spray buffer (m)	Nozzle reduction (%)	PEC (µg/L)	PEC/RAC	PEC/RAC
1 x 100 g a.s./ha, post-emergence	Step 4						
	D3/ditch (neutral scenario)	-	-	50	0.263	*	0.66
		-	5	-	0.172	*	0.43
		10 (L&M)	10	-	0.091	*	0.23
	D4/stream (acidic scenario)	-	-	50	0.227	*	0.57
		-	5	-	0.191	*	0.48
		10 (L&M)	10	-	0.102	*	0.26
	D5/stream (acidic scenario)	-	-	50	0.235	*	0.59
		-	5	-	0.199	*	0.50
		10 (L&M)	10	-	0.111	*	0.28
	D6/ditch (acidic scenario)	-	-	50	0.265	*	0.66
		-	5	-	0.175	*	0.44
		10 (L&M)	10	-	0.094	*	0.24
	R1/stream (acidic scenario)	-	-	50	1.60	*	**4.0**
		-	5	-	1.60	*	**4.0**
		10 (L&M)	10	-	0.724	*	**1.8**
		20 (L&M)	20	-	0.379	*	0.95
		5 (VFSmod)	5	-	0.150	*	0.38
	R2/stream (neutral scenario)	-	-	50	2.16	*	**5.4**
		-	5	-	2.16	*	**5.4**
		10 (L&M)	10	-	0.952	*	**2.4**
		20 (L&M)	20	-	0.493	*	**1.2**
		5 (VFSmod)	5	-	0.205	*	0.51
	R3/stream (neutral scenario)	-	-	50	3.94	**1.3**	**9.9**
		-	5	-	3.94	**1.3**	**9.9**

Group						Aquatic macrophyte E_rC_{50}	Aquatic macrophyte E_yC_{50}
		10 (L&M)	10	-	1.78	0.57	**4.5**
		20 (L&M)	20	-	0.931	0.30	**2.3**
		5 (VFSmod)	5	-	0.215	0.069	0.54
	R4/stream (neutral scenario)	-	-	50	4.16	**1.3**	**10**
		-	5	-	4.16	**1.3**	**10**
		10 (L&M)	10	-	1.89	0.61	**4.7**
		20 (L&M)	20	-	0.992	0.32	**2.5**
		5 (VFSmod)	5	-	0.153	0.049	0.38

[a] L&M = mitigation according to FOCUS Landscape and Mitigation V1 (2007); reduction for 10 / 20 m buffer is 60 / 80 % in runoff flux and volume and 85 / 95 % in sediment flux and mass
VFSmod = simulated using VFSMod tool included in SWAN v 4.0.1
PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**
* No further assessment is required as an acceptable risk has been demonstrated in a more conservative FOCUS scenario

The comparison of the refined PEC and RAC values indicates acceptable risk to aquatic macrophytes following 1 application of 100 g mesotrione/ha to maize post-emergence as follows:

- D scenarios, considering the E_yC_{50}: when a 5 m buffer or 50% drift-reducing nozzles are considered
- R scenarios:
  - Considering the E_rC_{50}:
    - R3 and R4: when a 10 m vegetated buffer zone according to FOCUS Landscape and Mitigation V1 (2007) buffer is considered, or a 5 m VFSmod buffer is used
  - Considering the E_yC_{50}:
    - R1: when a 20 m vegetated buffer zone according to FOCUS Landscape and Mitigation V1 (2007) is considered, or a 5 m VFSmod buffer is used
    - R2, R3, R4: when a 5 m VFSmod buffer is used.

zRMS comments:

The risk assessment presented in Tables 9.5-13 and 9.5-15 above is in general agreed by the zRMS, however the PEC/RAC values for aquatic macrophytes were amended accordingly with consideration of RAC based on endpoints agreed by the zRMS (for details, please refer to point 9.5.1 above). It is noted that in the risk assessment presented in tables mentioned, PEC_{SW} values calculated for mesotrione application at 75 g a.s./ha were considered, covering the intended rate of mesotrione in A18032E (i.e. 60 g a.s./ha).

For fish, aquatic invertebrates and algae acceptable acute and chronic risk could be concluded already for Step 1 PEC_{SW} values.

On the basis of the corrected calculations, acceptable risk for aquatic macrophytes could be concluded with Step 3 PEC_{SW} in scenarios D3, D4, D5, D6, R1 and R2, while for scenarios R3 and R4 further calculations based on Step 4 PEC_{SW} were deemed necessary.

Overall, following conclusions could be derived:

- acceptable risk to aquatic organisms with no need for risk mitigation measures was demonstrated in scenarios D3, D4, D5, D6, R1 and R2,
- acceptable risk to aquatic organisms with consideration of 10 m vegetated filter strip or 5 m VFSmod buffer was demonstrated in scenarios R3 and R4.

It is noted that additional risk assessment for application rate of 100 g mesotrione/ha was also presented by the Applicant in Tables 9.5-14 and 9.5-16. However, since calculations for application of 75 g mesotrione/ha are sufficient to cover intended rate of A18032E at 60 g mesotrione/ha, risk assessment for rate of 100 g a.s./ha was struck through above. It is further noted that PEC_{SW} calculations for application rate of 100 g a.s./ha were not presented in area of Section 8, where PEC_{SW} values for mesotrione and its metabolites were based on rate of 75 g a.s./ha (covering application rate of mesotrione in A18032E at 60 g/ha).

The Applicant is kindly reminded that exposure estimates considered in the risk assessment presented in area of Section 9 must correspond with PEC values presented in area of Section 8. It is further noted that there is no need to present the risk assessment based on exposure estimates available for all application rates for which the surface water modelling has been performed for the given substance, but evaluation should be focused either on the target rate or application rate forming a risk envelope (here: 75 g a.s./ha). Otherwise, the report is becoming less transparent since large parts of unnecessary evaluation must be struck through.

Calculations for primary producers based on RAC values derived using E_bC_{50} / E_yC_{50} are struck through in tables above, since in line with EFSA (2013) only E_rC_{50} values are relevant for the risk assessment.

zRMS comments:

It should be noted that most of the Central Zone Member States has concerns with use of the modified exposure studies due to uncertainties related to the exposure profiles modelled using FOCUS. Extensive discussion regarding this issue took place during the Central Zone harmonisation meetings and it was concluded that results of Tier 2C studies should be considered only when no acceptable risk may be demonstrated using standard approach (i.e. standard toxicity endpoints and exposure calculated with consideration of the risk mitigation measures). The same is stated in the document presenting the specific national requirements in area of environmental exposure and risk assessment in Poland.
For mesotrione applied as A18032E acceptable risk to aquatic organisms could be concluded using the endpoint required by EFSA, 2013 (i.e. E_rC_{50}) and applying standard risk mitigation measures (see commenting box above). In case of scenarios relevant for Poland (D3, D4 and R1), no mitigation measures were deemed necessary to resolve the risk to aquatic organisms from mesotrione. Taking this into account, no further assessment is deemed necessary at the zonal level and the provided below refinement based on Tier 2C studies was not evaluated by the zRMS and is thus struck through.

Refinement of risk for macrophytes considering realistic exposure

The R stream scenarios are characterised by short-lived peaks of exposure which are quickly dissipated. This exposure is demonstrated by EPAT for the FOCUS Step 3 and 4 outputs presented in the following report, which is referenced and summarised in the dRR Core Section 8 (environmental fate section):

Report:	Ibrahim L. (2017a) Mesotrione – A European Environmental Fate Assessment for Parent Using the FOCUS Surface Water Models at Steps 3 to 4 Following Spray Application to Maize and an Analysis of its FOCUS Step 3 and 4 Exposure Patterns Using the EPAT Tool. RIFCON GmbH, Report No. 1520528-2 (Syngenta File No. ZA1296_10482)

a) Considering the RAC based on the E_rC_{50}:

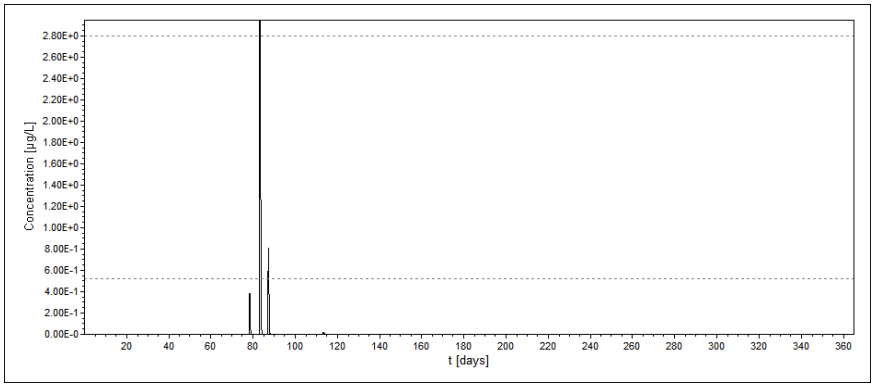
Please note that the EPAT work was carried out prior to the availability of the Myriophyllum endpoint; however the Lemna and Myriophyllum endpoints as so similar that in practice any differences in the EPAT analysis below will be minimal

Exposure events exceeding the threshold RAC of 2.8 µg a.s./L were identified and examined. The following table summarises the duration and frequency of exposure peaks exceeding the RAC:

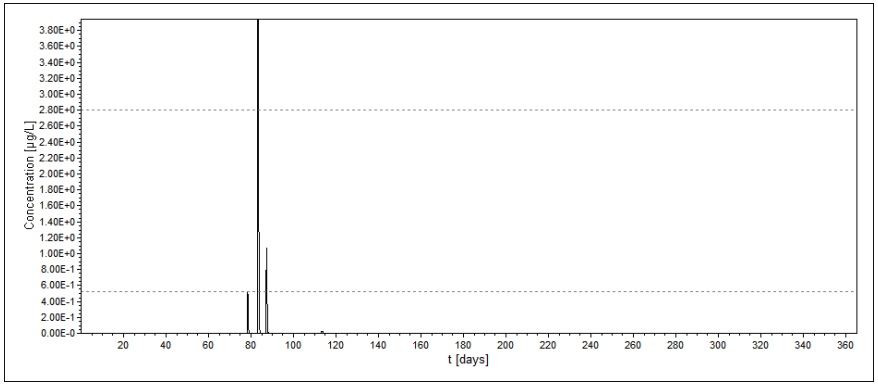
Table 9.5-17: Duration and frequency of exposure events exceeding the RAC of 2.8 µg a.s./L following post-emergence application to maize at 75 and 100 g a.s./ha as determined with EPAT v1.1 (risk envelope covering the proposed uses at 60, 67.5 and 90g a.s./ha)

FOCUS scenario	Scenario	Soil type giving worst-case	Event No.	Start date & time	Max conc. (µg/L)	Duration (days)	Interval (days)
Maize, 1 x 75 g a.s./ha, post-emergence							
FOCUS Step 3	R4/stream	Neutral soil	1	18/04/1984 01:00:00	3.12	0.666	-
Maize, 1 x 100 g a.s./ha, post-emergence							
FOCUS Step 3	R3/stream	Neutral soil	1	23/05/1980 02:00:00	3.94	0.500	-
	R4/stream	Neutral soil	1	18/04/1984 01:00:00	4.16	0.750	-

It is therefore clear that the maximum concentrations leading to an exceedance of the RAC are limited to a single occurrence lasting less than 24 hours. The profiles of exposure are illustrated in the figures below. Further details are provided in the dRR document Section 8.



~~Figure 9.5-1: Exposure profile for the R4 Stream Scenario for FOCUS Step 3 (1 × 75 g a.s./ha, post-emergence) – neutral soil. The upper dashed line indicates the relevant RAC of 2.8 µg a.s./L, the lower line indicates a RAC of 0.52 µg a.s./L.~~



~~Figure 9.5-2: Exposure profile for the R3 Stream Scenario for FOCUS Step 3 (1 × 100 g a.s./ha, post-emergence) – neutral soil. The upper dashed line indicates the relevant RAC of 2.8 µg a.s./L, the lower line indicates a RAC of 0.52 µg a.s./L.~~

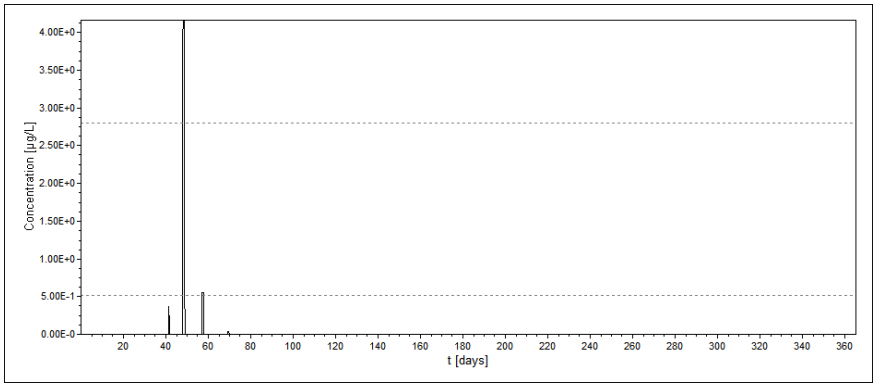


Figure 9.5-3: Exposure profile for the R4 Stream Scenario for FOCUS Step 3 (1 × 100 g a.s./ha, post-emergence) - neutral soil. The upper dashed line indicates the relevant RAC of 2.8 µg a.s./L, the lower line indicates a RAC of 0.52 µg a.s./L.

It is therefore clear that the standard Tier 1 *Lemna* test is the very worst case for assessment of these types of exposure, since the exposure concentrations were maintained over a period of 7 days within 20% of the nominal values, as stated in the EU summary presented at the end of this document. In accordance with the recommendations from E Link[22], an explicit link should be made between the exposure in the toxicity tests, and the realistic predicted exposure.

A new test was therefore carried out to specifically investigate the impact of a short pulse of exposure of mesotrione on the growth rate of *Lemna gibba*. This study is summarised in detail under Appendix 2. In this study (Hengsberger and Wydra, 2015a), *Lemna* were exposed to a single 24-h pulse of mesotrione at 60 µg a.s./L. Other exposure scenarios were also considered but are not relevant in the current assessment where the pulse of exposure exceeding the RAC is predicted to last less than 24 hours, and there are no further exceedances expected. Exposure to a single 24-h pulse of mesotrione at 60 µg a.s./L had less than 50% effects after 7 days, and this endpoint will be used (maximum 14% inhibition of growth rate) in order to further consider the R3 and R4 scenarios. Since this is still just a modified exposure Tier 1, 7-d study, the standard safety assessment of 10 will be retained giving a pulsed exposure (24 h) RAC = 6 µg/L.

A similar pulsed exposure test was carried out with *Myriophyllum* in the test by Gonsior (2017). In this test a 24h pulse of exposure to mesotrione at 70 and 120 µg/L had no significant inhibitory effect compared to the controls in any parameter measured after 14 days. Again considering a standard assessment factor of 10, this would give a pulsed exposure RAC of 12 µg/L. Since the result for *Lemna* is more conservative, this is considered in the calculations below.

Table 9.5-18: Aquatic macrophytes (*Lemna*): refined higher-tier risk assessment for acceptability of risk (PEC/RAC < 1) for mesotrione based on realistic pulsed exposure testing and/or mitigation at FOCUS Step 4 following application of mesotrione to maize at 1 x 75 g a.s./ha and 1 x 100 g a.s./ha, post-emergence (risk envelope covering the proposed uses at 60 a.s./ha)

Group						**Aquatic macrophyte**
RAC (µg/L)						Pulsed exposure 24 h RAC = 6.0
Application regime	**FOCUS Scenario**	**Vegetative filter strip (m)**	**No spray buffer (m)**	**Nozzle reduction (%)**	**PEC (µg/L)**	**PEC / Pulsed exposure RAC**
Maize, 1 x 75 g a.s./ha, post-emergence	**Step 3**					
	R4/stream	-	-	-	3.12	0.52
Maize, 1 x 100 g a.s./ha, post-emergence	**Step 3**					
	R3/stream	-	-	-	3.94	0.66
	R4/stream	-	-	-	4.16	0.69

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

Considering a realistic pulsed exposure RAC the comparison of the refined PEC and RAC values indicate acceptable risk to aquatic macrophytes following 1 application of 75 or 100 g mesotrione/ha to maize without mitigation requirements.

[22] Brock T et al. (2009) EXECUTIVE SUMMARY AND RECOMMENDATIONS. EU & SETAC Europe Workshop on Linking Aquatic Exposure and Effects in the Registration Procedure of Plant Protection Products (ELINK) SETAC Press & CRC Press, Taylor & Francis Group

b) Considering the RAC based on the $E_{b\ or\ y}C_{50}$:

Please note that the EPAT work was carried out prior to the availability of the Myriophyllum endpoint; however the Lemna and Myriophyllum endpoints as so similar that in practice any differences in the EPAT analysis below will be minimal.

Exposure events exceeding the threshold E_bC_{50} RAC of 0.52 µg/L were identified and examined. The following table summarises the duration and frequency of exposure peaks exceeding this RAC.

Table 9.5-19: Duration and frequency of exposure events exceeding the E_bC_{50} RAC of 0.52 µg a.s./L following post-emergence application to maize at 75 g a.s./ha and 100 g a.s./ha as determined with EPAT v1.1 (risk envelope covering the proposed uses at 60, 67.5 and 90 g a.s./ha)

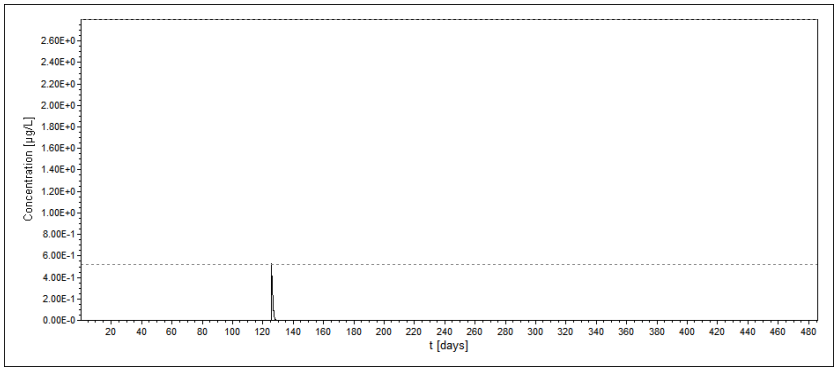
FOCUS scenario	Scenario	Soil type giving worst-case profile	Event No.	Start date & time	Max conc. (µg/L)	Duration (days)	Interval (days)
Maize, 1 x 75 g a.s./ha, post-emergence							
FOCUS Step 3	R1/stream	Acidic soil	1	14/05/1984 05:00:00	0.582	0.167	-
			2	20/05/1984 01:00:00	1.20	0.541	5.67
	R2/stream	Neutral soil	1	13/05/1977 02:00:00	1.61	0.625	-
	R3/stream	Neutral soil	1	23/05/1980 01:00:00	2.95	0.750	-
			2	27/05/1980 04:00:00	0.808	0.375	3.38
	R4/stream	Acidic soil	1	18/04/1984 01:00:00	2.67	0.875	-
			2	27/04/1984 01:00:00	0.797	0.750	8.13
FOCUS Step 4	R1/stream 10 (L&M)	Acidic soil	1	20/05/1984	0.544	0.375	-
	R3/stream 10 (L&M)	Neutral soil	1	23/05/1980	1.33	0.584	-
	R4/stream 10 (L&M)	Neutral soil	1	18/04/1984	1.42	0.833	-
Maize, 1 x 100 g a.s./ha, post-emergence							
FOCUS Step 3	D3/ditch	All soil scenarios	1	05/05/1992 09:00:00	0.525	0.042	-
	D4/stream	*Acidic soil [a]*	*1*	*30/05/1985 09:00:00*	*0.677*	*0.083*	-
	D5/stream	*Acidic soil [a]*	*1*	*11/05/1978 09:00:00*	*0.689*	*0.042*	-
	D6/ditch	Acidic soil	1	23/04/1986 09:00:00	0.527	0.042	-
	R1/stream	Acidic soil	1	14/05/1984 04:00:00	0.783	0.208	-
			2	20/05/1984 01:00:00	1.60	0.541	5.67
	R2/stream	Neutral soil	1	13/05/1977 01:00:00	2.16	0.666	-
	R3/stream	Neutral soil	1	23/05/1980 01:00:00	3.94	0.791	-
			2	27/05/1980 03:00:00	1.07	0.458	3.29
	R4/stream	Neutral soil	1	18/04/1984 01:00:00	4.16	0.875	-
			2	27/04/1984 01:00:00	0.555	0.666	8.13
FOCUS Step 4	R1/stream 10 (L&M)	Acidic soil	1	20/05/1984	0.724	0.500	-
	R3/stream		1	23/05/1980	1.41	0.625	-

	10 (L&M)	Acidic soil	2	27/05/1980	0.619	0.250	3.50
	R3/stream 20 (L&M)	Neutral soil	1	23/05/1980	0.931	0.542	-
	R4/stream 10 (L&M)	Neutral soil	1	18/04/1984	1.89	0.833	-

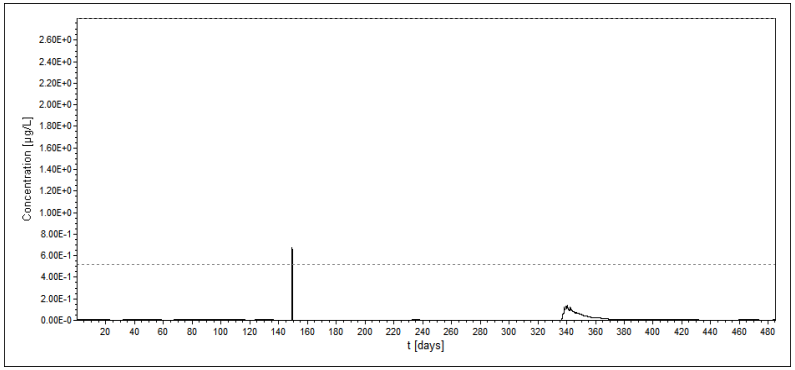
[a] Since these scenarios did not exceed the original EPAT exceedance figure of 0.52, EPATs were not calculated. However they would not pass the initial risk assessment with the marginally lowered geomean E_yC_{50} RAC considering the *Myriophyllum* endpoint and therefore the values for 150 g/ha are assessed here to represent a worst case.

Since the pulsed dose test as presented above only addressed the impact of a single RAC exceedance event, it cannot be used directly to assess the risk from multiple exceedance peaks. This approach is therefore not applicable for several R scenarios at Step 3; so in these cases mitigation at Step 4 has been taken into account to define the exceedance profiles. When considering 10 m to 20 m buffers according to FOCUS Landscape and Mitigation, it is clear that the maximum concentrations leading to an exceedance of the RAC are limited to less than 24 hours.

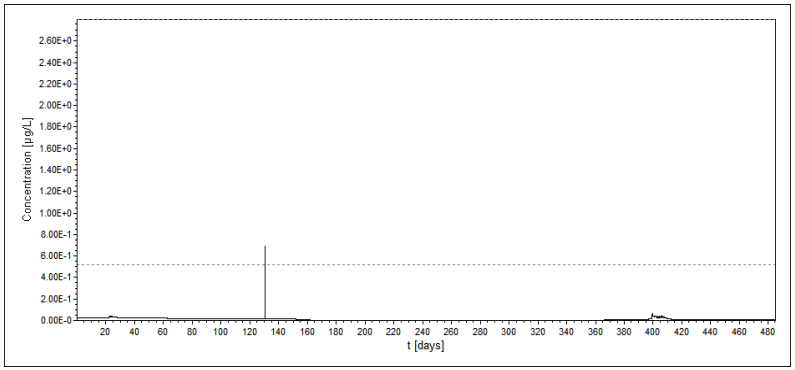
The profiles of exposure are illustrated in the figures below for 100 g/ha, since these are worst-case compared to 75 g/ha. Further details are provided in Ibrahim (2016) as referenced in the dRR document Section 8.



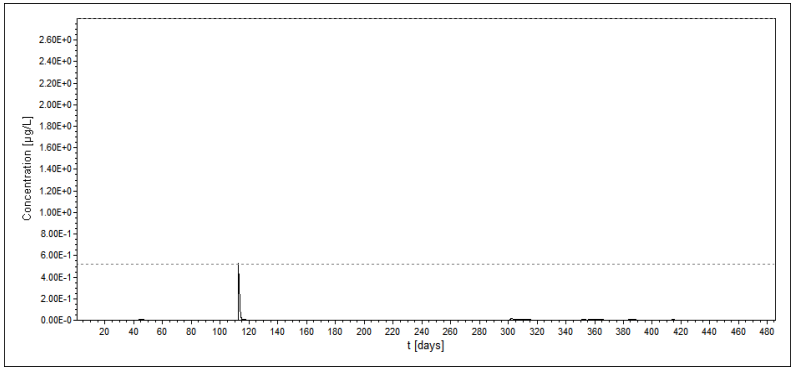
~~Figure 9.5-4: Exposure profile for the D3 Ditch Scenario for FOCUS Step 3 (1 × 100 g a.s./ha, post-emergence) – all soil scenarios. The upper dashed line indicates the relevant RAC of 2.8 µg a.s./L, the lower line indicates a RAC of 0.52 µg a.s./L.~~



~~Figure 9.5-5: Exposure profile for the D4 Stream Scenario for FOCUS Step 3 (1 × 150 g a.s./ha, post-emergence) – acidic soil. The upper dashed line indicates the relevant RAC of 2.8 µg a.s./L, the lower line indicates a RAC of 0.52 µg a.s./L.~~



~~Figure 9.5-6: Exposure profile for the D5 Stream Scenario for FOCUS Step 3 (1 × 150 g a.s./ha, post-emergence) – acidic soil. The upper dashed line indicates the relevant RAC of 2.8 µg a.s./L, the lower line indicates a RAC of 0.52 µg a.s./L.~~



~~Figure 9.5-7: Exposure profile for the D6 Ditch Scenario for FOCUS Step 3 (1 × 100 g a.s./ha, post-emergence) – acidic soil. The upper dashed line indicates the relevant RAC of 2.8 µg a.s./L, the lower line indicates a RAC of 0.52 µg a.s./L.~~

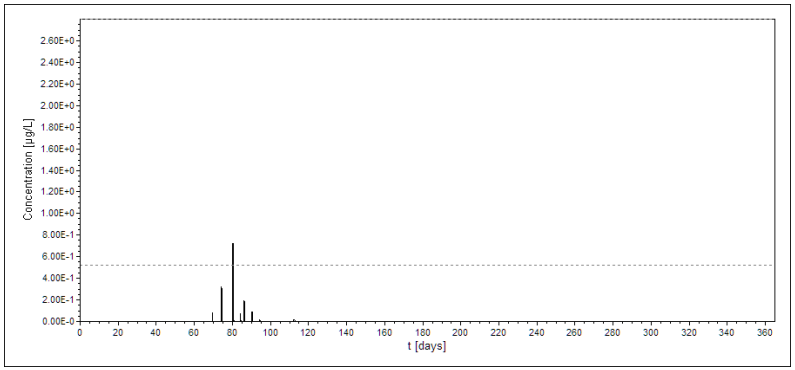


Figure 9.5-8: Exposure profile for the R1 Stream Scenario for FOCUS Step 4: 10 m spray drift + runoff (L&M) buffer (1 × 100 g a.s./ha, post-emergence) – acidic soil. The upper dashed line indicates the relevant RAC of 2.8 µg a.s./L, the lower line indicates a RAC of 0.52 µg a.s./L.

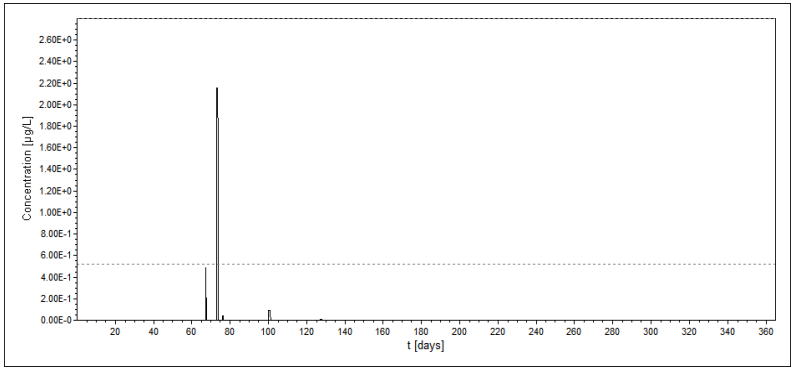


Figure 9.5-9: Exposure profile for the R2 Stream Scenario for FOCUS Step 3 (1 × 100 g a.s./ha, post-emergence) – neutral soil. The upper dashed line indicates the relevant RAC of 2.8 µg a.s./L, the lower line indicates a RAC of 0.52 µg a.s./L.

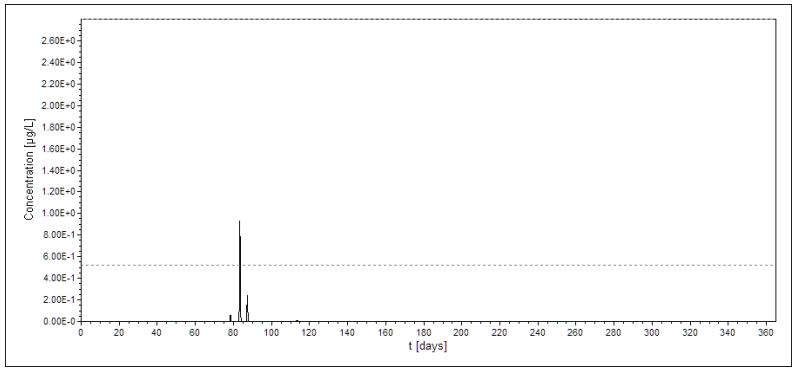


Figure 9.5-10: Exposure profile for the R3 Stream Scenario for FOCUS Step 4: 20 m spray drift + runoff (L&M) buffer (1 × 100 g a.s./ha, post-emergence) – neutral soil. The upper dashed line indicates the relevant RAC of 2.8 µg a.s./L, the lower line indicates a RAC of 0.52 µg a.s./L.

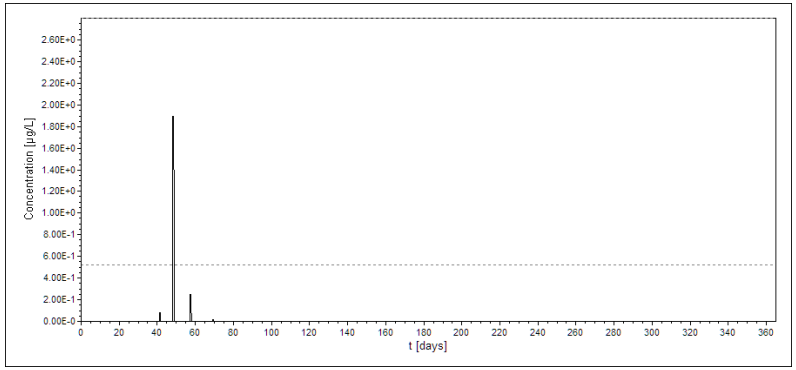


Figure 9.5-11: Exposure profile for the R4 Stream Scenario for FOCUS Step 4: 10 m spray drift + runoff (L&M) buffer (1 × 100 g a.s./ha, post-emergence) – neutral soil. The upper dashed line indicates the relevant RAC

Table 9.5-20: Aquatic macrophytes (*Lemna*): refined higher-tier risk assessment for acceptability of risk (PEC/RAC < 1) for mesotrione based on realistic pulsed exposure testing and/or mitigation at FOCUS Step 4 following application of mesotrione to maize at 1 x 75 g a.s./ha, post-emergence (risk envelope covering the proposed uses at 60 g a.s./ha)

Group						**Aquatic macrophyte**
RAC (µg/L)						Pulsed exposure 24 h RAC = 6.0
Application regime	**FOCUS Scenario**	**Vegetative filter strip (m)** [a]	**No spray buffer (m)**	**Nozzle reduction (%)**	**PEC (µg/L)**	**PEC / Pulsed exposure RAC**
1 x 75 g a.s./ha, post-emergence	**Step 3**					
	R2/stream	-	-	-	1.61	0.27
	Step 4					
	R1/stream	10 (L&M)	10	-	0.544	0.091
	R3/stream	10 (L&M)	10	-	1.33	0.22
	R4/stream	10 (L&M)	10	-	1.42	0.24
	R4/stream	10 (L&M)	10	-	1.89	0.32

[a] L&M = mitigation according to FOCUS Landscape and Mitigation V1 (2007); reduction for 10 / 20 m buffer is 60 / 80 % in runoff flux and volume and 85 / 95 % in sediment flux and mass

[b] Since these scenarios did not exceed the original EPAT exceedance figure of 0.52, EPATs were not calculated. However they would not pass the initial risk assessment with the marginally lowered geomean E_yC_{50} RAC considering the *Myriophyllum* endpoint and therefore the values for 150 g/ha are assessed here to represent a worst case

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

The comparison of the refined PEC and RAC values indicate acceptable risk to aquatic macrophytes following 1 application of 75 g mesotrione/ha to maize when a 10 m vegetated buffer zone according to FOCUS Landscape and Mitigation V1 (2007) is considered for the R scenarios.

Mesotrione metabolite MNBA

Table 9.5-21: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the mesotrione metabolite MNBA for each organism group based on maximum FOCUS PEC_{SW} calculations for the use of A18032E in maize (1 x 75 ~~100~~ g a.s./ha, post-emergence, risk envelope covering all proposed uses)

Group		Fish acute	Inverteb. acute	Algae	Aquatic macrophyte (based on E_rC_{50} or E_yC_{50})
RAC (µg/L)		>1200	1300	4200	>9700
FOCUS Scenario	**PEC gl-max (µg/L)**				
Step 1					
	11.6 ~~15.5~~	<0.010 ~~<0.013~~	0.009 ~~0.012~~	0.003 ~~0.0037~~	<0.001 ~~<0.0016~~

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

Mesotrione metabolite AMBA

Table 9.5-22: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the mesotrione metabolite AMBA for each organism group based on maximum FOCUS PEC_{SW} calculations for the use of A18032E in maize (1 x 75 ~~100~~ g a.s./ha, post-emergence, risk envelope covering all proposed uses)

Group		Fish acute	Inverteb. acute	Algae	Aquatic macrophyte (based on E_rC_{50} or E_yC_{50})
RAC (µg/L)		1500	1600	1400	>9000
FOCUS Scenario	**PEC gl-max (µg/L)**				
Step 1					
	5.39 ~~7.18~~	0.004 ~~0.0048~~	0.003 ~~0.0045~~	0.004 ~~0.0051~~	<0.001 ~~<0.00080~~

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

Mesotrione metabolite SYN546974

Table 9.5-23: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the mesotrione metabolite SYN546974 for each organism group based on maximum FOCUS PEC_{SW} calculations for the use of A18032E in maize (1 x 75 ~~100~~ g a.s./ha, post-emergence, risk envelope covering all proposed uses)

Group		Aquatic macrophyte (based on E_rC_{50})	~~Aquatic macrophyte (based on E_yC_{50})~~
RAC (µg/L)		>9500	~~9300~~
FOCUS Scenario	**PEC gl-max (µg/L)**		
Step 1			
	0.800 ~~1.07~~	<0.0001 ~~<0.00011~~	~~0.00012~~

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

zRMS comments:

It is noted that calculations for mesotrione metabolites were based on PEC_{SW} values derived for exaggerated application rate of 100 g a.s./ha, however PEC_{SW} calculations for this application rate were not presented in area of Section 8, where PEC_{SW} values mesotrione and its metabolites were based on rate of 75 g a.s./ha (covering application rate of mesotrione in A18032E at 60 g/ha). Taking this into account Tables above were amended with surface water exposure as agreed in Section 8.

Acceptable risk to aquatic organisms from exposure to mesotrione metabolites may be concluded following application of A18032E to maize (BBCH 12-14) at 0.4 kg/ha (corresponding to 125 g dicamba/ha) with no need for risk mitigation measures.

Nicosulfuron

Table 9.5-24: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for nicosulfuron for each organism group based on maximum FOCUS PEC_{SW} calculations for the use of A18032E in maize (1 x 40 g a.s./ha, post-emergence)

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Aquatic macrophyte - *Lemna*		~~Aquatic macrophyte – *Myriophyllum*~~	
							E_rC_{50}	~~E_bC_{50}~~	~~E_rC_{50}~~	~~E_bC_{50}~~
RAC (µg/L)		657	1000	900	520	840	0.27	~~0.17~~	~~>352.3~~	~~307.1~~
FOCUS Scenario	**PEC_{gl-max} (µg/L)**									
Step 1										
	13.3	0.020	0.013	0.015	0.026	0.016	**49**	~~**78**~~	~~<0.038~~	~~0.043~~
Step 2										
N-Europe	1.98	*	*	*	*	*	**7.3**	~~**12**~~	~~*~~	~~*~~
S-Europe	3.61	*	*	*	*	*	**13**	~~**21**~~	~~*~~	~~*~~
Step 3										
D3/Ditch	0.217	*	*	*	*	*	0.804	~~**1.3**~~	~~*~~	~~*~~
D4/Pond	0.026	*	*	*	*	*	0.096	~~0.15~~	~~*~~	~~*~~
D4/Stream	0.184	*	*	*	*	*	0.681	~~**1.1**~~	~~*~~	~~*~~
D5/Pond	0.019	*	*	*	*	*	0.070	~~0.11~~	~~*~~	~~*~~
D5/Stream	0.183	*	*	*	*	*	0.678	~~**1.1**~~	~~*~~	~~*~~
D6/Ditch	0.211	*	*	*	*	*	0.781	~~**1.2**~~	~~*~~	~~*~~
R1/Pond	0.017	*	*	*	*	*	0.063	~~0.10~~	~~*~~	~~*~~
R1/Stream	0.453	*	*	*	*	*	**1.7**	~~**2.7**~~	~~*~~	~~*~~
R2/Stream	1.16	*	*	*	*	*	**4.3**	~~**6.8**~~	~~*~~	~~*~~
R3/Stream	1.65	*	*	*	*	*	**6.1**	~~**9.7**~~	~~*~~	~~*~~
R4/Stream	1.79	*	*	*	*	*	**6.6**	~~**11**~~	~~*~~	~~*~~

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
* No further assessmentis required as an acceptable risk has been demonstrated in a more conservative FOCUS scenario

For *Lemna*, calculated PEC/RAC ratios did not indicate an acceptable risk to aquatic macrophytes in FOCUS Steps 1 and 2, and in the R1-R4/Stream scenarios in Step 3 considering the E_rC_{50}, or in all ditch and stream scenarios considering the E_bC_{50}. Therefore, further refinement is required.

Refinement of risk for macrophytes (*Lemna*) considering FOCUS Step 4

Table 9.5-25: Aquatic macrophytes (*Lemna*): refined higher-tier risk assessment for acceptability of risk (PEC/RAC < 1) for nicosulfuron based on mitigation at FOCUS Step 4 following application of nicosulfuron to maize at 1 x 40 g a.s./ha (post-emergence)

Group						Aquatic macrophyte - *Lemna* (based on E_rC_{50})	~~Aquatic macrophyte - *Lemna* (based on E_bC_{50})~~
RAC (µg/L)						0.27	~~0.17~~
Application regime	**Step 4 FOCUS Scenario**	**Vegetative filter strip (m)** [a]	**No spray buffer (m)**	**Nozzle reduction (%)**	**PEC (µg/L)**	**PEC/RAC**	~~**PEC/RAC**~~
1 x 40 g a.s./ha, post-emergence	D3/Ditch	-	-	50	nc	*	~~nc~~
		-	5	-	nc	*	~~nc~~
		10 (L & M)	10	-	0.043	*	~~0.25~~
		5 (VFSmod)	5	-	0.075	*	~~0.44~~
	D4/Stream	-	-	50	nc	*	~~nc~~
		-	5	-	nc	*	~~nc~~
		10 (L & M)	10	-	0.044	*	~~0.26~~
		5 (VFSmod)	5	-	0.080	*	~~0.47~~
	D5/Stream	-	-	50	nc	*	~~nc~~
		-	5	-	nc	*	~~nc~~
		10 (L & M)	10	-	0.044	*	~~0.26~~
		5 (VFSmod)	5	-	0.079	*	~~0.46~~
	D6/Ditch	-	-	50	nc	*	~~nc~~
		-	5	-	nc	*	~~nc~~
		10 (L & M)	10	-	0.037	*	~~0.22~~
		5 (VFSmod)	5	-	0.070	*	~~0.41~~
	R1/Stream	-	-	50	nc	nc	~~nc~~
		-	5	-	nc	nc	~~nc~~
		10 (L & M)	10	-	0.186	0.69	~~**1.1**~~
		20 (L & M)	20	-	0.094	0.35	~~0.55~~
		5 (VFSmod)	5	-	0.060	0.22	~~0.35~~
	R2/Stream	-	-	50	nc	nc	~~nc~~
		-	5	-	nc	nc	~~nc~~
		10 (L & M)	10	-	0.511	**1.9**	~~**3.0**~~

Group						Aquatic macrophyte - *Lemna* (based on E_rC_{50})	~~Aquatic macrophyte - *Lemna* (based on E_bC_{50})~~
		20 (L & M)	20	-	0.265	0.98	~~1.6~~
		5 (VFSmod)	5	-	0.082	0.30	~~0.48~~
	R3/Stream	-	-	50	nc	nc	~~nc~~
		-	5	-	nc	nc	~~nc~~
		10 (L & M)	10	-	0.745	**2.8**	~~4.4~~
		20 (L & M)	20	-	0.390	**1.4**	~~2.3~~
		5 (VFSmod)	5	-	0.086	0.32	~~0.51~~
	R4/Stream	-	-	50	nc	nc	~~nc~~
		-	5	-	nc	nc	~~nc~~
		10 (L & M)	10	-	0.815	**3.0**	~~4.8~~
		20 (L & M)	20	-	0.427	**1.6**	~~2.5~~
		5 (VFSmod)	5	-	0.061	0.23	~~0.36~~

nc = not calculated
[a] L & M = mitigation according to FOCUS Landscape and Mitigation V1 (2007); reduction for 10 / 20 m buffer is 60 / 80 % in runoff flux and volume and 85 / 95 % in sediment flux and mass
VFSmod = simulated using VFSMod tool included in SWAN v 4.0.1
PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
* No further assessment is required as an acceptable risk has been demonstrated in a more conservative FOCUS scenario

The comparison of the refined PEC and RAC values indicates acceptable risk to *Lemna* following 1 application of 40 g nicosulfuron/ha to maize post-emergence as follows:

- ~~D scenarios, considering the E_bC_{50}: when a 10 m vegetated buffer zone according to FOCUS Landscape and Mitigation V1 (2007) buffer is considered, or a 5 m VFSmod buffer is used~~
- R scenarios:
  - Considering the E_rC_{50}:
    - R1: when a 10 m vegetated buffer zone according to FOCUS Landscape and Mitigation V1 (2007) buffer is considered, or a 5 m VFSmod buffer is used
    - R2: when a 20 m vegetated buffer zone according to FOCUS Landscape and Mitigation V1 (2007) buffer is considered, or a 5 m VFSmod buffer is used
    - R3, R4: when a 5 m VFSmod buffer is used.
  - ~~Considering the E_bC_{50}:~~
    - ~~R1: when a 20 m vegetated buffer zone according to FOCUS Landscape and Mitigation V1 (2007) buffer is considered, or a 5 m VFSmod buffer is used~~
    - ~~R2, R3, R4: when a 5 m VFSmod buffer is used.~~

zRMS comments:

The risk assessment presented in Tables 9.5-24 and 9.5-25 above is agreed by the zRMS. The risk assessment presented was based on PEC_{SW} values calculated for the target rate of nicosulfuron in A18032E (i.e. 40 g a.s./ha).

For fish, aquatic invertebrates and algae acceptable acute and chronic risk could be concluded already for Step 1 PEC_{SW} values.

For aquatic macrophytes acceptable risk could be concluded with Step 3 PEC_{SW} in scenarios D3, D4, D5 and D6, while for scenarios R scenarios further calculations based on Step 4 PEC_{SW} were deemed necessary.

Overall, following conclusions could be derived:

- acceptable risk to aquatic organisms with no need for risk mitigation measures was demonstrated in scenarios D3, D4, D5 and D6,
- acceptable risk to aquatic organisms with consideration of 10 m vegetated filter strip or 5 m VFSmod buffer was demonstrated in scenario R1,
- acceptable risk to aquatic organisms with consideration of 20 m vegetated filter strip or 5 m VFSmod buffer was demonstrated in scenario R2,
- acceptable risk to aquatic organisms with consideration of 5 m VFSmod buffer was demonstrated in scenarios R3 and R4. No acceptable risk in these scenarios could be concluded for fractional reduction of run-off values relevant for 10 and 20 m vegetated filter strip.

Calculations for primary producers based on RAC values derived using E_bC_{50} / E_vC_{50} are struck through in tables above, since in line with EFSA (2013) only E_rC_{50} values are relevant for the risk assessment.

zRMS comments:

It should be noted that most of the Central Zone Member States has concerns with use of the modified exposure studies due to uncertainties related to the exposure profiles modelled using FOCUS. Extensive discussion regarding this issue took place during the Central Zone harmonisation meetings and it was concluded that results of Tier 2C studies should be considered only when no acceptable risk may be demonstrated using standard approach (i.e. standard toxicity endpoints and exposure calculated with consideration of the risk mitigation measures). The same is stated in the document presenting the specific national requirements in area of environmental exposure and risk assessment in Poland.
For nicosulfuron applied as A18032E acceptable risk to aquatic organisms could be concluded using the endpoint required by EFSA, 2013 (i.e. E_rC_{50}) and applying standard risk mitigation measures (see commenting box above). Taking this into account, no further assessment is deemed necessary at the zonal level and the provided below refinement based on Tier 2C studies was not evaluated by the zRMS and is thus struck through.

Refinement of risk for macrophytes considering realistic exposure

The R stream scenarios are characterised by short-lived peaks of exposure which are quickly dissipated. This exposure is demonstrated by EPAT for the FOCUS Step 3 and 4 outputs presented in the following report:

Report:	Carnall J. (2017): Nicosulfuron – A European Fate Assessment Using the FOCUS Surface Water Scenarios at Step 3 and Step 4 Following Spray Application to Maize. Report no. CEA.1864. Cambridge Environmental Assessments, UK. (Syngenta File No. ASF628_11312)

Exposure events exceeding the threshold RAC of 0.27 µg a.s./L were identified and examined. The following tables summarise the duration and frequency of exposure peaks exceeding the RAC.
Please note that exceedance events for the E_bC_{50} RAC of 0.17 µg a.s./L would be extremely similar as can be seen from the graphs for the profiles of exposure. Therefore, since the E_rC_{50} RAC is considered more robust, follows the aquatic guidance recommendations, and was proposed as the relevant endpoint in the EU review, further analysis based on the E_bC_{50} RAC is not provided below.

Table 9.5-26: Duration and frequency of exposure events exceeding the RAC of 0.27 µg a.s./L following application to maize at 1 x 40 g nicosulfuron/ha (post-emergence) as determined with EPAT v1.1

Step/ mitigation	Scenario	Event No.	Start date	Max conc. (µg/L)	Duration (days)	Interval (days)
Step 3	R1/Stream	1	14/05/1982	0.453	0.250	-
	R2/Stream	1	13/05/1977	1.16	0.666	-
	R3/Stream	1	23/05/1980	1.65	0.750	-
		2	27/05/1980	0.341	0.333	3.375
	R4/Stream	1	18/04/1984	1.79	0.875	-
Step 4: 10 m buffer + 10 m L&M	R3/stream	1	23/05/1980	0.745	0.625	-

It is therefore clear that the maximum concentrations leading to an exceedance of the RAC are limited to a single occurrence lasting less than 24 hours in all cases at Step 3, except for the R3 scenario. For the latter the impact of a 10 m buffer at Step 4 was therefore investigated. When considering a10 m buffer, only a single exceedance lasting less than 24 hours is also expected for R3. The profiles of exposure are illustrated in the figures below. Further details are provided in the dRR document Section 8.

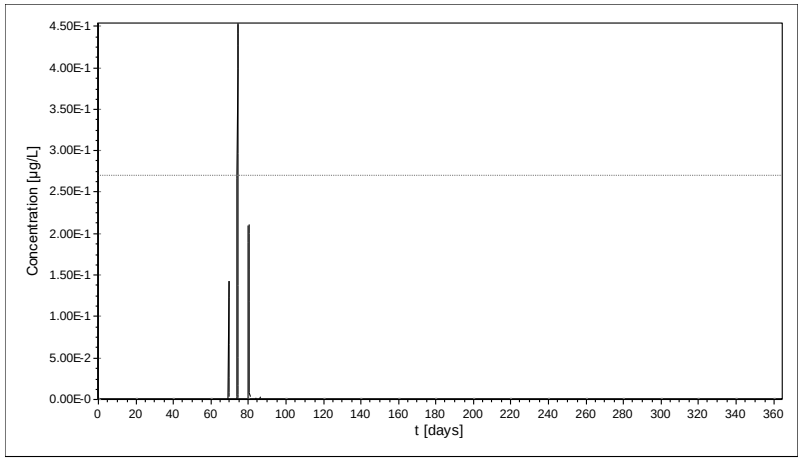


Figure 9.5-12: Exposure profile for Step 3; R1 Stream Scenario (1 × 40 g a.s./ha, post-emergence). The dashed line indicates the RAC.

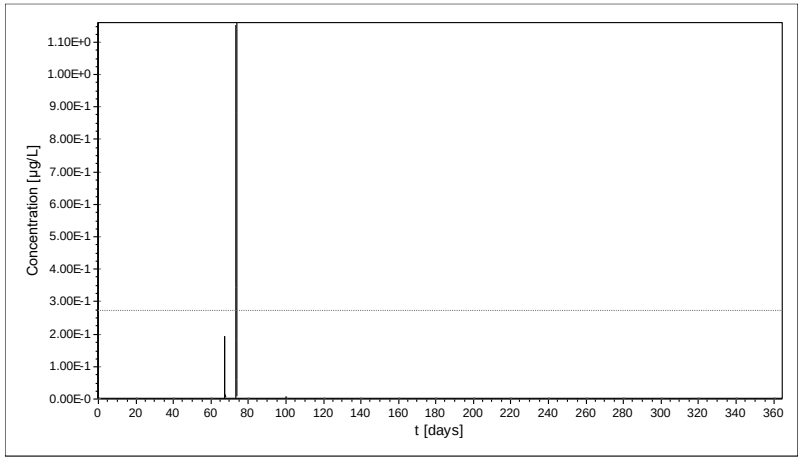


Figure 9.5-13: Exposure profile for Step 3; R2 Stream Scenario (1 × 40 g a.s./ha, post-emergence). The dashed line indicates the RAC.

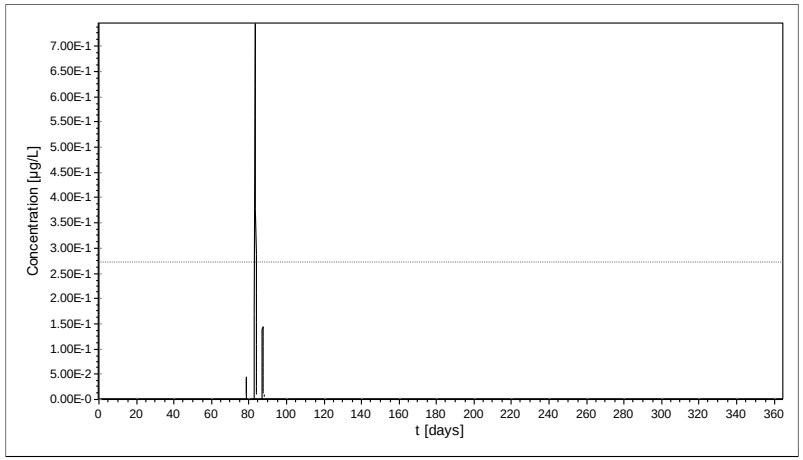


Figure 9.5-14: Exposure profile for Step 4; 10 m buffer + 10 m fractional runoff reduction; R3 Stream Scenario (1 × 40 g a.s./ha, post-emergence). The dashed line indicates the RAC.

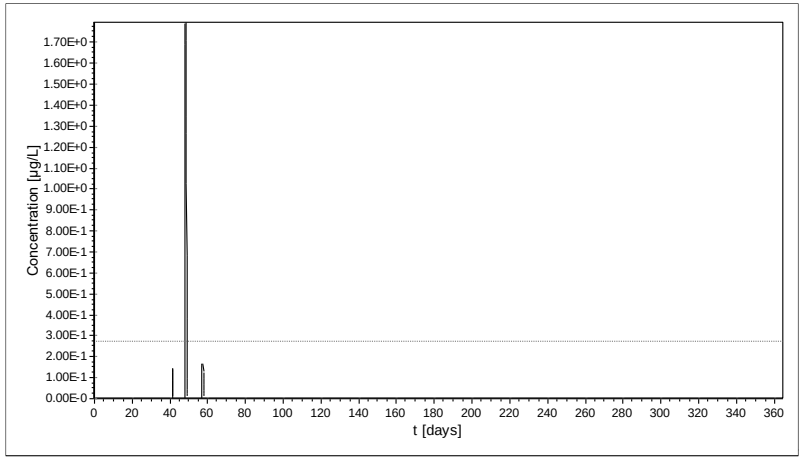


Figure 9.5-15: Exposure profile for Step 3; R4 Stream Scenario (1 × 40 g a.s./ha, post-emergence). The dashed line indicates the RAC.

Pulsed exposure studies and potential of recovery

Three additional studies with *Lemna gibba* investigating the effects of pulsed exposures were presented in the DAR addendum for nicosulfuron, and these can be used as higher tier information for the risk assessment.

1) The effects on growth in *Lemna* after a 24 hour pulse of exposure to nicosulfuron was investigated, showing full recovery at 27 µg a.s./L by 14 days after exposure. This can be compared to the realistic exposure pattern predicted for most of the D and R ditch and stream scenarios, which are characterised by a short lived peak of exposure lasting less than 24 hours.
2) The potential for recovery of growth in *Lemna* after 7 days of exposure to nicosulfuron was investigated, showing full recovery at up to 50 µg a.s./L after 5 weeks. This is very worst case compared to the predicted exposure for the R scenarios, even where repeated exceedances are predicted to occur when considering the FOCUS Step 3 profiles.
3) The potential for recovery of growth in *Lemna* after 14 days of exposure to nicosulfuron was investigated showing no significant effects at 0.8 µg a.s./L. Again, this is very worst case considering the predicted pattern of exposure for the R scenarios where repeated exceedances are predicted to occur.

The pulsed dose studies summarised in the DAR addendum clearly demonstrate that there is a rapid time to onset of effects, but that the exposure needs to be sustained in order for effects to continue; when fronds are transferred to clean water it is clear that the effects are reversible and recovery of growth rate is seen. This is similar to the situation that will happen in the field for the ditch and stream scenarios where exposure above the RAC will be limited to less than 24 hours.

The exposure profiles presented in Section 8, and illustrated above, indicate that in the D ditch and stream scenarios and in the R1-4 scenarios the peaks of exposure are limited to a single short lived (<24h) exceedance event. Therefore the NOEAEC endpoint of 27 µg a.s./L from the single 24 hour pulse of exposure study can be used to refine the potential risk from these scenarios. When applying the Tier 1 10-fold safety factor to account for inter-species differences, the RAC_{pulse} is 2.7 µg a.s./L.

Where multiple exceedance events are predicted at Step 3, these can be refined considering mitigation at FOCUS Step 4. When considering a 10 m buffer only a single exceedance event is expected in all cases, and therefore the refined RAC of 2.7 µg a.s./L is also applicable.

Table 9.5-27: Aquatic macrophytes (*Lemna*): refined higher-tier risk assessment for acceptability of risk (PEC/RAC < 1) for nicosulfuron based on realistic pulsed exposure testing and/or mitigation at FOCUS Step 4 following application of nicosulfuron to maize at 1 x 40 g a.s./ha, post-emergence

Group					Aquatic macrophyte
RAC (µg/L)					Pulsed exposure 24 h RAC = 2.7
Application regime	FOCUS Scenario	Vegetative filter strip (m) [a]	No spray buffer (m)	PEC (µg/L)	PEC / Pulsed exposure RAC
FOCUS Step 3 scenario					
1 x 40 g a.s./ha, post-emergence	R1/Stream	-	-	0.453	0.17
	R2/Stream	-	-	1.16	0.43
	R4/Stream	-	-	1.79	0.63
FOCUS Step 4 scenario					
1 x 40 g a.s./ha, post-emergence	R3/stream	10 (L&M)	10	0.745	0.28

[a] L&M = mitigation according to FOCUS Landscape and Mitigation V1 (2007); reduction for 10 m buffer is 60 % in runoff flux and volume and 85 % in sediment flux and mass

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

The comparison of the FOCUS Step 3 and 4 PEC values and the refined pulsed exposure RAC value of 2.7 µg a.s./L indicates acceptable risk to aquatic macrophytes exposed to nicosulfuron following application of 40, 45 and 60 g a.s./ha when a 10 m vegetated buffer strip according to FOCUS Landscape and Mitigation V1 (2007) is considered.

Nicosulfuron metabolite ASDM

Table 9.5-28: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the nicosulfuron metabolite ASDM for each organism group based on maximum FOCUS PEC_{SW} calculations for the maximum use of A18032E in maize (1 x 40 ~~60~~ g a.s./ha, post-emergence; risk envelope approach covering all proposed uses)

Group		Fish acute	Inverteb. acute	Algae	Aquatic macrophyte (based on E_rC_{50} and E_bC_{50})
RAC (µg/L)		>1000	>9540	>33600	>10000
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**				
Step 1					
	5.42 ~~8.14~~	<0.005 ~~<0.0081~~	<0.001 ~~<0.00085~~	<0.0002 ~~<0.00024~~	<0.001 ~~<0.00081~~

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

Nicosulfuron metabolite AUSN

Table 9.5-29: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the nicosulfuron metabolite AUSN for each organism group based on maximum FOCUS PEC_{SW} calculations for the maximum use of A18032E in maize (1 x 40 ~~60~~ g a.s./ha, post-emergence; risk envelope approach covering all proposed uses)

Group		Fish acute	Inverteb. acute	Algae	Aquatic macrophyte (based on E_rC_{50} and E_bC_{50})
RAC (µg/L)		>1000	>1000	>10000	>10000
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**				
Step 1					
	3.84 ~~5.75~~	<0.004 ~~<0.0058~~	<0.004 ~~<0.0058~~	<0.0004 ~~<0.00058~~	<0.0004 ~~<0.00058~~

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

Nicosulfuron metabolite HMUD

Table 9.5-30: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the nicosulfuron metabolite HMUD for each organism group based on maximum FOCUS PEC_{SW} calculations for the maximum use of A18032E in maize (1 x 40 ~~60~~ g a.s./ha, post-emergence; risk envelope approach covering all proposed uses)

Group		Fish acute	Inverteb. acute	Algae	Aquatic macrophyte (based on E_rC_{50} and E_bC_{50})
RAC (µg/L)		>1000	>1000	>10000	>100
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**				
Step 1					
	4.39 ~~6.58~~	<0.004 ~~<0.0066~~	<0.004 ~~<0.0066~~	<0.0004 ~~<0.00066~~	<0.044 ~~<0.066~~

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

Nicosulfuron metabolite ADMP

Table 9.5-31: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the nicosulfuron metabolite ADMP for each organism group based on maximum FOCUS PEC_{SW} calculations for the maximum use of A18032E in maize (1 x 40 ~~60~~ g a.s./ha, post-emergence; risk envelope approach covering all proposed uses)

Group		Fish acute	Inverteb. acute	Algae
RAC (µg/L)		>1000	>1000	>10000
FOCUS Scenario	**$PEC_{gl\text{-}max}$ (µg/L)**			
Step 1				
	0.340 ~~0.510~~	<0.0003 ~~<0.00051~~	<0.0003 ~~<0.00051~~	<0.00003 ~~<0.000051~~

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

Nicosulfuron metabolite UCSN

Table 9.5-32: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the nicosulfuron metabolite UCSN for each organism group based on maximum FOCUS PEC_{SW} calculations for the maximum use of A18032E in maize (1 x 40 ~~60~~ g a.s./ha, post-emergence; risk envelope approach covering all proposed uses)

Group		~~Fish acute~~	Inverteb. acute	Algae	Aquatic macrophyte (based on E_rC_{50} and E_bC_{50})
RAC (µg/L)		~~>1000~~	>1000	>10000	>10000
FOCUS Scenario	**$PEC_{gl\text{-}max}$ (µg/L)**				
Step 1					
	1.80 ~~2.71~~	~~<0.0027~~	<0.002 ~~<0.0027~~	<0.0002 ~~<0.00027~~	<0.0002 ~~<0.00027~~

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

zRMS comments:

It is noted that calculations for nicosulfuron metabolites were based on PEC_{SW} values derived for exaggerated application rate of 60 g a.s./ha, however PEC_{SW} calculations for this application rate were not presented in area of Section 8, where PEC_{SW} values for nicosulfuron and its metabolites were based on rate of 40 g a.s./ha (intended rate of nicosulfuron in A18032E). Taking this into account Tables 9.5-28 to 9.5-32 were amended with surface water exposure as agreed in Section 8.

The acute risk assessment for fish from metabolites UCSN was struck through since no relevant endpoint is reported in EFSA Scientific Report (2007) 120.

Acceptable risk to aquatic organisms from exposure to nicosulfuron metabolites may be concluded following application of A18032E to maize (BBCH 12-14) at 0.4 kg/ha (corresponding to 40 g nicosulfuron/ha) with no need for risk mitigation measures.

The Applicant is kindly reminded that exposure estimates considered in the risk assessment presented in area of Section 9 must correspond with PEC values presented in area of Section 8.

A18032E

Table 9.5-33: Aquatic organisms: initial and higher-tier risk assessment for acceptability of risk (PEC/RAC < 1) for A18032E considering exposure mitigation measures

Group				Fish acute	Inverteb. acute	Algae	Aquatic macrophyte (based on E_rC_{50})	~~Aquatic macrophyte (based on E_yC_{50})~~
RAC (µg/L)				34.4	12.2	7.28	1.81	~~0.643~~
Application rate	**Spray drift buffer (m)**	**Drift-reducing nozzles (%)**	**PEC (µg/L)**	PEC/RAC	PEC/RAC	PEC/RAC	PEC/RAC	~~PEC/RAC~~
1 x 400 g A18032E/ha	1	-	3.69	0.11	0.30	0.51	**2.0**	~~5.7~~
	1	50	1.85	-	-	-	**1.0**	~~2.9~~
	1	75	0.923	-	-	-	0.51	~~1.4~~
	1	90	0.369	-	-	-	-	~~0.57~~
	5	-	0.760	-	-	-	0.42	~~1.2~~
	5	50	0.380	-	-	-	-	~~0.59~~
	10	-	0.387	-	-	-	-	~~0.60~~

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

The comparison of the refined PEC and RAC values indicate acceptable risk to aquatic organisms following application to maize when considering the following mitigation options:

<u>0.4 A18032E/ha</u>

- E_rC_{50}: 75% drift reducing nozzles; or a 5 m buffer
- E_yC_{50}: 90% drift reducing nozzles; or a 5 m buffer with 50% drift reducing nozzles; or a 10 m buffer

zRMS comments:

The risk assessment for A180352E based on formulation endpoints and exposure data calculated for migration of formulation to surface water bides via spray drift is agreed by the zRMS. It is considered to cover the risk from toxic co-formulants, for which no efate data are available and hence estimation of exposure based on sum of active substances and toxic compounds was not possible.
Based on above calculations, acceptable risk for the formulated product could be concluded provided that in order to protect aquatic organisms an unsprayed buffer zone of 5 m to surface water bodies is respected or the spray drift is reduced by 75% using appropriate drift reducing techniques.

The risk assessment based on $PEC_{SW,MIX}$ for individual active compounds compared with formulation endpoints expressed in terms of sum of active substances is presented below for completeness. Please note that for dicamba no Step 3 PEC_{SW} values were calculated by the Applicant and respective modelling was performed by the zRMS. Since Poland is the only cMS indicated in the GAP table, only scenarios relevant for Poland (D3, D4 and R1) were included in these simulations.

Group		Fish acute	Inverteb. acute	Algae	Aquatic macrophyte (based on E_rC_{50})
RAC (µg/L)		19.7	6.9	4.1	1.03
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**				
Step 1					
	83.1	**4.2**	**12.0**	**20.3**	**80.7**
Step 2					
N-Europe	14.03	0.712	**2.0**	**3.4**	**13.6**

Step 3					
D3/Ditch	1.267	*	0.184	0.309	**1.2**
D4/Pond	0.094	*	0.014	0.023	0.091
D4/Stream	1.085	*	0.157	0.265	**1.1**
R1/Pond	0.644	*	0.093	0.157	0.625
R1/Stream	1.258	*	0.182	0.307	**1.2**
Step 4 (5 m buffer + including 5 m vegetated filter strip, VFSmod)					
D3/Ditch	0.860 [1)]	*	*	*	0.835
D4/Stream	0.785 [1)]	*	*	*	0.762
R1/Stream	0.361	*	*	*	0.350

[1)] In scenarios D3 ditch and D4 stream for dicamba Step 3 PEC_{SW} was summed with Step 4 PEC_{SW} for mesotrione and nicosulfuron (worst case since PEC_{SW} for dicamba at 5 m buffer would be lower)

Above calculations demonstrated acceptable risk to aquatic organisms from the mixture of dicamba, mesotrione and nicosulfuron, provided that 5 m vegetated filter strip is respected. Please note, that the width of the filter strip was derived using VFSmod, as consideration of this tool is acceptable in Poland.

9.5.3 Overall conclusions

The PEC/RAC ratios for all aquatic organisms other than macrophytes using worst-case PEC_{SW} values for A18032E, dicamba, mesotrione, nicosulfuron and their metabolites are below the trigger of 1, indicating acceptable risk for these organisms following use of A18032E according to the proposed use pattern when considering the following mitigation measures as presented in the tables below. Since ADAMA ~~Syngenta~~ proposes that the E_rC_{50} values should be used for macrophyte risk assessment in accordance with the Aquatic Guidance Document, these endpoints have been used to summarise the mitigation below. ~~Mitigations addressing the use of the $E_{b\ or\ y}C_{50}$ are available in the main text.~~

Table 9.5-34: Aquatic organisms: Overall proposed mitigation measures for A18032E applied at 1 x 0.4 kg/ha in maize (125 g dicamba/ha, 60 g mesotrione/ha and 40 g nicosulfuron/ha)

Test substance	Appl. rate (g/ha)	Organism	A or C	Scenario									
				D1	D2	D3	D4	D5	D6	R1	R2	R3	R4
A18032E	400	Fish	A			- [a]							
Dicamba	132	Fish	A			-	-	-	-	-	-	-	-
Mesotrione	75	Fish	A			-	-	-	-	-	-	-	-
Nicosulfuron	40	Fish	A			-	-	-	-	-	-	-	-
Dicamba	132	Fish	C			-	-	-	-	-	-	-	-
Mesotrione	75	Fish	C			-	-	-	-	-	-	-	-
Nicosulfuron	40	Fish	C			-	-	-	-	-	-	-	-
A18032E	400	Aq inverts	A			- [a]							
Dicamba	132	Aq inverts	A			-	-	-	-	-	-	-	-
Mesotrione	75	Aq inverts	A			-	-	-	-	-	-	-	-
Nicosulfuron	40	Aq inverts	A			-	-	-	-	-	-	-	-
Dicamba	132	Aq inverts	C			-	-	-	-	-	-	-	-
Mesotrione	75	Aq inverts	C			-	-	-	-	-	-	-	-
Nicosulfuron	40	Aq inverts	C			-	-	-	-	-	-	-	-
A18032E	400	Algae	C			- [a]							
Dicamba	132	Algae	C			-	-	-	-	-	-	-	-
Mesotrione	75	Algae	C			-	-	-	-	-	-	-	-
Nicosulfuron	40	Algae	C			-	-	-	-	-	-	-	-
A18032E	400	Macrophytes	C			75% DR; or 5 m SD [a]							
Dicamba	132	Macrophytes	C			-	-	-	-	-	-	-	-

Test substance	Appl. rate (g/ha)	Organism	A or C	Scenario									
				D1	D2	D3	D4	D5	D6	R1	R2	R3	R4
Mesotrione	75	Macrophytes	C			-	-	-	-	-	-	E_rC_{50}: 10 m VFS (L&M) or 5 m VFS (VFSmod)	E_rC_{50}: 10 m VFS (L&M) or 5 m VFS (VFSmod) ~~EAC_{pulse}: none~~
Nicosulfuron	40	Macrophytes	C			-	-	-	-	E_rC_{50}: 10 m VFS (L&M) or 5 m VFS (VFSmod) ~~EAC_{pulse}: none~~ [b]	E_rC_{50}: 20 m VFS (L&M) or 5 m VFS (VFSmod) ~~EAC_{pulse}: none~~ [b]	E_rC_{50}: 5 m VFS (VFSmod) ~~EAC_{pulse}: 10 m VFS (L&M)~~ [b]	E_rC_{50}: 5 m VFS (VFSmod) ~~EAC_{pulse}: none~~ [b]

A = acute, C = chronic
An empty/grey field means that the scenario is not relevant to the crop group
"-"mitigation measures are not required for this scenario
SD = spray drift buffer
VFS (L&M) = vegetative filter strip according with FOCUS Landscape and Mitigation V1 (2007)
DR = drift reducing nozzles
EAC_{pulse} = Environmentally Acceptable Concentration derived from pulsed exposure study
[a] spray drift entry; drift value according to Rautmann at al. (2001)
[b] Considering refined RAC of 2.7 µg a.s./L (based on pulsed exposure studies and potential of recovery)

zRMS comments:
Table 9.5-34 above was amended accordingly with consideration of the outcome of the risk assessment performed for particular active compounds.

It is noted that additionally also risk assessment for the formulated product based on $PEC_{SW,MIX}$ for individual active compounds compared with formulation endpoints expressed in terms of sum of active substances has been performed by the zRMS for completeness. However, it included only scenarios relevant for Poland (D3, D4 and R1) since PL is the only cMS indicated in GAP table. Acceptable risk could be concluded provided that 5 m vegetated filter strip (VFSmod) to surface water bodies is respected..

9.6 Effects on bees (KCP 10.3.1)

9.6.1 Toxicity data

Studies on the toxicity to bees have been carried out with dicamba, mesotrione and nicosulfuron. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on bees of A18032E were not evaluated as part of the EU assessment of dicamba, mesotrione and nicosulfuron. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process.

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

Species	Substance	Exposure system	Results	Reference
Apis mellifera	Dicamba	Oral	LD_{50} > 100 µg a.s./bee	EFSA Conclusion 2011 Hillesheim, 1993b SAN837/5339
Apis mellifera	Dicamba	Contact	LD_{50} > 100 µg a.s./bee	EFSA Conclusion 2011 Hillesheim, 1993a SAN837/5680
~~*Apis mellifera*~~	~~Dicamba (formulated as A7254B)~~	~~Larval toxicity (8-day study)~~	~~LD_{50} = 301.7 µg a.s./larva~~ ~~NOED = 125 µg a.s./larva~~	~~Kleebaum, 2015 A7254B_10377~~ [a]
~~*Apis mellifera*~~	~~Dicamba (formulated as A7254B)~~	~~Chronic adult toxicity (10 days)~~	~~LD_{50} > 194.7 µg a.s./bee/day~~ ~~NOED = 194.7 µg a.s./bee/day~~	~~Ruhland, 2015 A7254B_10378~~ [a]
Higher-tier studies (tunnel test, field studies)				
Not relevant				

[a] These studies have been submitted to fulfil the data requirements under Commission Regulation (EU) No 283/2013; but they are not used in this risk assessment as they are not considered under the currently notified risk assessment guidance.

zRMS comments:

Acute endpoints for dicamba presented in Table 9.6-1 are in line with the EU agreed endpoints reported in EFSA Journal 2011;9(1):1965.

Studies on chronic adult and larvae toxicity of dicamba solo-formulation (A7254B) were not validated by the zRMS since in case of A18032E, containing 3 active compounds, respective larvae and chronic toxicity studies should be performed with the formulated product in order to fulfil data requirements, while active substance endpoints should be generated at the EU level.

Table 9.6-2: Endpoints and effect values relevant for the risk assessment for bees - mesotrione

Species	Substance	Exposure system	Results	Reference
Apis mellifera	Mesotrione	48h Oral	LD_{50} >11 µg a.s./bee	EFSA Conclusion 2016 Jackson and Gough, 1995 ZA1296/0540
Apis mellifera	Mesotrione	48h Contact	LD_{50} >100 µg a.s./bee	EFSA Conclusion 2016 Jackson and Gough, 1995 ZA1296/0540
~~*Apis mellifera*~~	~~Mesotrione (formulated as A12739A)~~	~~Semi-chronic larval toxicity (7-day study)~~	~~LD_{50} = 118.5 µg a.s./larva NOED = 57.8 µg a.s./larva~~	~~EFSA Conclusion 2016 Kleebaum, 2013 A12739A_10464~~ [a]
~~*Apis mellifera*~~	~~Mesotrione (formulated as A12739A)~~	~~Chronic adult toxicity (10 days)~~	~~LD_{50} = 19.2 µg a.s./bee/day NOED = 8.1 µg a.s./bee/day~~	~~EFSA Conclusion 2016 Kleebaum, 2013a A12739A_10465~~ [a]
~~*Apis mellifera*~~	~~Mesotrione~~	~~Chronic adult toxicity (10 days)~~	~~LDD_{50} > 21.9 µg a.s./bee/day NOEDD = 21.9 µg a.s./ bee/ day~~	~~xxxxxxxxxxxx ZA1296_10608~~
~~*Apis mellifera*~~	~~Mesotrione~~	~~22 day chronic larval toxicity, repeat exposure~~	~~8d LD_{50} = >46 µg a.s./ larva /developmental period 8d NOED = 46 µg a.s./larva /developmental period 22d NOED = 46µg a.s./larva/developmental period~~	~~xxxxxxxxx ZA1296_10465~~
Higher-tier studies (tunnel test, field studies)				
Not relevant				

[a] These studies have been submitted to fulfil the data requirements under Commission Regulation (EU) No 283/2013; but they are not used in this risk assessment as they are not considered under the currently notified risk assessment guidance.

zRMS comments:

Acute endpoints for mesotrione presented in Table 9.6-2 are in line with the EU agreed endpoints reported in EFSA Journal 2016;14(3):4419. Chronic and larvae toxicity data for solo formulation A12739A are also in line with the LoEP, however they are struck through in Table 9.6-2 as being generated with other product and thus not relevant for evaluation performed for A18032E.

Studies on chronic adult and larvae toxicity of mesotrione were not validated by the zRMS since in case of A18032E, containing 3 active compounds, respective larvae and chronic toxicity studies should be performed with the formulated product in order to fulfil data requirements, while active substance endpoints should be generated at the EU level.

Table 9.6-3: Endpoints and effect values relevant for the risk assessment for bees - nicosulfuron

Species	Substance	Exposure system	Results	Reference
Apis mellifera	Nicosulfuron (formulated in SL-950 4% SC)	Oral	LD_{50} = 5.24 µg a.s./bee	EFSA Conclusion 2007 Petto, 1994 480400
Apis mellifera	Nicosulfuron	Contact	LD_{50} = 76 µg a.s./bee	EFSA Conclusion 2007 Winter et al., 1991 272-102
~~*Apis mellifera*~~	~~Nicosulfuron~~	~~Larval toxicity (7 day study)~~	~~NOED = 20 µg a.s./larva~~	~~Klank, 2014 S14-00341~~ [a]
~~*Apis mellifera*~~	~~Nicosulfuron~~	~~Chronic adult toxicity (10 days)~~	~~LDD_{50} > 11.43 µg a.s./bee/day NOEDD = 11.43 µg a.s./bee/day~~	~~Schmitt, 2014 S 14-00413~~ [a]
Higher-tier studies (tunnel test, field studies)				
Not relevant				

[a] These studies have been submitted to fulfil the data requirements under Commission Regulation (EU) No 283/2013; but they are not used in this risk assessment as they are not considered under the currently notified risk assessment guidance.

zRMS comments:

Acute endpoints for nicosulfuron presented in Table 9.6-3 are in line with the EU agreed endpoints reported in EFSA Scientific Report (2007) 120. It is noted that acute oral endpoint was generated in study performed with solo-formulation of nicosulfuron and it may be thus questioned if it is relevant to address toxicity of the active substance itself due to presence of co-formulants which may have impact on the toxicity. Nevertheless, in absence of respective endpoint for the active substance, this endpoint was used in the risk assessment performed for nicosulfuron.

Studies on chronic adult and larvae toxicity of nicosulfuron were not validated by the zRMS since in case of A18032E, containing 3 active compounds, respective larvae and chronic toxicity studies should be performed with the formulated product in order to fulfil data requirements, while active substance endpoints should be generated at the EU level.

Table 9.6-4: Endpoints and effect values relevant for the risk assessment for bees - A18032E

Species	Substance	Exposure system	Results	Reference
Apis mellifera	A18032E	Oral	LD_{50} = 170 µg/bee	Kling, 2012 A18032E_10005
Apis mellifera	A18032E	Contact	LD_{50} > 227 µg/bee	Kling, 2012 A18032E_10005
Higher-tier studies (tunnel test, field studies)				
Not relevant				

zRMS comments:

Studies on acute toxicity of A18032E to bees were evaluated by the zRMS and considered acceptable. For details of evaluation, please refer to Appendix 2. Endpoints reported in Table 9.6-4 are confirmed to be correct.

Since formulation A18032E is intended to be applied exclusively with the adjuvant, all formulation studies were performed with the recommended adjuvant Adigor (A12127R).

It is noted that no study on chronic toxicity of A18032E to adult bees and bee larvae were provided by the Applicant. Since A18032E contains three active compounds, testing of chronic and larvae toxicity is mandatory, in line with data requirements set by the Commission Regulation (EU) No 284/2013 and a data gap in this area is identified. Nevertheless, as the results of the chronic and larvae toxicity studies are not considered in the risk assessment based on indications of the current guidance document (SANCO 10329/2002 rev 2 final), the studies must be submitted not later than the date of entry into force of EFSA bee guidance (2013). Please note that the larvae study must be performed in line with OECD TG 239.

9.6.1.1 Justification for new endpoints

Studies with A18032E

New studies are available for A18032E which are required to fulfil the data requirements for plant protection products in accordance with Regulation (EC) No 1107/2009. The endpoints are summarised in Table 9.6-4.

~~**Chronic adult 10d study with mesotrione**~~

~~A new 10d chronic oral study with the honey bee has been carried out with mesotrione TGAI since the EU review, as this was requested by the US EPA. The study gives a marginally higher endpoint than the EU endpoint coming from the test with the lead formulation A12739A. The EU endpoint has been used in the risk assessments below.~~

~~**Chronic 22d study with mesotrione**~~

~~A new 22d repeat exposure larval study with the honey bee has been carried out with mesotrione since the EU review, as this was requested by the US EPA.~~

~~**Chronic studies with dicamba and nicosulfuron**~~

~~Since Annex I Submission/inclusion new bee chronic and larval studies with dicamba and nicosulfuron have been performed in order to fulfil new data requirements in accordance with Regulation (EC) No 1107/2009, and as a result there are new endpoints. These studies are summarised in Table 9.6-3 and Table 9.6-1, listed in Appendix 1 and summarised in Appendix 2. The new studies for dicamba have been carried out with the respective solo a.s. formulations *in lieu* of the active substances, as these are easier to provide in solution to the bees and in addition, any chronic effects from exposure will be due to the active substance rather than the formulation. Furthermore, there is no indication from current non-target arthropod studies that this herbicide has any insecticidal activity.~~

~~**Acute and chronic mixture toxicity**~~

~~According to the draft (EFSA Journal 2014;11(7):3295) combined action of several toxicants must be specifically considered in the risk assessment when it is obvious that such exposure situations will occur for bees.~~

~~For the assessment of effects, surrogate endpoints can be calculated. The EFSA Guidance Document indicates that the following equation should be used for deriving a surrogate endpoint for a mixture of active substances with known toxicity assuming dose additivity:~~

$$LD_{50}\,(\text{mix}) = \left(\sum_i \frac{X(a.s._i)}{LD_{50}(a.s._i)} \right)^{-1}$$

where:
$X_{(a.s._i)}$ = fraction of active substance (i) in the formulation mixture
$LD_{50(a.s._i)}$ = acute toxicity for the active substance (i)

The mixture endpoints are summarised in the table below.

Table 9.6-5: Endpoints for the dicamba/mesotrione/nicosulfuron mixture

Exposure system	Test substance	Concentration of active substance in formulation A18032E (g/L)	Fraction of active substance in the formulation mixture [A]	Toxicity endpoint (µg a.s./bee)	Predicted endpoint for mixture (µg a.s./bee)	Predicted endpoint for mixture (µg product/bee) [B]	Measured endpoint (µg product/bee)
Acute contact	Dicamba	312.5	0.556	>100	>94.7	>168	>227
	Mesotrione	150	0.267	>100			
	Nicosulfuron	100	0.178	76			
	Total	562.5	1	-			
Acute oral	Dicamba	312.5	0.556	>100	15.7	27.9	170
	Mesotrione	150	0.267	>11			
	Nicosulfuron	100	0.178	5.24			
	Total	562.5	1	-			
Adult chronic	Dicamba	312,5	0.556	194,7	31.0	55.1	n.a.
	Mesotrione	150	0.267	19.2			
	Nicosulfuron	100	0.178	11.43			
	Total	562.5	1	-			
Larval chronic	Dicamba	312.5	0.556	125	52.3	92.9	n.a.
	Mesotrione	150	0.267	46			
	Nicosulfuron	100	0.178	20			
	Total	562.5	1	-			

[A] Concentration of an active substance in the formulation divided by the total concentration of all active substances in the formulation.
[B] Used for comparison with measured acute toxicity of product

The acute oral and contact measured LD_{50} of the formulation is more than 6 and 1.4 fold lower than the predicted respective LD_{50} of the active substances in the mixture, indicating that the formulation is less toxic than expected from additive toxicity.

For the purposes of risk assessment the formulation and predicted endpoints will be used in the acute risk assessments, and the predicted mixture endpoints will be used in the chronic adult and larval risk assessment.

zRMS comments:

Consideration of new acute toxicity studies performed with A18032E is justified, since the formulation contains three active compounds and testing with the formulated product in such case is mandatory.

The new studies on chronic toxicity of dicamba (solo formulation), mesotrione and nicosulfuron to adult bees and bee larvae were not evaluated by the zRMS since in order to fulfil the data requirements set by the Commission Regulation (EU) No 284/2013 studies on chronic and larvae toxicity performed with A18032E should have been submitted due to presence of 3 active compounds in the product, while studies addressing data requirements set by Commission Regulation (EU) No 283/2013 should be evaluated at the EU level.

Mixture toxicity assessment provided by the Applicant above was not validated by the zRMS since its results will not be used in the risk assessment performed in line with SANCO 10329/2002 rev 2 final. Furthermore, as already indicated above, respective studies on chronic adult and larvae toxicity of A18032E must be generated since testing is mandatory in case of formulations with 2 or more active substances.

Not validated information has been struck through in text above.

9.6.2 Risk assessment

For the purposes of this risk assessment, the evaluation of the risk for honeybees was performed in accordance with the principles of the "EFSA Guidance Document on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees)" (EFSA Journal 2013;11(7):3295 – updated 2014).
The applicant considers that risk assessment to the EFSA guidance is not appropriate for regulatory decision making at EU level as the guidance is not agreed by all member states and as such has not been noted. However, given recent requests by EFSA and many Member States, an assessment has been provided by the applicant below. Areas where a lot of uncertainty in approach still exist (e.g. water exposure, HPG assessment and bumble and solitary bee assessments) have not been addressed.

The risk assessment guidance is structured in a stepwise manner beginning with a screening step assessment. Those scenarios which pass the screening step are considered to demonstrate acceptable risk and as such will not be considered at higher tiers of assessment.

All calculations were performed using the EFSA Bee calculator Tool (Bee Tool v.3; Date accessed 24/5/2017) available at http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2013.3295/full.

Where the screening step indicates a potential risk for acute or chronic exposure to bees, a Tier I risk assessment will be performed. For acute contact and oral a Tier I risk assessment will be conducted using the EFSA bee calculator Tool. The treated crop scenario is considered by the applicant to represent the worst-case exposure. All other scenarios are considered to have lower exposure e.g. field margins, adjacent crop etc. Therefore only contact and oral exposure in the treated crop is considered at Tier 1 and where this indicates ETR values below the triggers, acceptable risk to bees is considered to be demonstrated.

For chronic exposure to adult bees and honey bee larvae, the Tier I risk assessment is conducted following the EFSA Bee Guidance Document (2013) modified according to the ECPA approach[23]. A detailed explanation of the methods is provided under the 'Tier 1 – Chronic Risk Assessment' utilising the EFSA bee calculator tool.

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the worst-case application rate of 0.6 kg A18032E/ha (corresponding to 187.5 g dicamba/ha, 90 g mesotrione/ha and 60 g nicosulfuron/ha) is used in the risk assessment.

Table 9.6-6: Crop groupings and critical use patterns relevant to the use of A12739A

Test substance	GAP crop species	Application category	Critical use pattern		
			Rate (kg/ha)	No. of apps	App. Interval (days
A18032E	Maize BBCH 12-18	Downward spray	0.6	1	N/A

zRMS comments:

According to conclusions of the Central Zone Steering Committee, recommendations of EFSA (2013) should not be considered for the zonal evaluations until the guidance is noted at the EU level. Taking this into account, the risk assessment in the Core Assessment should be performed in line with recommendations of the current guidance, i.e. SANCO/10329/2002 rev 2 final.
In addition to that it is noted that the Applicant considered recommendations of EFSA (2013), but with some modifications as proposed by ECPA. This is approach is not agreed by the zRMS, as the guidance should be

[23] ECPA (2017) Proposal for a protective and workable regulatory European bee risk assessment scheme based on the EFSA bee guidance and other new data and available approaches http://www.ecpa.eu/sites/default/files/document_policy/28028_ECPA%20Proposal%20for%20a%20protective%20and%20workable%20EU%20Bee%20Risk%20Assessment%20-%20Version%2009%20June%2017.pdf

followed entirely and not selectively. Furthermore, modifications of ECPA are not part of the guidance and have to be implemented into the risk assessment scheme to be accepted.

Consideration of the risk envelope approach as indicated in Table 9.6-5 is agreed by the zRMS, however risk assessment performed by the zRMS was based on the intended GAP of A18032E in Poland (see commenting box in point 9.6.2.1 below).

9.6.2.1 Hazard quotients for bees

Screening Step Acute and Chronic Risk Assessment

Acute, chronic adult and larval honey bee studies have been conducted with A18032E and/or dicamba, mesotrione or nicosulfuron, according to the data requirements under 1107/2009. The endpoints from these studies have been assessed by using EFSA Bee Guidance (2013) and EFSA Bee Tool.

Table 9.6-7: Screening step assessment of the risk for bees due to the use of formulation A18032E in maize – dicamba

Intended use **Active substance** **Application rate (g a.s./ha)**	Downward Spray dicamba 1 × 187.5				
Test design	**Endpoint (lab.) (µg/bee)**	**Single application rate**	**Shortcut Value (downward spray)**	**HQ/ ETR**	**Trigger**
Acute contact toxicity LD_{50}	>100	187.5 g/ha	(1)	<1.9	42
Acute oral toxicity LD_{50}	>100	0.1875 kg/ha	7.6	0.01	0.2
Chronic adult oral toxicity LDD_{50}	194.7 µg a.i./bee/day	0.1875 kg/ha	7.6	0.007	0.03
Larval development oral toxicity NOED	125 µg a.i./ larva/ development period	0.1875 kg/ha	4.4	0.010	0.2

HQ (hazard quotients) and ETR (exposure toxicity ratio) for oral and contact exposure. HQ/ETR values shown in bold breach the relevant trigger.

The HQ / ETR values for dicamba are all less than the screening step trigger values for downward sprays indicating that the acute and chronic risk to honeybees is acceptable following use of dicamba according to the proposed use pattern. The risk envelope approach is applied and calculations are valid for all proposed uses.

Table 9.6-8: Screening step assessment of the risk for bees due to the use of formulation A18032E in maize – mesotrione

Intended use **Active substance** **Application rate (g a.s./ha)**	Downward Spray mesotrione 1 × 90				
Test design	**Endpoint (lab.) (µg/bee)**	**Single application rate**	**Shortcut Value (downward spray)**	**HQ/ ETR**	**Trigger**
Acute contact toxicity LD_{50}	>100	90 g/ha	(1)	<0.9	42
Acute oral toxicity LD_{50}	11	0.09 kg/ha	7.6	0.06	0.2
Chronic adult oral toxicity LDD_{50}	19.2 µg a.i./bee/day	0.09 kg/ha	7.6	**0.036**	0.03
Larval development oral toxicity NOED	46 µg a.i./ larva/ development period	0.09 kg/ha	4.4	0.010	0.2

HQ (hazard quotients) and ETR (exposure toxicity ratio) for oral and contact exposure. HQ/ETR values shown in bold breach the relevant trigger.

The HQ / ETR values for mesotrione are less than the screening step trigger values for downward sprays indicating that the acute and chronic risk to honeybees is acceptable following use of mesotrione according to the proposed use pattern. However, the screening step risk assessment above has indicated a potential chronic oral risk and therefore a Tier 1 assessment for the treated crop has been provided.

Tier 1 – Chronic Risk Assessment

Table 9.6-9: First-tier assessment of the chronic risk for bees due to the use of A18032E in maize for the treated crop – mesotrione

Intended use **Active substance** **Application rate (g/ha)**		Downward spray Mesotrione 1 × 90					
Test design	**LDD_{50} (lab.) (µg/bee)**	**Single application rate**	**Shortcut Value (downward spray)**	**TWA**	**fDep/ Ef**	**HQ/ ETR**	**Trigger**
Adult chronic oral toxicity	19.2	0.09 kg/ha	0.92	0.72	1	0.003	0.03

HQ (hazard quotients) and ETR (exposure toxicity ratio) for oral and contact exposure. HQ/ETR values shown in bold breach the relevant trigger.

The Tier 1 HQ/ETR value for mesotrione is less than the trigger for downward sprays, indicating that the chronic risk to honeybees is acceptable following use of A18032E according to the proposed use pattern.

Table 9.6-10: Screening step assessment of the risk for bees due to the use of formulation A18032E in maize – nicosulfuron

Intended use **Active substance** **Application rate (g a.s./ha)**	Downward Spray nicosulfuron 1 × 60				
Test design	**Endpoint (lab.) (µg/bee)**	**Single application rate**	**Shortcut Value (downward spray)**	**HQ/ ETR**	**Trigger**
Acute contact toxicity LD_{50}	76	60 g/ha	(1)	0.8	42
Acute oral toxicity LD_{50}	5.24	0.06 kg/ha	7.6	0.09	0.2
Chronic adult oral toxicity LDD_{50}	11.43 µg a.i./bee/day	0.06 kg/ha	7.6	**0.04**	0.03
Larval development oral toxicity NOED	20 µg a.i./ larva/ development period	0.06 kg/ha	4.4	0.01	0.2

HQ (hazard quotients) and ETR (exposure toxicity ratio) for oral and contact exposure. HQ/ETR values shown in bold breach the relevant trigger.

The HQ / ETR values for nicosulfuron are less than the screening step trigger values for downward sprays indicating that the acute and chronic risk to honeybees is acceptable following use of nicosulfuron according to the proposed use pattern. However, the screening step risk assessment above has indicated a potential chronic oral risk and therefore a Tier 1 assessment for the treated crop has been provided.

Tier 1 – Chronic Risk Assessment

Table 9.6-11: First-tier assessment of the chronic risk for bees due to the use of A18032E in maize for the treated crop – nicosulfuron

Intended use **Active substance** **Application rate (g/ha)**		Downward spray Nicosulfuron 1 × 60					
Test design	**LDD_{50} (lab.) (µg/bee)**	**Single application rate**	**Shortcut Value (downward spray)**	**TWA**	**fDep/ Ef**	**HQ/ ETR**	**Trigger**
Adult chronic oral toxicity	11.43	0.06 kg/ha	0.92	0.72	1	0.003	0.03

HQ (hazard quotients) and ETR (exposure toxicity ratio) for oral and contact exposure. HQ/ETR values shown in bold breach the relevant trigger.

The Tier 1 HQ/ETR value for mesotrione is less than the trigger for downward sprays, indicating that the chronic risk to honeybees is acceptable following use of A18032E according to the proposed use pattern.

Combination mixture assessment

Acute and Chronic Mixture Assessment – Screening Step

To assess a worst-case both the measured and predicted mixture toxicity values for the formulation are assessed below.

Table 9.6-12: Screening step assessment of the risk for bees due to the use of formulation A18032E in maize – A18032E

Intended use **Active substance** **Application rate (g product/ha)**	Downward Spray A18032E 1 × 600[a]				
Test design	**Endpoint (lab.) (µg/bee)**	**Single application rate**	**Shortcut Value (downward spray)**	**HQ/ ETR**	**Trigger**
Acute contact toxicity LD_{50}	>227 (measured) >168 (predicted)	600 g/ha	(1)	2.6 3.6	42
Acute oral toxicity LD_{50}	170 (measured) 27.9 (predicted)	0.6 kg/ha	7.6	0.03 0.16	0.2
Chronic adult oral toxicity LDD_{50}	55.1 (predicted)	0.6 kg/ha	7.6	**0.083**	0.03
Larval development oral toxicity NOED	92.9 (predicted)	0.6 kg/ha	4.4	0.03	0.2

HQ (hazard quotients) and ETR (exposure toxicity ratio) for oral and contact exposure. HQ/ETR values shown in bold breach the relevant trigger.

[a] A18032E is applied as 0.6kg/ha; density is not relevant it is a WG

The HQ / ETR values for A18032E are less than the screening step trigger values for downward sprays indicating that the acute and chronic risk to honeybees is acceptable following use according to the proposed use pattern. However, the screening step risk assessment above has indicated a potential chronic oral risk from the worst case predicted toxicity values, and therefore a Tier 1 assessment for the treated crop has been provided below.

Tier 1 – Chronic Risk Assessment

Table 9.6-13: First-tier assessment of the chronic risk for bees due to the use of A18032E in maize for the treated crop – A18032E

Intended use **Active substance** **Application rate (g/ha)**		Downward spray A18032E 1 × 600 [a]					
Test design	**LDD_{50} (lab.) (µg/bee)**	**Single application rate**	**Shortcut Value (downward spray)**	**TWA**	**fDep/ Ef**	**HQ/ ETR**	**Trigger**
Adult chronic oral toxicity	217 (predicted)	0.6 kg/ha	0.92	0.72	1	0.007	0.03

HQ (hazard quotients) and ETR (exposure toxicity ratio) for oral and contact exposure. HQ/ETR values shown in bold breach

~~the relevant trigger.~~
~~[a] A18032E is applied as 0.6kg/ha; density is not relevant it is a WG~~

~~The HQ/ETR values for A18032E are less than the trigger for downward sprays, indicating that the chronic risk to honeybees is acceptable following use of A18032E according to the proposed use pattern.~~

Risk from metabolites

In accordance with the **EFSA Guidance Document on the risk assessment of plant protection products on bees (EFSA, 2013)** as the identified metabolites of Dicamba, mesotrione and nicosulfuron are formed in amounts of <10% then the risk assessment is considered covered by the parent and so further assessment is not required.

zRMS comments:

The risk assessment performed by the Applicant in line with EFSA (2013) was not validated by the zRMS, as according to conclusions of the Central Zone Steering Committee, recommendations of EFSA (2013) should not be considered for the zonal evaluations until the guidance is noted at the EU level. Taking this into account, the risk assessment in the Core Assessment should be performed in line with recommendations of the current guidance, i.e. SANCO/10329/2002 rev 2 final. Respective calculations are thus presented in table below. The Applicants' calculations were based on the risk envelope approach, but in below calculations the intended application rate was considered (0.4 kg product/ha).

Intended use	Maize, BBCH 12-18, 1 x 0.4 kg product/ha		
Active substance	dicamba		
Application rate (g a.s./ha)	1 × 125 g a.s./ha		
Test design	**LD_{50} (lab.) (µg a.s/bee)**	**Single application rate (g a.s./ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	>100	125	<1.25
Contact toxicity	>100		<1.25
Product	mesotrione		
Application rate (g product/ha)	1 × 60 g a.s./ha		
Test design	**LD_{50} (lab.) (µg a.s/bee)**	**Single application rate (g a.s./ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	>11.0	60	<5.45
Contact toxicity	>100		<0.60
Product	nicosufuron		
Application rate (g product/ha)	1 × 40 g a.s./ha		
Test design	**LD_{50} (lab.) (µg a.s/bee)**	**Single application rate (g a.s./ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	5.24	40	7.63
Contact toxicity	76		0.53

Product	A18032E		
Application rate (g product/ha)	1 × 400 g product/ha		
Test design	**LD_{50} (lab.) (µg product/bee)**	**Single application rate (g product/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	170	400	2.35
Contact toxicity	>227		<1.76

All calculated HQ values are below the trigger of 50 indicating acceptable risk to bees from intended uses of A18032E.

Information regarding exposure to metabolites provided by the Applicant is agreed by the zRMS. It should be also noted that neither of active compounds exhibit insecticidal MoA and available data indicate that metabolites formed from dicamba, mesotrione and nicosulfuron are not more toxic than the parent compounds.

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

Not relevant.

9.6.3 Effects on bumble bees

No data or information is currently available for bumble bees.

Bumblebee acute oral and contact studies, chronic toxicity to adults and risk to larvae are not required under Regulation (EC) No 1107/2009 therefore currently there is no data requirement for these study types.

9.6.4 Effects on solitary bees

No data or information is currently available for solitary bees.

For solitary bees there are currently no validated accepted test guidelines or guidance documents available. Ring tests are ongoing for the *Osmia* acute (contact and oral) study and semi field test design. Studies on solitary bees are not required under Regulation (EC) No 1107/2009, therefore currently there is no data requirement for these study types.

9.6.5 Overall conclusions

zRMS comments:

The acute risk of A18032E posed to honeybees following the intended uses in maize was re-assessed by the zRMS in line with indications of SANCO/10329/2002 rev 2 final. Respective hazard quotients were calculated with consideration of acute oral and contact studies with A18032E, dicamba, mesotrione and nicosulfuron and the maximum single application rate of the product (0.4 kg/ha) and corresponding rates of active compounds.

All the calculated hazard quotients were less than the relevant trigger of 50, indicating that the acute oral and contact risk to bees is acceptable following use of A18032E according to the proposed use pattern.

~~The acute risk of A18032E to honeybees was assessed from hazard quotients and Exposure Toxicity Ratios (ETRs) following EFSA (2014), estimated from acute oral and contact studies with A18032E, dicamba, mesotrione and nicosulfuron, and exposure rates following application at the maximum single application rate of 0.6 kg A18032E/ha, equivalent to 187.5 dicamba/ha, 90 g mesotrione/ha and 60 g nicosulfuron/ha. All the hazard quotients and Exposure Toxicity Ratios (ETRs) for A18032E are less than the relevant triggers, indicating that the acute oral and contact risk to bees is acceptable following use of A18032E according to the proposed use pattern.~~

~~The chronic adult and larval risk of A18032E to honeybees was assessed from ETRs and toxicity exposure ratios (TERs) following the principles of EFSA (2014), estimated from chronic adult and larval studies with mesotrione, Dicamba and nicosulfuron. All the ETR and TER values are less/greater than respectively the relevant trigger values, indicating that the chronic risk to adult and larval honeybees is acceptable following use of A18032E according to the proposed use pattern.~~

9.7 Effects on arthropods other than bees (KCP 10.3.2)

9.7.1 Toxicity data

Studies on the toxicity to non-target arthropods have been carried out with the representative solo formulation of dicamba, mesotrione and nicosulfuron. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on non-target arthropods of A18032E were not evaluated as part of the EU assessment of dicamba, mesotrione and nicosulfuron. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

The selection of studies and endpoints for the risk assessment deviates from the results of the EU review process as endpoints and risk assessments were related to the formulated product A18032E. Further justifications are provided below.

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

Species	Substance	Exposure system	Results	Reference
Typhlodromus pyri (protonymphs)	Banvel 480 SL	Laboratory test glass plates (2D)	LR_{50} = 232.6 g a.s./ha	EFSA Conclusion 2011 Grimm, 2000a SAN837/5962
Aphidius rhopalosiphi (adults)	Banvel 480 SL	Laboratory test glass plates (2D)	LR_{50} = 356 g a.s./ha No effects on fecundity at 182 g a.s./ha	EFSA Conclusion 2011 Grimm, 2000b SAN837/5961
Typhlodromus pyri (protonymphs)	Banvel 480 SL	Extended laboratory test leaves (2D)	LR_{50} > 460 g a.s./ha No effects on fecundity at 57.5 g a.s./ha	EFSA Conclusion 2011 Zenz, 2002 SAN837/6039
Chrysoperla carnea (larvae)	Banvel 480 SL	Extended laboratory test maize leaves (2D)	LR_{50} > 960 g a.s./ha No effects on fecundity at 960 g a.s./ha	EFSA Conclusion 2011 Hargreaves and Weyman, 2003 SAN837/6110
Aloechara bilineata (adult)	Banvel 480 SL	Extended laboratory test sand (2D)	LR_{50} > 363 g a.s./ha No effects on fecundity at 363 g a.s./ha	EFSA Conclusion 2011 Taruza, 2001 SAN837/5967
Poecilus cupreus (adults)	Banvel 480 SL	Extended laboratory test sand (2D)	LR_{50} > 360 g a.s./ha No effects on predation rate at 960 g a.s./ha	EFSA Conclusion 2011 Römbke, 1990 SAN837/0140

Table 9.7-2: Endpoints and effect values relevant for the risk assessment for non-target arthropods - mesotrione

Species	Substance	Exposure system	Results	Reference
Typhlodromus pyri (protonymphs)	A12739A	Laboratory test glass plates (2D)	LR_{50} = 93.11 g/ha	EFSA Conclusion 2016 Fallowfield, 2012 A12739A_10010
Aphidius rhopalosiphi (adults)	A12739A	Laboratory test glass plates (2D)	LR_{50} = 43.56 g/ha	EFSA Conclusion 2016 Stevens, 2012 A12739A_10008
Typhlodromus pyri (protonymphs)	A12739A	Extended laboratory test maize leaves (2D)	LR_{50} >300 g a.s./ha ER_{50} >150 g a.s./ha	EFSA Conclusion 2016 Fallowfield, 2013 A12739A_10020

Species	Substance	Exposure system	Results	Reference
Aphidius rhopalosiphi (adults)	A12739A	Extended laboratory test barley leaves (3D)	LR_{50} >225 g a.s./ha ER_{50} >225 g a.s./ha	EFSA Conclusion 2016 Stevens, 2013 A12739A_10276
Aleochara bilineata (adults)	A12739A	Extended laboratory test sand (2D)	ER_{50} >200 g a.s./ha	EFSA Conclusion 2016 Tew, 2013 A12739A_10275
Pardos sp. (adults)	A12739A	Extended laboratory test soil (2D)	LR_{50} >150 g a.s./ha ER_{50} >150 g a.s./ha	EFSA Conclusion 2016 Vaughan, 2013 A12739A_10388

Table 9.7-3: Endpoints and effect values relevant for the risk assessment for non-target arthropods - nicosulfuron

Species	Substance	Exposure system	Results	Reference
Typhlodromus pyri (protonymphs)	SL 950 4% SC	Laboratory test glass plates (2D)	LR_{50} > 60 g a.s./ha	EFSA Conclusion 2007 Goßmann, 1998 4270063
Aphidius rhopalosiphi (adults)	SL 950 4% SC	Laboratory test glass plates (2D)	LR_{50} > 60 g a.s./ha	EFSA Conclusion 2007 Moll and Groer, 1998 2900001
Aphidius rhopalosiphi (adults)	SL 950 4% SC	Extended laboratory test barley plants (3D)	LR_{50} > 60 g a.s./ha ER_{50} > 60 g a.s./ha	EFSA Conclusion 2007 Moll and Bienert, 1998 3420002
Poecilus cupreus (adults)	SL 950 4% SC	Extended laboratory test sand (2D)	LR_{50} > 60 g a.s./ha ER_{50} > 60 g a.s./ha	EFSA Conclusion 2007 Petto, 1993 324617
Coccinella septempunctata (larvae)	SL 950 4% SC	Extended laboratory test glass plates (2D)	LR_{50} > 120 g a.s./ha ER_{50} > 120 g a.s./ha	EFSA Conclusion 2007 Klepka, 1994 457602
Aloechara bilineata (adult)	SL 950 4% SC	Extended laboratory test sand (2D)	LR_{50} > 60 g a.s./ha ER_{50} > 60 g a.s./ha	EFSA Conclusion 2007 Goßmann, 1997 2450070

zRMS comments:

Toxicity data for non-target arthropods provided in Tables 9.7-1 to 9.7-3 are in line with data reported in:

- EFSA Journal 2011;9(1):1965 for dicamba,
- EFSA Journal 2016;14(3):4419 for mesotrione,
- EFSA Scientific Report (2007) 120 for nicosulfuron.

However, results of these studies were struck through in Tables 9.7-1 to 9.7-3 as being generated with solo-formulations of particular active substances and thus not relevant for evaluation performed for A18032E.

Table 9.7-4: Endpoints and effect values relevant for the risk assessment for non-target arthropods - A18032E

Species	Substance	Exposure system	Results	Reference
Typhlodromus pyri (protonymphs)	A18032E	Laboratory test glass plates (2D)	LR_{50} = 1028.2 g/ha ER_{50} >600 g/ha	Fallowfield, 2012 A18032E_10003
Aphidius rhopalosiphi (adults)	A18032E	Laboratory test glass plates (2D)	LR_{50} = 23.3 g/ha ER_{50} >25 g/ha	Stevens, 2012 A18032E_10000
Aphidius rhopalosiphi (adults)	A18032E	Extended laboratory test barley plants (3D)	LR_{50} > 1200 g/ha ER_{50} >1200 g/ha	Stevens, 2012a A18032E_10010
Aloechara bilineata (adult)	A18032E	Extended laboratory test sand (2D)	LR_{50} >600 g/ha ER_{50} >600 g/ha	Tew, 2013 A18032E_10015

zRMS comments:

Studies on toxicity of A18032E to non-target arthropods were evaluated by the zRMS and considered acceptable. For details of evaluation, please refer to Appendix 2. Endpoints reported in Table 9.7-4 are confirmed to be correct.

Since formulation A18032E is intended to be applied exclusively with the adjuvant, all formulation studies were performed with the recommended adjuvant Adigor (A12127R).

9.7.1.1 Justification for new endpoints

Studies with non-target arthropods are always conducted with a formulated product and no testing is carried out with unformulated technical material. Therefore it may not be appropriate to rely on the data for the individual solo formulations, submitted as representative formulations for the EU review, for the risk assessment for non-target arthropods.

The testing and risk assessment strategy used here follows the approach recommended in the ESCORT 2 guidance document (***Candolfi et al., 2001***)[24] as proposed by **EC Guidance Document on Terrestrial Ecotoxicology** [25].

The toxicity of A18032E to non-target arthropods has been investigated by carrying out a Tier I test on *Aphidius rhopalosiphi* and *Typhlodromus pyri*. These two species are tested, in accordance with ESCORT 2, as representative non-target arthropods since they have been found to be particularly sensitive species, and therefore can be considered as indicators of potential effects to the most sensitive non-target arthropods in the field. Since the Tier 1 risk assessment indicated a potential risk to *Aphidius*, in accordance with ESCORT 2 an additional Tier II test on *Aphidius rhopalosiphi* was conducted and the soil dwelling arthropod *Aleochara bilineata* was tested as an additional species relevant for applications to nearly bare soil since A18032E is recommended for use early post-emergence.

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.

[24] Candolfi MP, Barrett KL, Campbell PJ, Forster R, Grandy N, Huet M-C, Lewis G, Oomen PA, Schmuck R, Vogt H (2000) 'Guidance Document on regulatory testing procedures for plant protection products with non-target arthropods' From the workshop, European Standard Characteristics of Non-target Arthropod Regulatory Testing (ESCORT 2) 21-23 March 2000.

[25] EC Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC, SANCO/10329, 17 October 2002.

9.7.2.1 Risk assessment for in-field exposure

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the worst-case application rate (600 g A18032E/ha) is used in the risk assessment.

The $PER_{in\text{-}field}$ values according to ESCORT 2 were calculated as:

Application rate × MAF

Table 9.7-5: First- and higher-tier assessment of the in-field risk for non-target arthropods due to the maximum use of A18032E in maize

Intended use	Maize		
Product	A18032E		
Application rate (g/ha)	1 × 600		
MAF	1 (foliar) / 1 (soil)		
Test species Tier I	**LR_{50} (lab.) (g/ha)**	**$PER_{in\text{-}field}$ (g/ha)**	**$HQ_{in\text{-}field}$ criterion: HQ ≤ 2**
Typhlodromus pyri	1028.2	600(foliar) 600 (soil)	0.58 (foliar) 0.58 (soil)
Aphidius rhopalosiphi	23.3		**26** (foliar) **26** (soil)
Test species Higher-tier	**Rate with ≤ 50 % effect (g/ha)**	**$PER_{in\text{-}field}$ (g/ha)**	**$PER_{in\text{-}field}$ below rate with ≤ 50 % effect?**
Aphidius rhopalosiphi	>1200	600 (foliar) 600 (soil)	Yes
Aloechara bilineata	>600	600 (foliar) 600 (soil)	Yes

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient; Criteria values shown in bold breach the relevant trigger.

The HQ values for *A. rhopalosiphi* are greater than the trigger of 2 for Tier I tests indicating a potential risk to sensitive non-target arthropods within the field, which needs to be assessed further.
A Tier II test has been carried out with *A. rhopalosiphi*. A test with an additional species is triggered by the Tier 1 in-field risk assessment for *A. rhopalosiphi*, and therefore a test has been carried out on the relevant ground-dwelling beetle *Aleochara bilineata* since applications are early post-emergence and therefore a representative ground-dweller is more relevant than a foliage-dwelling arthropod.
Higher tier fecundity assessments for *A. rhopalosiphi* and *A. bilineata* indicated that exposure to 1200 g/ha and 600 g/ha, respectively, did not cause an unacceptable (>50%) reduction in fecundity relative to the control.

In conclusion the risk assessment for non-target arthropods indicates that there will be no unacceptable effects in-field from the proposed uses of A18032E.

zRMS comments:

The risk assessment presented in Table 9.7-5 is agreed by the zRMS. Evaluation was performed for the exaggerated application rate of 0.6 kg product/ha, being protective for the rate intended in Poland (0.4 kg product/ha).

Selection of *Aleochara bilineata* for Tier II testing was based on recommendations of ESCORT 2 and is agreed by the zRMS.

Based on above evaluation, acceptable risk may be concluded for in-field populations of non-target arthropods from the intended uses of A18032E.

9.7.2.2 Risk assessment for off-field exposure

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the worst-case application rate (600 g A18032E/ha) is used in the risk assessment.

The $PER_{off\text{-}field}$ value according to ESCORT 2 was calculated as:

Application rate × MAF × (drift factor/VDF (vegetation distribution factor)).

Note - The model used to estimate spray drift was developed for drift onto a two-dimensional water surface and, as such, does not account for interception and dilution by three-dimensional vegetation in off-crop areas. Therefore, a vegetation distribution factor (or dilution factor) is incorporated into the equation when calculating PERs to be used in conjunction with toxicity endpoints derived from two-dimensional (glass plate or leaf disc) studies. A VDF of 10 is recommended by ESCORT 2. For 3-dimensional studies, i.e. where spray treatment is applied onto whole plants, the VDF of 10 is not used, as any dilution over the 3-dimensional vegetation surface is accounted for in the study design.

The corrected $PER_{off\text{-}field}$ values according to ESCORT 2 was calculated as:

corr. $PER_{off\text{-}field}$ = $PER_{off\text{-}field}$ * correction factor

Note - ESCORT 2 recommends that a correction factor is applied to study data to account for extrapolation from testing just two representative species, to the species diversity expected in off-crop areas. A correction factor of 10 is applied for Tier I data. A correction factor of 5 is applied to Tier II data.

Table 9.7-6: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the maximum use of A18032E in maize

Intended use		Maize				
Product		A18032E				
Application rate (g/ha)		1 × 600				
MAF		1				
Drift rate		2.77%				
VDF		10 (2D) / none (3D)				
Test species Tier I	**LR_{50} (lab.) (g/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (g/ha)**	**CF**	**Corrected $PER_{off\text{-}field}$ (g/ha)**	**$HQ_{off\text{-}field}$ criterion: HQ ≤ 2**
Typhlodromus pyri	1028.2	0.0277	1.662	10	16.62	0.016
Aphidius rhopalosiphi	23.3					0.71
Test species Higher-tier	**Rate with ≤ 50 % effect (g/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (g/ha)**	**CF**	**Corrected $PER_{off\text{-}field}$ (g/ha)**	**corr. $PER_{off\text{-}field}$ below rate with ≤ 50 % effect?**
Aphidius rhopalosiphi	>1200	0.0277	16.62	5	83.1	Yes
Aloechara bilineata	>600	0.0277	1.662	5	8.31	Yes

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.

zRMS comments:

The risk assessment presented in Table 9.7-6 is agreed by the zRMS. Evaluation was performed for the exaggerated application rate of 0.6 kg product/ha, being protective for the rate intended in Poland (0.4 kg product/ha).

Based on above evaluation, acceptable risk to off-field populations of non-target arthropods from the intended uses of A18032E may be concluded with no need for risk mitigation measures.

It is noted that in calculation of the off-field exposure for 2-dimensional systems the Applicant considered VDF of 10, while currently some Member States require consideration of AF of 5, in line with discussion held during the general meeting in area of ecotoxicology in 2019. However, in line with implementation schedule indicated in the Bullet points in area of ecotoxicology agreed by the CZSC in November 2021, VDF of 5 should be considered since 1st of July 2022. Furthermore, Bullet point 4 presented in this document indicates that:

The majority of MSs agreed to be in line with the EFSA Technical Report (2019) and use a VDF of 5

It should be pointed out that the EFSA Technical Report (EFSA Supporting publication 2019:EN-1673) does not indicate that currently VDF of 5 must be used in evaluations, but that VDF of 5 should be considered as an interim solution that will be reflected in the SANCO/10329/2002-rev.2 guidance document with its implementation considered further. However, the SANCO guidance document was not amended yet and this is acknowledged in the most recent version of the Working document on Risk Assessment of Plant Protection Products in the Central Zone (May 2021):

The CZSC will make an urgent request to the Commission to adjust this issue in the guidance document as soon as possible.

Therefore, from the formal point of view, VDF of 10 is still applicable and may be used for purposes of calculation of the off-field exposure.

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

At Tier I, the in-field and off-field HQ values for *Typhlodromus pyri* were below the trigger value for the worst-case use scenario (1 x 600 g A18032E/ha in maize) indicating that the risk to non-target arthropods is acceptable following the use of A18032E according to the proposed use pattern.

At Tier I the off-field HQ value for *Aphidius rhopalosiphi* was below the trigger value for the worst-case use scenario (1 x 600 g A18032E/ha in maize) indicating acceptable off-field risk to this species following the use of A18032E according to the proposed use pattern. However, the in-field HQ values for *Aphidius rhopalosiphi* were above the trigger value and required further refinement. The Tier II, extended laboratory studies showed acceptable foliar in-field and off-field effects from foliar applications of A18032E for *Aphidius rhopalosiphi* and *Aloechara bilineata* for the worst-case use scenario (1 x 600 g A18032E/ha in maize).

Overall, the risk to non-target arthropods is therefore acceptable following use of A18032E according to the proposed use pattern with no need for risk mitigation measures.

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

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 dicamba, mesotrione, nicosulfuron and their 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).

Effects on earthworms and other non-target soil organisms (meso- and macrofauna) of A18032E were not evaluated as part of the EU assessment of dicamba, mesotrione and nicosulfuron. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process. Justifications are 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) - dicamba and relevant soil metabolites

Species	Substance	Exposure system	Results	Reference
~~*Eisenia fetida*~~	~~Dicamba~~	~~Mixed into substrate 14 d, acute 10 % peat content~~	~~LC_{50} > 1000 mg a.s./kg dw~~	~~EFSA Conclusion 2011 Barth, 2001a SAN837/5970~~
~~*Eisenia fetida*~~	~~Dicamba (formulated as Banvel 480 SL)~~	~~Mixed into substrate 14 d, acute 10 % peat content~~	~~LC_{50} > 480 mg a.s./kg dw~~	~~EFSA Conclusion 2011 Rombke and Vickus, 1990 SAN837/0141~~
~~*Eisenia fetida*~~	~~NOA414746~~	~~Mixed into substrate 14 d, acute 10 % peat content~~	~~LC_{50} > 1000 mg/kg dw~~	~~EFSA Conclusion 2011 Barth, 2001b NOA414746/0007~~
Field studies				
Not relevant				
Litter bag test				
Not relevant				

zRMS comments:

Although endpoints presented in Table 9.8-1 are in line with the EU agreed endpoints reported in EFSA Journal 2011;9(1):1965, they were struck through in Table 9.8-1 as originating from the acute toxicity studies which are no longer a data requirement and are thus not considered in the risk assessment.

No chronic toxicity data for dicamba were generated in the course of the EU review, however, the risk to soil organisms from dicamba is considered to be sufficiently addressed in the risk assessment performed for A18032E since according to indications of Commission Regulation (EU) No 283/2013 testing with the formulation may be more appropriate than studies performed with the active substance.

Table 9.8-2: Endpoints and effect values relevant for the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) – mesotrione and relevant soil metabolites

Species	Substance	Exposure system	Results	Reference
~~Acute~~				
~~*Eisenia fetida*~~	~~Mesotrione~~	~~Mixed into substrate 28 d, acute 10 % peat content~~	~~LC_{50} >2000 mg a.s./kg dw~~	~~EFSA Conclusion 2016 Bembridge and Jackson, 1996 ZA1296/0554~~
~~*Eisenia fetida*~~	~~MNBA~~	~~Mixed into substrate 28 d, acute 10 % peat content~~	~~LC_{50} >1000 mg/kg dw~~	~~EFSA Conclusion 2016 Travis and Gough, 1999 ZA1296/0528~~
Chronic				
Eisenia fetida	Mesotrione (formulated as A12739A)	Mixed into substrate 56 d, chronic 10 % peat content	NOEC = 10.85 mg a.s./kg dw EC_{10} = 5.91 mg a.s./kg dw	EFSA Conclusion 2016 Friedrich, 2011 A12739A_10000
Eisenia fetida	MNBA	Mixed into substrate 56 d, chronic 10 % peat content	NOEC = 1050 mg/kg dw EC_{10} >1050 mg/kg dw	EFSA Conclusion 2016 Friedrich, 2013a CA3511_10002
Eisenia fetida	AMBA	Mixed into substrate 56 d, chronic 10 % peat content	NOEC = 1050 mg/kg dw EC_{10} = 1050 mg/kg dw	EFSA Conclusion 2016 Friedrich, 2013b R044276_10002
Folsomia candida	Mesotrione (formulated as A12739A)	Mixed into substrate 28 d, chronic 5 % peat content	NOEC = 50.5 mg a.s./kg dw [a] EC_{10} = 37.5 mg a.s./kg dw [a]	EFSA Conclusion 2016 Friedrich, 2013c A12739A_10013
Folsomia candida	MNBA	Mixed into substrate 28 d, chronic 5 % peat content	NOEC = 100 mg/kg dw ~~NOEC = 180 mg/kg dw~~	Dickinson, 2015 CA3511_10011
Hypoaspis aculeifer	Mesotrione (formulated as A12739A)	Mixed into substrate 14 d, chronic 5 % peat content	NOEC = 90.9 mg a.s./kg dw [a] EC_{10} > 90.9 mg a.s./kg dw [a]	EFSA Conclusion 2016 Schulz, 2013 A12739A_10014
Hypoaspis aculeifer	MNBA	Mixed into substrate 14 d, chronic 5 % peat content	NOEC = 1050 mg/kg dw EC_{10} could not be calculated	Ramsden, 2015 CA3511_10010
Field studies				
Not relevant				
Litter bag test				
Not relevant				

[a] The endpoints are reported as NOEC (reproduction) Collembola = 556 mg A12739A/kg and NOEC for *Hypoaspis* = 1000 mg A12739A/kg; these have been converted to the mesotrione content considering it is present as 9.09% w/w

zRMS comments:

Endpoints presented in Table 9.8-2 are in line with the EU agreed endpoints reported in EFSA Journal 2016;14(3):4419. Information regarding acute toxicity to earthworms, although in line with the LoEP, has been struck through as being no longer a data requirement.

It should be noted that the chronic toxicity of mesotrione to soil macro- and meso-fauna was addressed in studies performed with the representative solo formulation (A12739A) and for this reason derived endpoints may be not necessarily representative for the active substance itself due to presence of co-formulants in the test item. Nevertheless, in absence of the toxicity data for the parent, endpoints from studies with A12739A were agreed as a surrogate solution. Risk from mesotrione in A18032E is covered by the risk assessment performed for the

formulated product.

Additional studies on toxicity of metabolite MNBA to *Folsomia candida* and *Hypoaspis aculeifer* were submitted by the Applicant. It is noted that in general, new Annex II studies should not be evaluated at the zonal level and the risk assessment should be based on the EU agreed endpoints. As no data gap regarding further testing of toxicity of mesotrione metabolites to soil macro- and meso-fauna was identified in the EFSA Journal 2016;14(3):4419, newly submitted studies are deemed not necessary to finalisation of the soil risk assessment. Nevertheless, it is noted by the zRMS that formation of metabolite MNBA from mesotrione is high with maximum occurrence of nearly 60% of AR observed on day 28 in soil degradation studies. Taking this into account, the exposure of soil macro- and meso-fauna to this compound is high and potential risk is not covered by the performed studies. Therefore it was decided by the zRMS to evaluate the studies and consider their results in the risk assessment for precautionary reasons. For details of the studies evaluation, please refer to respective points in Appendix 2.

It is noted that no additional studies with metabolite AMBA were provided, although formation of this compound in soil degradation studies was >5% at more than two consecutive sampling points thus triggering the exposure and risk assessment. In absence of the experimentally derived endpoints, the risk assessment was performed with assumption that this metabolite is 10 times more toxic than the parent.

The lower of NOEC and EC_{10} value should be used in the risk assessment.

Table 9.8-3: Endpoints and effect values relevant for the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) - nicosulfuron and relevant soil metabolites

Species	Substance	Exposure system	Results	Reference
~~*Eisenia fetida*~~	~~Nicosulfuron~~	~~Mixed into substrate 14 d, acute 10 % peat content~~	~~LC_{50} > 1000 mg a.s./kg dw~~	~~EFSA Conclusion 2007 Ross et al, 1991 272-104C~~
~~*Eisenia fetida*~~	~~ASDM~~	~~Mixed into substrate 14 d, acute 10 % peat content~~	~~LC_{50} > 1000 mg/kg dw~~	~~EFSA Conclusion 2007 Buchanan and Knight, 1997b 15139~~
~~*Eisenia fetida*~~	~~ADMP~~	~~Mixed into substrate 14 d, acute 10 % peat content~~	~~LC_{50} > 1250 mg/kg dw~~	~~EFSA Conclusion 2007 Imai, 1996b 96026-I-02~~
~~*Eisenia fetida*~~	~~AUSN, HMUD, MU-466, UCSN~~	~~Mixed into substrate 14 d, acute 10 % peat content~~	~~LC_{50} > 1250 mg/kg dw~~	~~EFSA Conclusion 2007 Imai, 1996a 96026-I-01~~
Eisenia fetida	AUSN	Mixed into substrate 56 d, chronic 10 % peat content	NOEC = 0.100 mg/kg dw	EFSA Conclusion 2007 Lührs, 2006a 31611022
Eisenia fetida	UCSN	Mixed into substrate 56 d, chronic 10 % peat content	NOEC = 0.050 mg/kg dw	EFSA Conclusion 2007 Lührs, 2006a 31611022
Eisenia fetida	ASDM	Mixed into substrate 56 d, chronic 10 % peat content	NOEC = 0.350 mg/kg dw	EFSA Conclusion 2007 Lührs, 2006a 31611022
Folsomia candida	AUSN	Mixed into substrate 28 d, chronic 10 % peat content	NOEC = 0.100 mg/kg dw	EFSA Conclusion 2007 Lührs, 2006b 31612016
Folsomia candida	UCSN	Mixed into substrate 28 d, chronic	NOEC = 0.050 mg/kg dw	EFSA Conclusion 2007

Species	Substance	Exposure system	Results	Reference
		10 % peat content		Lührs, 2006b 31612016
Folsomia candida	ASDM [a]	Mixed into substrate 28 d, chronic 10 % peat content	NOEC = 0.350 mg/kg dw	EFSA Conclusion 2007 Lührs, 2006b 31612016
Field studies				
Not relevant				
Litter bag test				
Not relevant				

[a] EFSA Scientific Report (2007) 120, 1-91 does not state this endpoint but twice the same endpoint for AUSN. Applicant believes this is an error in the review documents as the addendum to the DAR presents a NOEC of 0.35 mg/kg soil for metabolite ASDM.

zRMS comments:

Endpoints presented in Table 9.8-3 are in line with the EU agreed endpoints reported in EFSA Scientific Report (2007) 120. Information regarding acute toxicity to earthworms, although in line with the LoEP, has been struck through as being no longer a data requirement.

No chronic toxicity data for nicosulfuron were generated in the course of the EU review, however, the risk to soil organisms from nicosulfuron is considered to be sufficiently addressed in the risk assessment performed for A18032E since according to indications of Commission Regulation (EU) No 283/2013 testing with the formulation may be more appropriate than studies performed with the active substance. Risk from nicosulfuron in A18032E is covered by the risk assessment performed for the formulated product.

The Applicant correctly recognised incorrectly reported endpoints for *Folsomia candida* in the EFSA conclusion - in line with information available in the final addendum to nicosulfuron monograph (July 2007), for metabolite ADSM the NOEC of 0.350 mg pm/kg dws has been derived in the respective study with F. candida.

Since EC_{10} values are not reported in nicosulfuron monograph and in the LoEP, the risk assessment should be based on NOEC values.

Table 9.8-4: Endpoints and effect values relevant for the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) - A18032E

Species	Substance	Exposure system	Results	Reference
Eisenia fetida	A18032E	Overspray 56 d, chronic 10 % peat content	NOEC = 62.5 mg/kg dw EC_{10} = 48.55 mg/kg dw	Friedrich, 2012 A18032E_10007
Folsomia candida	A18032E	Mixed into substrate 28 d, chronic 5% peat content	NOEC = 100 mg/kg dw EC_{10} = 112 mg/kg dw	Friedrich, 2013 A18032E_10011
Hypoaspis aculeifer	A18032E	Mixed into substrate 14 d, chronic 5% peat content	NOEC = 50 mg/kg dw EC_{10} = 74 mg/kg dw	Schulz, 2013 A18032E_10012
Field studies				
Not relevant				
Litter bag test				
Not relevant				

zRMS comments:

Studies on toxicity of A18032E to soil macro- and meso-fauna were evaluated by the zRMS and considered acceptable. For details of evaluation, please refer to Appendix 2. Endpoints reported in Table 9.8-4 are confirmed to be correct with some additional information added by the zRMS.

Since formulation A18032E is intended to be applied exclusively with the adjuvant, all formulation studies were performed with the recommended adjuvant Adigor (A12127R).

The lower of NOEC and EC_{10} value should be used in the risk assessment.

9.8.1.1 Justification for new endpoints

A18032E

New studies are available for A18032E which are required to fulfil the data requirements for plant protection products in accordance with Regulation (EC) No 1107/2009. The endpoints are summarised in Table 9.8-4.

Mesotrione

Since Annex I submission new studies with mesotrione metabolites have been performed to fulfil the new data requirements under 1107/2009, and as a result there are new endpoints for use in the risk assessment. These studies are summarised in Table 9.8-2.

zRMS comments:

As mentioned in point 9.8.1 above, the new studies performed with metabolite MNBA were considered in the risk assessment for precautionary reasons, although they were formally not required. For evaluation of the studies, please refer to Appendix 2.

Studies performed with A18032E were submitted to fulfil data requirements and their use in the risk assessment is not deviation for the EU agreed endpoints.

9.8.2 Risk assessment

The evaluation of the risk to 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), Chapter 8.7.2. According to the assessment of environmental-fate data, multi-annual accumulation in soil does not need to be considered for dicamba, mesotrione and nicosulfuron.

~~To achieve a concise risk assessment, the risk envelope approach is applied. Here, the worst-case application rates (187.5 g dicamba/ha, 90 g mesotrione/ha and 60 g nicosulfuron/ha) are used in the risk assessment.~~

The relevant endpoints for A18032E, dicamba, mesotrione, nicosulfuron and relevant metabolites are compared to the maximum PEC_{soil}.

Table 9.8-5: First-tier assessment of the acute and chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the maximum use of A18032E in maize - dicamba and relevant metabolites

~~Intended use~~	~~Maize~~		
~~Acute effects on earthworms~~			
~~Test substance~~	~~LC_{50} (mg/kg dw)~~	~~PEC_{soil} (mg/kg dw)~~	~~TER_a (criterion TER ≥ 10)~~
~~Dicamba (formulated as Banvel 480 SL)~~	~~>480~~	~~0.188~~	~~>2600~~
~~NOA414746~~	~~>1000~~	~~0.070~~	~~>14000~~

PEC: Predicted environmental concentration; TER: toxicity to exposure ratio

Table 9.8-6: First-tier assessment of the acute and chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the maximum use of A18032E in maize - mesotrione and relevant metabolites

Intended use	Maize		
~~Acute effects on earthworms~~			
~~Test substance~~	~~LC_{50} (mg/kg dw)~~	~~PEC_{soil} (mg/kg dw)~~	~~TER_a (criterion TER ≥ 10)~~
~~Mesotrione~~	~~>2000~~	~~0.090~~	~~>22000~~
~~MNBA~~	~~>1000~~	~~0.018~~	~~>56000~~
Chronic effects on earthworms			
Test substance	**NOEC / EC_{10} (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
Mesotrione (tested as A12739A)	5.91 ~~10.85 *(EC_{10} = 5.91)*~~	0.060 ~~0.090~~	98.5 ~~120 *(66)*~~
MNBA	1050	0.0248 ~~0.018~~	42339 ~~58000~~
AMBA	1050	0.004 ~~0.007~~	262500 ~~150000~~
Chronic effects on other soil macro- and mesofauna *(Folsomia)*			
Test substance	**NOEC / EC_{10} (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
Mesotrione (tested as A12739A)	37.5 ~~50.5~~	0.060 ~~0.090~~	625 ~~560~~
MNBA	100 ~~180~~	0.0248 ~~0.018~~	4032 ~~10000~~
AMBA	3.75 [a] ~~50.5[a]~~	0.004 ~~0.007~~	938 ~~7200~~
Chronic effects on other soil macro- and mesofauna *(Hypoaspis)*			
Test substance	**NOEC / EC_{10} (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
Mesotrione (tested as A12739A)	90.9	0.060 ~~0.090~~	1515 ~~1000~~
MNBA	1050	0.0248 ~~0.018~~	42339 ~~58000~~
AMBA	9.09 [a] ~~90.9[a]~~	0.004 ~~0.007~~	2273 ~~13000~~

PEC: Predicted environmental concentration; TER: toxicity to exposure ratio

[a] Tests with AMBA have not been carried out, and the risk assessment has been conservatively performed on the basis of the endpoint for the parent divided by 10.

Table 9.8-7: First-tier assessment of the acute and chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the maximum use of A18032E in maize - nicosulfuron and relevant metabolites

Intended use	Maize		
~~**Acute effects on earthworms**~~			
~~**Test substance**~~	~~**LC_{50} (mg/kg dw)**~~	~~**PEC_{soil} (mg/kg dw)**~~	~~**TER_a (criterion TER ≥ 10)**~~
~~Nicosulfuron~~	~~>1000~~	~~0.060~~	~~>17000~~
~~ASDM~~	~~>1000~~	~~0.0056 [a]~~	~~>180000~~
~~ADMP~~	~~>1250~~	~~0.0006~~	~~>2100000~~
~~HMUD~~	~~>1250~~	~~0.0064~~	~~>200000~~
~~AUSN~~	~~>1250~~	~~0.0095 [a]~~	~~>130000~~
~~UCSN~~	~~>1250~~	~~0.0052 [a]~~	~~>240000~~
Chronic effects on earthworms			
Test substance	**NOEC / EC_{10} (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
AUSN	0.100	0.0091 [a] ~~0.0095 [a]~~	11
UCSN	0.050	0.0040 [a] ~~0.0052 [a]~~	12.5 ~~9.6~~
ASDM	0.350	0.0164 [a] ~~0.0056 [a]~~	21.3 ~~63~~
HMUD [1)]	0.49	0.0056	87.5
ADMP [1)]	0.49	0.0015	327
Chronic effects on other soil macro- and mesofauna *(Folsomia)*			
Test substance	**NOEC / EC_{10} (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
AUSN	0.100	0.0091 [a] ~~0.0095 [a]~~	11
UCSN	0.050	0.0040 [a] ~~0.0052 [a]~~	12.5 ~~9.6~~
ASDM	0.350	0.0164 [a] ~~0.0056 [a]~~	21.3 ~~63~~
HMUD [1)]	1.01	0.0056	180
ADMP [1)]	1.01	0.0015	673
Chronic effects on other soil macro- and mesofauna *(Hypoaspis)*			
Test substance	**NOEC / EC_{10} (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
AUSN [1)]	0.51	0.0091 [a]	56.04
UCSN [1)]	0.51	0.0040 [a]	128
ASDM [1)]	0.51	0.0164 [a]	31.1
HMUD [1)]	0.51	0.0056	91.1
ADMP [1)]	0.51	0.0015	340

PEC: Predicted environmental concentration; TER: toxicity to exposure ratio

[a] $PEC_{s,\ accumulation}$

[1)] Endpoint for A18032E expressed in terms of nicosulfuron, divided by 10 to represent 10 times toxicity of the parent

Table 9.8-8: First-tier assessment of the acute and chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the maximum use of A18032E in maize - A18032E

Intended use	Maize		
Chronic effects on earthworms			
Test substance	**NOEC / EC_{10} (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
A18032E	48.55 ~~62.5~~	0.40 ~~0.60~~	121 ~~100~~
Chronic effects on other soil macro- and mesofauna *(Folsomia)*			
Test substance	**NOEC / EC_{10} (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
A18032E	100	0.40 ~~0.60~~	250 ~~170~~
Chronic effects on other soil macro- and mesofauna *(Hypoaspis)*			
Test substance	**NOEC / EC_{10} (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
A18032E	50	0.40 ~~0.60~~	125 ~~83~~

PEC: Predicted environmental concentration; TER: toxicity to exposure ratio

zRMS comments:

The risk assessment provided by the Applicant above has been corrected by the zRMS with consideration of the agreed endpoints (EC_{10} values were used in case lower than the NOEC). It is noted that the Applicant considered the risk envelope approach and used PEC_{SOIL} values calculated for the higher application rate, however in area of Section 8 only soil exposure calculated for the intended rate of 400 g product/ha was presented and PEC_{SOIL} for higher rate were not validated. For this reason respective corrections were provided in tables above, so the TER values are based on validated soil exposure data.

Correction of the endpoints was not necessary since log Pow of neither compound considered in the risk assessment was >2.

The following is also noted for particular compounds:

1. <u>*Dicamba*</u>

No chronic toxicity endpoints were available for the active compound and its relevant soil metabolite DCSA. However, as already indicated in point 9.8.1 above, the risk from the active substance is considered to be covered by the evaluation performed for the formulation A18032, since in line with data requirements set by the Commission Regulation (EU) No 283/2013, in case of soil organisms testing with the formulation is more appropriate. With regard to the soil metabolite no separate risk assessment based on extrapolated endpoints was deemed necessary since in soil metabolism studies maximum formation of DCSA was observed on day 8 and for this reason toxicity of this metabolite was also accounted for in studies performed with formulation, lasting 56, 28 and 14 days for earthworms, *F. candida* and *H. aculeifer*, respectively.

2. <u>*Mesotrione*</u>

In the risk assessment for metabolite AMBA the Applicant considered the parent endpoint with no justification. However, in absence of any data 10 times toxicity of the parent is assumed as a worst case approach. TER values for AMBA were thus recalculated by the zRMS.

3. <u>*Nicosulfuron*</u>

- No chronic toxicity endpoints were available for the active compound and its relevant soil metabolite DCSA. However, as already indicated in point 9.8.1 above, the risk from the active substance is considered to be covered by the evaluation performed for the formulation A18032, since in line with data requirements set by the Commission Regulation (EU) No 283/2013, in case of soil organisms testing with the formulation is more appropriate.
- No toxicity endpoint were available for metabolites HMUD and ADMP. As maximum formation of these compounds was observed late in the soil metabolism studies, their toxicity to earthworms and *F.*

candida was not covered in the studies performed with the formulated product. Therefore the zRMS performed the risk assessment using the formulation endpoint expressed in terms of nicosulfuron divided by 10 as a worst case compared with respective PEC_{SOIL} agreed in area of Section 8.

- No toxicity endpoint for *H. aculeifer* were available for all nicosulfuron soil metabolites. As maximum formation of these compounds was observed late in the soil metabolism studies, their toxicity to *H. aculeifer* was not covered in the study performed with the formulated product. Therefore the zRMS performed the risk assessment using the formulation endpoint expressed in terms of nicosulfuron divided by 10 as a worst case compared with respective PEC_{SOIL} agreed in area of Section 8.

4. *Formulation A18032E*

In the risk assessment for A18032E the soil exposure calculated for the formulated product was considered since none of the active compounds has potential to accumulate in soil and initial PEC_{SOIL} values were relevant for the evaluation.

Overall, based on the above calculations acceptable risk to soil macro- and meso-fauna may be concluded from the intended uses of A18032E.

The acute risk assessment for earthworms was struck through as being no longer a data requirement.

9.8.2.2 Higher-tier risk assessment

Not relevant.

9.8.3 Overall conclusions

Earthworms

The ~~acute and~~ long-term risk of A18032E to earthworms was assessed from ~~acute and~~ long-term TERs between the selected toxicity endpoints for A18032E, dicamba, mesotrione, nicosulfuron and relevant metabolites, and the maximum PEC_{soil}. The ~~acute and~~ chronic TER values for A18032E, dicamba, mesotrione, nicosulfuron and their metabolites are greater than the Regulation (EU) 546/2011 trigger ~~of 10 and~~ 5 ~~respectively~~, indicating that the risk to earthworms is acceptable following use of A18032E according to the proposed use pattern.

Other soil macro-organisms

The risk of A18032E, mesotrione, mesotrione metabolites and nicosulfuron metabolites to other non-target soil macro-organisms, as represented by Collembola and *Hypoaspis*, was assessed from long-term TERs between the selected no-effect concentrations, derived from laboratory tests on A18032E, mesotrione, mesotrione metabolites and nicosulfuron metabolites, and the maximum PEC_{soil}. The TER_{LT} values for A18032E, mesotrione, mesotrione metabolites and nicosulfuron metabolites are all greater than the recommended trigger value of 5, indicating that the risk to soil macro-organisms, as represented by Collembola and *Hypoaspis*, is acceptable following use of A18032E according to the proposed use pattern.

9.9 Effects on soil microbial activity (KCP 10.5)

9.9.1 Toxicity data

Studies of the effects on soil microorganisms have been carried out with dicamba, mesotrione, nicosulfuron and their relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on soil microorganisms of A18032E were not evaluated as part of the EU assessment of dicamba, mesotrione and nicosulfuron. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process.

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

Endpoint	Substance	Exposure system	Results	Reference
N-mineralisation	Dicamba	28 d, aerobic	<25% effect at 6.4 mg a.s./kg soil dw	EFSA Conclusion 2011 Seyfried, 2002 SAN837/5991
~~C-mineralisation~~	~~Dicamba~~	~~28 d, aerobic~~	~~<25% effect at 6.4 mg a.s./kg soil dw~~	~~EFSA Conclusion 2011~~ ~~Seyfried, 2002~~ ~~SAN837/5991~~

Table 9.9-2: Endpoints and effect values relevant for the risk assessment for soil microorganisms – mesotrione and relevant soil metabolites

Endpoint	Substance	Exposure system	Results	Reference
N-mineralisation	Mesotrione (tested as formulation A12739A)	28 d, aerobic	Nitrate formation rate 5.84 mg A12739A/kg soil dw 7.8% (<25% effect at up to 0.53 mg a.s./kg soil dw)	EFSA Conclusion 2016 Schulz, 2013a A12739A_10024
~~C-mineralisation*~~	~~Mesotrione (tested as formulation A12739A)~~	~~28 d, aerobic~~	~~CO_2 formation~~ ~~21.9 mg A12739A/kg soil dw~~ ~~8.7%~~ ~~(<25% effect at up to 1.99 mg a.s./kg soil dw)~~	~~EFSA Conclusion 2016~~ ~~Schulz, 2013a~~ ~~A12739A_10024~~
N-mineralisation	MNBA	28 d, aerobic	Nitrate formation rate 1.13 mg/kg soil dw -7.6% (<25% effect at up to 1.13 mg a.s./kg soil dw)	EFSA Conclusion 2016 Schulz, 2013b CA3511_10000
~~C-mineralisation*~~	~~MNBA~~	~~28 d, aerobic~~	~~CO_2 formation~~ ~~1.13 mg/kg soil dw~~ ~~2.8%~~ ~~(<25% effect at up to 1.13 mg a.s./kg soil dw)~~	~~EFSA Conclusion 2016~~ ~~Schulz, 2013b~~ ~~CA3511_10000~~
N-mineralisation	AMBA	28 d, aerobic	Nitrate formation rate 1.13 mg/kg soil dw -4.8% (<25% effect at up to 1.13 mg a.s./kg soil dw)	EFSA Conclusion 2016 Schulz, 2013b CA3511_10000
~~C-mineralisation*~~	~~AMBA~~	~~28 d, aerobic~~	~~CO_2 formation~~ ~~1.13 mg/kg soil dw~~ ~~-4.8%~~ ~~(<25% effect at up to 1.13 mg a.s./kg soil dw)~~	~~EFSA Conclusion 2016~~ ~~Schulz, 2013b~~ ~~CA3511_10000~~

~~*Please note that Carbon transformation is no longer a data requirement~~

Table 9.9-3: Endpoints and effect values relevant for the risk assessment for soil microorganisms - nicosulfuron and relevant soil metabolites

Endpoint	Substance	Exposure system	Results	Reference
N-mineralisation	Nicosulfuron	29 d, aerobic	<25% effect at 0.8 mg a.s./kg soil dw	EFSA Conclusion 2007 Müller-Kallert, 1992 301195
~~C-mineralisation~~	~~Nicosulfuron~~	~~29 d, aerobic~~	~~<25% effect at 0.8 mg a.s./kg soil dw~~	~~EFSA Conclusion 2007 Müller-Kallert, 1992 301195~~
N-mineralisation	AUSN	28 d, aerobic	<25% effect at 0.082 mg/kg soil dw	EFSA Conclusion 2007 Völkel, 2003 848319
~~C-mineralisation~~	~~AUSN~~	~~28 d, aerobic~~	~~<25% effect at 0.082 mg/kg soil dw~~	~~EFSA Conclusion 2007 Völkel, 2003 848319~~
N-mineralisation	UCSN	28 d, aerobic	<25% effect at 0.034 mg/kg soil dw	EFSA Conclusion 2007 Völkel, 2003 848319
~~C-mineralisation~~	~~UCSN~~	~~28 d, aerobic~~	~~<25% effect at 0.034 mg/kg soil dw~~	~~EFSA Conclusion 2007 Völkel, 2003 848319~~
N-mineralisation	ASDM	28 d, aerobic	<25% effect at 0.191 mg/kg soil dw	EFSA Conclusion 2007 Völkel, 2003 848319
~~C-mineralisation~~	~~ASDM~~	~~28 d, aerobic~~	~~<25% effect at 0.191 mg/kg soil dw~~	~~EFSA Conclusion 2007 Völkel, 2003 848319~~

Table 9.9-4: Endpoints and effect values relevant for the risk assessment for soil microorganisms - A18032E

Endpoint	Substance	Exposure system	Results	Reference
N-mineralisation	A18032E	28 d, aerobic	<25% effect at 4 mg/kg soil dw	Schulz, 2012 A18032E_10004
~~C-mineralisation~~	~~A18032E~~	~~28 d, aerobic~~	~~<25% effect at 4 mg/kg soil dw~~	~~Schulz, 2012 A18032E_10004~~

zRMS comments:

Toxicity data for non-target arthropods provided in Tables 9.9-1 to 9.9-3 are in line with data reported in:

- EFSA Journal 2011;9(1):1965 for dicamba,
- EFSA Journal 2016;14(3):4419 for mesotrione,
- EFSA Scientific Report (2007) 120 for nicosulfuron.

It should be noted that the effects of mesotrione on soil microbial activity investigated in study performed with the representative solo formulation (A12739A) and for this reason derived endpoint may be not necessarily representative for the active substance itself due to presence of co-formulants in the test item. Nevertheless, in absence of the respective data for the parent, endpoint from studies with A12739A was agreed as a surrogate solution. Risk from nicosulfuron in A18032E is covered by the risk assessment performed for the formulated product.

Study on effects of A18032E on soil nitrogen transformation was evaluated by the zRMS and considered acceptable. Since formulation A18032E is intended to be applied exclusively with the adjuvant, all formulation studies were performed with the recommended adjuvant Adigor (A12127R). For details of evaluation, please refer to Appendix 2. The endpoint reported in Table 9.9-4 is confirmed to be correct.

Endpoints for effects on C-mineralisation were struck through as being no longer a data requirement.

9.9.1.1 Justification for new endpoints

New studies are available for A18032E which are required to fulfil the data requirements for plant protection products in accordance with Regulation (EC) No 1107/2009. The endpoints are summarised in Table 9.9-4. A study summary is presented in Appendix 2.

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, and were already used in the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) (see 0).

~~To achieve a concise risk assessment, the risk envelope approach is applied. Here, the worst-case application rates (187.5 g dicamba/ha, 90 g mesotrione/ha and 60 g nicosulfuron/ha) are used in the risk assessment.~~

For A18032E, dicamba, mesotrione, nicosulfuron and relevant metabolites, the maximum concentration with effects ≤25% are compared to the maximum PEC_{soil}.

Table 9.9-5: Assessment of the risk for effects on soil micro-organisms due to the use of A18032E in maize – dicamba and relevant soil metabolites

Intended use	Maize		
N- mineralisation ~~and C-mineralisation~~			
Test substance	**Max. conc. with effects ≤ 25 % (mg a.s./kg dw)**	**PEC_{soil} (mg a.s./kg dw)**	**Risk acceptable?**
Dicamba	6.4 (at 28 d)	0.125 ~~0.188~~	Yes
DCSA	0.64 [1)]	0.0688	Yes

[1)] 10 times toxicity of the parent assumed as a worst case

Table 9.9-6: Assessment of the risk for effects on soil micro-organisms due to the use of A18032E in maize – mesotrione and relevant soil metabolites

Intended use	Maize		
N-mineralisation			
Test substance	**Max. conc. with effects ≤ 25 % (mg a.s./kg dw)**	**PEC_{soil} (mg a.s./kg dw)**	**Risk acceptable?**
Mesotrione	0.53 (at 28d)	0.060 ~~0.090~~	Yes
MNBA	1.13 (at 28 d)	0.0248 ~~0.018~~	Yes
AMBA	1.13 (at 28 d)	0.004 ~~0.007~~	Yes
~~**C-mineralisation**~~			
~~**Test substance**~~	~~**Max. conc. with effects ≤ 25 % (mg a.s./kg dw)**~~	~~**PEC_{soil} (mg a.s./kg dw)**~~	~~**Risk acceptable?**~~
~~Mesotrione~~	~~1.99 (at 28 d)~~	~~0.090~~	~~Yes~~
~~MNBA~~	~~1.13 (at 28 d)~~	~~0.018~~	~~Yes~~
~~AMBA~~	~~1.13 (at 28 d)~~	~~0.007~~	~~Yes~~

Table 9.9-7: Assessment of the risk for effects on soil micro-organisms due to the use of A18032E in maize – nicosulfuron and relevant soil metabolites

Intended use	Maize		
N- mineralisation ~~and C-mineralisation~~			
Test substance	**Max. conc. with effects ≤ 25 % (mg a.s./kg dw)**	**PEC_{soil} (mg a.s./kg dw)**	**Risk acceptable?**
Nicosulfuron	0.8 (at 29 d)	0.040 ~~0.060~~	Yes
AUSN	0.082 (at 28 d)	0.0091 [a] ~~0.0095[a]~~	Yes
UCSN	0.034 (at 28 d)	0.0040 [a] ~~0.0052[a]~~	Yes
ASDM	0.191 (at 28 d)	0.0164 [a] ~~0.0056[a]~~	Yes
HMUD	0.08 [1)]	0.0056	
ADMP	0.08 [1)]	0.0015	

[a] $PEC_{s,\ accumulation}$
[1)] 10 times toxicity of the parent assumed as a worst case

Table 9.9-8: Assessment of the risk for effects on soil micro-organisms due to the use of A18032E in maize – A18032E

Intended use	Maize		
N- and C-mineralisation			
Test substance	**Max. conc. with effects ≤ 25 % (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**Risk acceptable?**
A18032E	4 (at 28 d)	0.40 ~~0.60~~	Yes

zRMS comments:

The risk assessment for soil micro-organisms was performed by the Applicant with consideration of the risk envelope approach using PEC_{SOIL} values calculated for the higher application rate. However, in area of Section 8 only soil exposure calculated for the intended rate of 400 g product/ha was presented and PEC_{SOIL} for higher rate were not validated. For this reason respective corrections were provided in tables above, so the risk assessment is based on validated soil exposure data.

For dicamba and nicosulfuron metabolites, for which no toxicity data were available, the risk assessment was performed with assumption of 10 times toxicity of the parent, as a worst case.

In the risk assessment for A18032E the soil exposure calculated for the formulated product was considered since none of the active compounds has potential to accumulate in soil and initial PEC_{SOIL} values were relevant for the evaluation.

Overall, based on the above calculations no unacceptable effects on soil microbial activity are from the intended uses of A18032E.

The risk assessment for C-mineralisation was struck through as being no longer a data requirement.

9.9.3 Overall conclusions

The risk of A18032E, dicamba, mesotrione, nicosulfuron and relevant metabolites to soil micro-organisms was evaluated by comparison of the maximum concentrations with effects ≤25% derived from laboratory tests, with maximum PEC_{soil} or $PEC_{soil,\ accumulation}$ where applicable.

All no-effect levels of A18032E, dicamba, mesotrione, nicosulfuron and relevant metabolites exceeded the relevant PEC_{soil} values, indicating that the risk to soil micro-organisms is acceptable following the use of A18032E according to the proposed use pattern.

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

9.10.1 Toxicity data

Studies on the toxicity to non-target terrestrial plants have been carried out with dicamba, mesotrione and nicosulfuron. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on non-target terrestrial plants of A18032E were not evaluated as part of the EU assessment of dicamba, mesotrione and nicosulfuron. New data submitted with this application are listed in Appendix 1 summarised in Appendix 2.

The selection of studies and endpoints for the risk assessment deviates from the results of the EU review process as endpoints and risk assessments were related to the formulated product A18032E. Further justifications are provided below.

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

Species	Substance	Exposure system	Results	Reference
Beta vulgaris d	Banvel 480 SL	21 d Vegetative vigour	ER_{50} = 24.2 g a.s./ha	EFSA Conclusion 2011 Baluff, 2003b SAN837/6109
Beta vulgaris d	Banvel 480 SL	21 d Seedling emergence	ER_{50} = 97 g a.s./ha	EFSA Conclusion 2011 Baluff, 2003a SAN837/6108

d: dicotyledonous

Table 9.10-2: Endpoints and effect values relevant for the risk assessment for non-target terrestrial plants - mesotrione

Species	Substance	Exposure system	Results	Reference
9 – 20 NTP species	AMBA and MNBA	Screening study	NOEC = 4000 g/ha	Renewal Assessment Report, 11 Vol.3 CA B-9 (11/11/2015) Shribbs, 1997 ZA1296/0189
Lactuca sativa d	A12739A	21 d Seedling emergence	ER_{50} biomass = 13.8 g a.s./ha	EFSA Conclusion 2016 Porch et al., 2003a ZA1296/1144
Lactuca sativa d	A12739A	21 d Vegetative vigour	ER_{50} biomass = 0.883 g a.s./ha	EFSA Conclusion 2016 Porch et al., 2003b ZA1296/1145
Species Sensitivity Distribution (SSD)	A12739A	21 d Vegetative vigour	HC_5 = 0.173 g a.s./ha	EFSA Conclusion 2016

d: dicotyledonous

~~**Table 9.10-3: Endpoints and effect values relevant for the risk assessment for non-target terrestrial plants – nicosulfuron**~~

~~Species~~	~~Substance~~	~~Exposure system~~	~~Results~~	~~Reference~~
~~Rice m~~	~~SL-950 4% SC~~	~~21 d Vegetative vigour~~	~~ER_{50} = 0.47 g a.s./ha~~	~~EFSA Conclusion 2007 ISK Ltd, 1993 300193~~
~~*Zea mays* m *Avena sativa* m *Allium cepa* m *Daucus carota* d *Brassica napus* d *Pisum sativum* d~~	~~SL-950 4% SC~~	~~21 d Seedling emergence~~	~~ER_{50} > 20 g a.s./ha~~	~~EFSA Conclusion 2007 Porch and Krueger, 2000a 147-189~~

~~m: monocotyledonous; d: dicotyledonous~~

Table 9.10-4: Endpoints and effect values relevant for the risk assessment for non-target terrestrial plants - A18032E

Species	Substance	Exposure system	Results	Reference
Beta vulgaris d [1)] *Brassica napus* d [2)] *Cucumis sativus* d [3)] *Daucus carota* d [4)] *Lactuca sativa* d [5)] ***Lycopersicon esculentum* d** [6)] *Raphanus sativus* d [7)] *Avena sativa* m [8)] *Lolium perenne* m [9)] *Oryza sativa* m [10)]	A18032E (+A12127R)	21 d Vegetative vigour	[1)] ER_{50} biomass = 16.32 g/ha [2)] ER_{50} biomass = 23.52 g/ha [3)] ER_{50} biomass = 36.50 g/ha [4)] ER_{50} biomass = 34.19 g/ha [5)] ER_{50} biomass = 8.22 g/ha [6)] **ER_{50} biomass = 1.30 g/ha** [7)] ER_{50} biomass = 18.76 g/ha [8)] ER_{50} biomass = 93.63 g/ha [9)] ER_{50} biomass = 48.51 g/ha [10)] ER_{50} biomass = 44.84 g/ha	Bramby-Gunary, 2013 A18032E_10025
SSD (10 species)	A18032E (+A12127R)	21 d Vegetative vigour	HC_5 = 2.8 g/ha	see 9.10.1.1
Beta vulgaris d [1)] *Brassica napus* d [2)] *Cucumis sativus* d [3)] *Daucus carota* d [4)] ***Lactuca sativa* d** [5)] *Lycopersicon esculentum* d [6)] *Raphanus sativus* d [7)] *Avena sativa* m [8)] *Lolium perenne* m [9)] *Oryza sativa* m [10)]	A18032E (+A12127R)	21 d Seedling emergence	[1)] ER_{50} dry weight = 7.72 g/ha [2)] ER_{50} dry weight = 53.85 g/ha [3)] ER_{50} dry weight = 60.33 g/ha [4)] ER_{50} dry weight = 13.22 g/ha [5)] **ER_{50}** dry weight **= 6.97 g/ha** [6)] ER_{50} dry weight = 43.91 g/ha [7)] ER_{50} dry weight = 33.61 g/ha [8)] ER_{50} dry weight = 913.22 g/ha [9)] ER_{50} dry weight = 79.51 g/ha [10)] ER_{50} dry weight = 28.62 g/ha	Bramby-Gunary, 2013a A18032E_10024
SSD (10 species)	A18032E (+A12127R)	21 d Seedling emergence	HC_5 = 3.6 g/ha	see 9.10.1.1

m: monocotyledonous; d: dicotyledonous
Values in **bold** indicate most sensitive species and endpoints

zRMS comments:

Toxicity data for non-target arthropods provided in Tables 9.10-1 to 9.10-3 are in line with data reported in:

- EFSA Journal 2011;9(1):1965 for dicamba,
- EFSA Journal 2016;14(3):4419 for mesotrione,
- EFSA Scientific Report (2007) 120 for nicosulfuron.

However, results of these studies were struck through in tables above as being generated with solo-formulations of particular active substances and thus not relevant for evaluation performed for A18032E.

Information regarding herbicidal activity of metabolites MNBA and AMBA presented in Table 9.10-2 has been taken from the mesotrione RAR (Vol. 3CA, B.9 and Vol. 3CP, B.99 of 2015) and is confirmed to be correct.

Studies on toxicity of A18032E to non-target terrestrial plants were evaluated by the zRMS and considered

acceptable. Since formulation A18032E is intended to be applied exclusively with the adjuvant, all formulation studies were performed with the recommended adjuvant Adigor (A12127R). For details of evaluation, please refer to Appendix 2. Endpoints reported in Table 9.10-4 are confirmed to be correct.

The HC_5 values for vegetative vigour and seedling emergence were validated by the zRMS using ETX 2.3 by RIVM and are confirmed to be correct.

9.10.1.1 Justification for new endpoints

Studies with non-target terrestrial plants are always conducted with a formulated product and no testing is carried out with unformulated technical material. Therefore it may not be appropriate to rely on the data from the individual solo formulations submitted as representative formulations for the EU review for the risk assessment for non-target terrestrial plants.

Refined HC_{05} using a probabilistic approach
The risk of A18032E to non-target plants has been refined using the probabilistic HC_{05} approach (***Aldenberg & Jaworska 2000***[26]) to investigate the distribution of sensitivities of all the tested plant species. This approach considers the whole sensitivity distribution of species in an ecosystem, represented by the tested species, to derive a hazard concentration protective of 95% of the species (HC_{05}) instead of just using the lowest ER_{50} value. Because of the large data set (10 species of varying classes and morphologies) the uncertainty in extrapolation of the data to the natural environment is reduced, and accordingly the assessment factor can also be reduced.

The vegetative vigour and seedling emergence data for the 10 species of plants, as summarised in Table 9.10-4, are used with the species sensitivity distribution (SSD) method for evaluating the risk. The statistical analysis was conducted to investigate the distribution of sensitivities of the tested species, estimate the proportion of species affected at a range of concentrations, and derive the HC_{05} value from laboratory to be protective of ecosystems in the field. This method has been proposed and accepted by leading authorities and ecotoxicologists and is a clearly defined probabilistic risk assessment method, with supporting software (RIVM program *ETX* 2.0[27]; or Webfram[28]).

For vegetative vigour and seedling emergence the data for all 10 species were normally distributed.

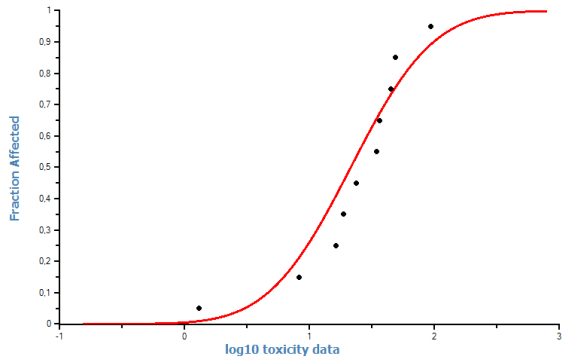
The results of the species sensitivity distributions for A18032E for vegetative vigour and seedling emergence are presented below, calculated using RIVM program *ETX* 2.0.

[26] Aldenberg T & Jaworska JS (2000) Estimation of the hazardous concentration and fraction affected from normally distributed species sensitivity distributions. Ecotoxicology and Environmental Safety 46, 1-18.

[27] Van Vlaardingen PLA, Traas TP, Wintersen AM, Aldenberg T. 2004 *ETX 2.0*. A program to calculate hazardous concentrations and fraction affected, based on normally distributed toxicity data. Bilthoven, the Netherlands: National Institute for Public Health and the Environment (RIVM). Report no. 601501028/2004, 68pp.

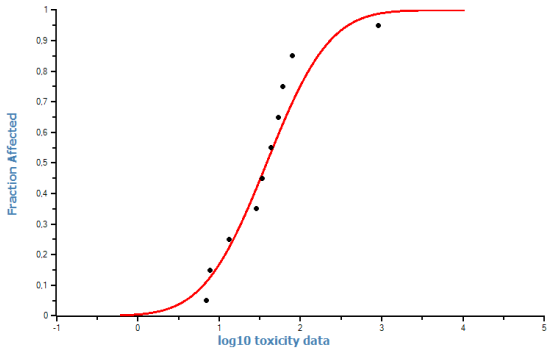
[28] Pesticide Risk Assessment Tool. Framework for Addressing Uncertainty and Variability in Pesticide Risk Assessment (https://webfram.com/home.aspx)

Figure 9.10-1: Species Sensitivity Distribution for Plants in the Vegetative Vigour Study (10 species)



The calculated HC_{05} value was:
Median HC_{05} for plants = 2.81 g A18032E /ha
The lower and upper 90% confidence limits were: 0.666 g/ha and 6.35 g/ha.
The mean (log 10) for the plant data was 1.33 with a sample deviation of 0.517.
Tests for normality were all accepted at the 0.1 significance level.

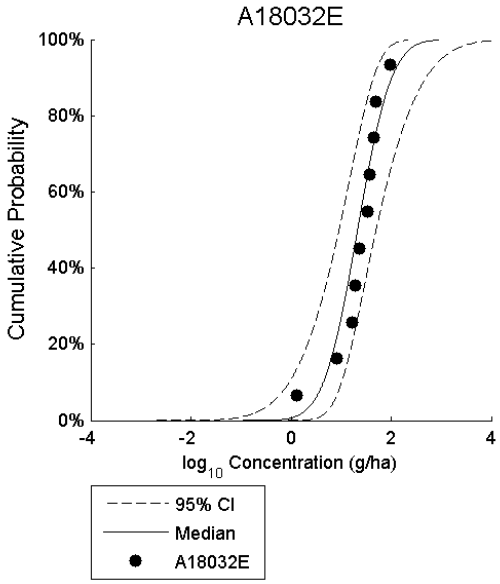
Figure 9.10-2: Species Sensitivity Distribution for Plants in the Seedling Emergence Study (10 species)



The calculated HC_{05} value was:
Median HC_{05} for plants = 3.55 g A18032E/ha
The lower and upper 90% confidence limits were 0.654 g/ha and 9.26 g/ha.
The mean (log 10) for the plant data was 1.58 with a sample deviation of 0.608.
Tests for normality were all accepted at the 0.1 significance level.

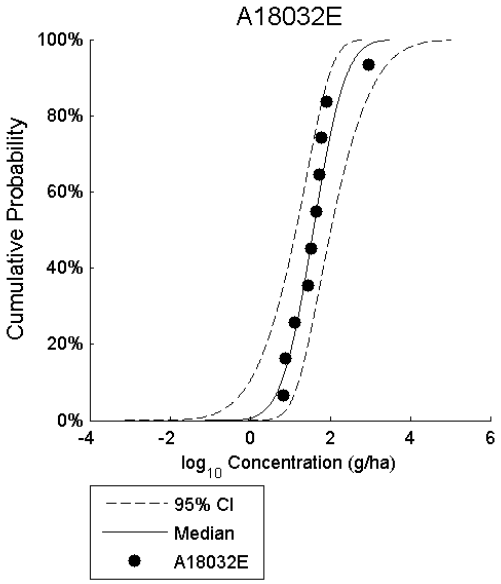
The model Webfram was also used to run these data inputs, and the results are summarised below:

Figure 9.10-3: Species Sensitivity Distribution for Plants in the Vegetative Vigour Study (10 species)



The calculated HC_{05} value was:
Median HC_{05} for plants = 2.81 g A18032E/ha
The lower and upper 90% confidence limits were: 0.666 g/ha and 6.35 g/ha.
Tests for normality were all accepted at the 0.1 significance level.

Figure 9.10-4: Species Sensitivity Distribution for Plants in the Seedling Emergence Study (10 species)



The calculated HC_{05} value was:
Median HC_{05} for plants = 3.55 g A18032E/ha
The lower and upper 90% confidence limits were 0.653 g/ha and 9.25 g/ha.
Tests for normality were all accepted at the 0.1 significance level.

The results from both models are very similar and therefore the results to 2 significant figures will be used.

zRMS comments:

The HC_5 values for vegetative vigour and seedling emergence were validated by the zRMS using ETX 2.3 by RIVM. Considered data passed all tests for normality (Anderson-Darling, Kolmogorov-Smirnov and Cramer von Mises) demonstrating normal distribution. Results obtained by the zRMS were the same as Applicants' values.

9.10.2 Risk assessment

9.10.2.1 Tier-1 risk assessment (based on 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.

The $PER_{off\ field}$ was calculated as Application rate × drift factor.

Since dicamba is volatile (please see dRR Section 8 for details), deposition rates for dicamba from volatilisation should also be considered. This has been calculated with EVA 2.1 and gives a deposition of 0.11% at 1 m. As a worst-case, it will be assumed that the % deposition applies to the whole formulation rather than just the dicamba component.

Table 9.10-5: Assessment of the risk for non-target plants due to the use of A18032E in maize (1 x 400 g A18032E/ha)

Intended use	Maize			
Product	A18032E			
Application rate (g/ha)	1 × 400			
Drift rate (%)	2.77% at 1 m + 0.11% deposition from volatilisation of dicamba			
MAF	1			
Test species	**ER_{50} (g/ha)**	**Drift + deposition factor**	**$PER_{off\text{-}field}$ (g/ha)**	**TER criterion: TER ≥ 5**
Lycopersicon esculentum (most sensitive, vegetative vigour) ~~*Lactuca sativa* (most sensitive, seedling emergence)~~	1.30	0.0277 + 0.0011 = 0.0288	11.52	**0.11**
Lycopersicon esculentum (most sensitive, vegetative vigour) ~~*Lactuca sativa* (most sensitive, seedling emergence)~~	6.97	0.0277 + 0.0011 = 0.0288	11.52	**0.61**
	HC_{05} (g/ha)	Drift + deposition factor	**$PER_{off\text{-}field}$ (g/ha)**	**TER criterion: TER ≥ 1**
HC_{05} vegetative vigour	2.8	0.0277 + 0.0011 = 0.0288	11.52	**0.24**
HC_{05} seedling emeregence	3.6	0.0277 + 0.0011 = 0.0288	11.52	**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.

zRMS comments:

Deterministic and probabilistic risk assessment presented in Table 9.10-5 above is in general agreed by the zRMS. It is noted that endpoints for deterministic assessment were switched. Respective corrections were thus made by the zRMS in Table 9.10-5.

The Applicants' approach to apply the deposition of dicamba to the whole formulation represents worst case and is agreed by the zRMS.

No acceptable risk could be concluded for either of the parameters and further calculations with assumption of risk mitigation measures are presented in point 9.10.2.3.

9.10.2.3 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, 10 or 20 m; drift-reducing nozzles with reduction by 50 %, 75 %, or 90 %) are summarised in the following table.

Since dicamba is volatile (please see dRR Section 8 for details), the following deposition rates for dicamba from volatilisation have been calculated with EVA 2.1. As a worst-case, it will be assumed that the % deposition applies to the whole formulation rather than just the dicamba component.

Deposition rate (%)					
1 m	3 m	5 m	10 m	15 m	20 m
0.11	0.10	0.09	0.07	0.05	0.04

Table 9.10-6: Risk assessment for non-target terrestrial plants due to the use of A18032E (1 x 400 g A18032E/ha) in maize considering risk mitigation measures (in-field no-spray buffer zones, and drift-reducing nozzles)

Intended use		Maize			
Product		A18032E			
Application rate (g/ha)		1 × 400			
MAF		1			
Buffer strip (m)	**Drift + deposition rate (%)**	**$PER_{off\text{-}field}$ (g/ha)**	**$PER_{off\text{-}field}$ 50 % drift red. (g/ha)**	**$PER_{off\text{-}field}$ 75 % drift red. (g/ha)**	**$PER_{off\text{-}field}$ 90 % drift red. (g/ha)**
1	2.77 + 0.11 = 2.88	11.52	5.76	2.88	1.15
5	0.57 + 0.09 = 0.66	2.64	1.32	0.66	0.26
10	0.29 + 0.07 = 0.36	1.44	0.72	0.36	0.14
20	0.15 + 0.04 = 0.19	0.76	0.38	0.19	0.08
Toxicity value ER_{50} = 1.30 g/ha (most sensitive distribution, vegetative vigour) ~~*Lactuca sativa* (most sensitive, seedling emergence)~~		**TER** **criterion: TER ≥ 5**			
1		**0.11**	**0.23**	**0.45**	**1.1**
5		**0.49**	**0.98**	**2.0**	**4.92** ~~5.0~~
10		**0.90**	**1.8**	**3.6**	9.3

20	**1.7**	**3.4**	6.8	16
Toxicity value	**TER**			
HC_{05} = 2.8 g/ha (most sensitive distribution, vegetative vigour)	**criterion: TER ≥ 1**			
1	**0.24**	**0.49**	**0.97**	2.4
5	1.1	2.1	4.2	11
10	1.9	3.9	7.8	20
20	3.7	7.4	15	35

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

zRMS comments:
Deterministic and probabilistic risk assessment presented in Table 9.10-8 above is in general agreed by the zRMS. It is noted that the lowest endpoint was determined for vegetative vigour and not for seedling emergence as incorrectly reported in Table 9.10-8. Provided information was thus amended accordingly.

Evaluation was based on more sensitive endpoints derived in vegetative vigour test being protective also for seedling emergence.

Presented above calculations were validated by the zRMS using unrounded values and obtained results were in good agreement with Applicants' values. Respective corrections were made in Table 9.10-8 in case consideration of unrounded values had impact on the outcome of the risk assessment, which was the case for deterministic evaluation for 5 m buffer combined with 90% drift reduction, at which the TER was <5, indicating that more restrictive mitigation is required.

The Applicants' approach to apply the deposition of dicamba to the whole formulation represents worst case and is agreed by the zRMS.
Performed evaluation demonstrated that in case of deterministic risk assessment, following risk mitigation measures are necessary:

- 20 m unsprayed buffer zone to non-agricultural land combined with 75% drift reduction using appropriate drift reducing techniques, or
- 10 m unsprayed buffer zone to non-agricultural land combined with 90% drift reduction using appropriate drift reducing techniques.

In case of probabilistic risk assessment, following risk mitigation measures are necessary:

- 5 m unsprayed buffer zone to non-agricultural land, or
- 90% drift reduction using appropriate drift reducing techniques.

For purposes of authorisation of the product in Poland results of probabilistic risk assessment may be taken into account.

9.10.3 Overall conclusions

The risk of A18032E to non-target terrestrial plants was assessed from TERs using the A18032E toxicity data from Tier II studies, and the maximum off-field predicted environmental residues (PERs). TER values, calculated from worst-case endpoints from seedling emergence and vegetative vigour studies with 10 species and a $PER_{off\text{-}field}$ value at 1 m from the treated crop, indicated a potential risk to off-field non-target plants. The risk was refined using a probabilistic risk assessment and considering mitigation with buffers and spray drift reduction technology.

The risk to non-target terrestrial plants in off-crop areas is acceptable following use of A18032E according to the proposed use pattern, provided the following mitigation is implemented:

1 x 400 g A18032E/ha:

- 1 m buffer and 90% drift reduction mitigation or
- 5 m buffer

The risk to terrestrial non-target plants in off-crop areas is therefore acceptable following use of A18032E according to the proposed use pattern when the appropriate mitigation measures are used.

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

Tests on other non-target species are not required.

9.12 Monitoring data (KCP 10.8)

There are no other relevant data for the active substance or product on organisms in the environment generated from monitoring schemes.

9.13 Classification and Labelling

Based upon all the available aquatic endpoints for A18032E, the proposed classification and labelling of A18032E is driven by effects on *Lemna* (E_rC_{50} = 0.0181 mg A18032E/L for the acute classification, and the *Lemna* NOEC = 0.00123 mg A18032E/L for the chronic classification).

Acute Category 1
H400 'Very toxic to aquatic life'

Chronic Category 1
H410 'Very toxic to aquatic life with long lasting effects'

zRMS comments:

The CLP classification presented above is agreed by zRMS.

Following labelling is proposed with regard to effects on aquatic environment:

Hazard pictograms:	GHS09 
Signal word:	Warning
Hazard statement(s):	H410 - Very toxic to aquatic life with long lasting effects
Precautionary statement(s):	P391: Collect spillage P501: Dispose of contents/container to hazardous or special waste collection point, in accordance with local, regional, national and/or international regulation

Appendix 1 Lists of data considered in support of the evaluation

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	Vertebrate study Y/N	Owner
KCP 7.1.1	xxxxxxxxxxxx	2013	Mesotrione/Dicamba/Nicosulfuron WG (A18032E) – Acute Oral Toxicity Study in the Rat (Up and Down Procedure). xxxxxxxxxxxxx Report No. 12/309-001P GLP, Unpublished Syngenta File No. A18032E_10018	Y	SYN (ADAMA has access)
KCP 10.1.2	Alvarez T.	2019	Mesotrione: refined risk assessments for mammals. Syngenta, Jealott's Hill, Bracknell, United Kingdom. Syngenta Unpublished Report (Syngenta File No. A18032E_10335) **This is CONFIDENTIAL INFORMATION***	N	SYN (ADAMA has access)
KCP 10.1.2.2/07	Grimm T & Katzschner I	2019	Generic monitoring of European hares to determine proportion of time spent foraging in early maize in Central Europe. RIFCON GmbH, Goldbeckstr. 13, 69493 Hirschberg, Germany Report No. R1740045 GLP, Unpublished Syngenta File No. NA_14950	N	SYN (ADAMA has LOA)
KCP 10.1.2.2	North, L.	2016	Mesotrione – Foliage Decline Study with A12739A on Maize in Northern France and the United Kingdom in 2015. Eurofins Agroscience Services Ltd., Slade Lane, Wilson, Melbourne, Derbyshire, DE73 8AG, UK Report No. S15-02057 GLP, Unpublished Syngenta File No. A12739A_11065	N	SYN
KCP 10.1.2.2/12	Allen L.	2019	Mesotrione – Mesotrione – Study on Clover in Hungary, Germany, United Kingdom, Northern France and Belgium in 2018. CEMAS, Imperial House, Oaklands Park, Wokingham, Berkshire, RG41 2FD, , UK Report No. CEMR-8397 GLP, Unpublished Syngenta File No. A12738A_10535	N	SYN (ADAMA has LOA)

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 10.2.1	Falk, S.	2012	Mesotrione/nicosulfuron/dicamba WG (A18032E) plus Adigor (A12127R) - Testing of Effects on the Single Cell Green Alga *Pseudokirchneriella subcapitata* Eurofins Agroscience Services EcoChem GmbH, Eutinger Str.24, 75223 Niefern-Öschelbronn, Germany Report No. S12-02296 GLP, Unpublished Syngenta File No. A18032E_10002	N	SYN (ADAMA has LOA)
KCP 10.2.1	Gonsior, G.	2017	Mesotrione – Growth Inhibition of *Myriophyllum spicatum* in a Water/Sediment System Eurofins Agroscience Services EcoChem GmbH / Eurofins Agroscience Services Ecotox GmbH Eutinger Strasse 24, 75223 Niefern-Öschelbronn, Germany Report No. S16-06273 GLP, Unpublished Syngenta File No. ZA1296_10504	N	SYN (ADAMA has LOA)
KCP 10.2.1	Hengsberger A., Wydra V. (amendment 2; Kosak L., Wydra V.	2015 (amend.2 2016)	Mesotrione Wet Paste (ZA1296) - Toxicity to the Aquatic Plant *Lemna gibba* in a Semi-Static Growth Inhibition Test with a Subsequent Recovery Period. Ibacon GmbH Arheilger Weg 17 64380 Rossdorf Germany. Report No. 105732240 GLP, Unpublished Syngenta File No. ZA1296_10438	N	SYN (ADAMA has LOA)
KCP 10.2.1	Weber, K.	2012	Mesotrione/nicosulfuron/dicamba WG (A18032E) plus Adigor (A12127R) – Assessment of Toxic Effects on *Daphnia magna* using the 48 h Acute Immobilisation Test Eurofins Agroscience Services EcoChem GmbH, Eutinger Strasse 24, 75223 Niefern-Öschelbronn, Germany Report No. S12-02294 GLP, Unpublished Syngenta File No. A18032E_10008	N	SYN (ADAMA has LOA)
KCP 10.2.1	Weber, K.	2012a	Mesotrione/nicosulfuron/dicamba WG (A18032E) plus Adigor (A12127R) – Assessment of Toxic Effects on the duckweed *Lemna gibba* in a Semi-Static Test Eurofins Agroscience Services EcoChem GmbH, Eutinger Str. 24, 75223 Niefern-Öschelbronn, Germany Report No. S12-02297 GLP, Unpublished Syngenta File No. A18032E_10009	N	SYN (ADAMA has LOA)

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 10.2	xxxxxxxxxxx	2012	Mesotrione/nicosulfuron/dicamba WG (A18032E) plus Adigor (A12127R) – Acute Toxicity Testing in Rainbow Trout (*Oncorhynchus mykiss*) (Teleostei, Salmonidae) xxxxxxxxxxxxxxx Report No. S12-02295 GLP, Unpublished Syngenta File No. A18032E_10001	Y	SYN (ADAMA has LOA)
KCP 10.2 (report available from data owner)	Wenzel, A.	2010	Macrophyte Growth Test with Nicosulfuron Technical. Test with a subsequent Recovery Period. Fraunhofer IME study No. CHE-009/4-80 Report No. 185 NIS GLP, Unpublished	N	Cheminova (ADAMA has LoA from FMC, owner of Cheminova) ~~(ADAMA has equivalent data)~~
KCP 10.3.1.1.1 and 10.3.1.1.2	Kling, A.	2012	Mesotrione/nicosulfuron/dicamba WG (A18032E) plus Adigor (A12127R) – Acute Oral and Contact Toxicity to the Honeybee *Apis mellifera* L. in the Laboratory Eurofins Agroscience Services EcoChem GmbH, Eutinger Str. 24, 75223 Niefern-Öschelbronn, Germany Report Number S12-02293 GLP, Unpublished Syngenta File No. A18032E_10005	N	SYN (ADAMA has LOA)
KCP 10.3.2.1	Fallowfield, L.	2012	Mesotrione/Nicosulfuron/Dicamba WG (A18032E) plus A12127R (Adigor adjuvant) – A rate-response laboratory bioassay of the effects of fresh residues on the predatory mite, *Typhlodromus pyri* (Acari: Phytoseiidae) Mambo-Tox Ltd., 2 Venture Road, Chilworth Science Park, Southampton, SO16 7NP, UK Report No. SYN-12-28 GLP, Unpublished Syngenta file No. A18032E_10003	N	SYN (ADAMA has LOA)
KCP 10.3.2.1	Stevens, J.	2012	Mesotrione/Nicosulfuron/Dicamba WG (A18032E) plus Adigor (A12127R) – A rate-response laboratory bioassay of the effects of fresh residues on the parasitic wasp *Aphidius rhopalosiphi* (Hymenoptera, Braconidae) Mambo-Tox Ltd., 2 Venture Road, Chilworth Science Park, Southampton SO16 7NP, United Kingdom Report No. SYN-12-29 GLP, Unpublished Syngenta File No. A18032E_10000	N	SYN (ADAMA has LOA)

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 10.3.2.2	Stevens, J.	2012a	Mesotrione/Nicosulfuron/Dicamba WG (A18032E) plus Adigor (A12127R) – A rate-response extended laboratory bioassay of the effects of fresh residues on the parasitic wasp *Aphidius rhopalosiphi* (Hymenoptera, Braconidae) Mambo-Tox Ltd., 2 Venture Road, Chilworth Science Park, Southampton SO16 7NP, United Kingdom Report No. SYN-12-45 GLP, Unpublished Syngenta File No. A18032E_10010	N	SYN (ADAMA has LOA)
KCP 10.3.2.2	Tew, G.	2013	Mesotrione/Nicosulfuron/Dicamba WG (A18032E) plus Adigor A12127R – A rate-response extended laboratory bioassay of the effects of fresh residues on the rove beetle, *Aleochara bilineata* (Coleoptera; Staphylinidae) Mambo-Tox Ltd., 2 Venture Road, Chilworth Science Park, Southampton SO16 7NP, United Kingdom Report No. SYN-12-46 GLP, Unpublished Syngenta File No. A18032E_10015	N	SYN (ADAMA has LOA)
KCP 10.4.1.1	Friedrich, S.	2012	Friedrich S, (2012). Mesotrione/Dicamba/Nicosulfuron WG (A18032E) plus Adigor (A12127R) – Sublethal Toxicity to the Earthworm *Eisenia fetida* in Artificial Soil BioChem agrar Labor für biologische und chemische Analytik GmbH, Kupferstraße 6 04827 Gerichshain, Germany Report Number 12 10 48 147 S GLP, Unpublished Syngenta File No. A18032E_10007	N	SYN (ADAMA has LOA)
KCP 10.4.2.1	Dickinson, R.A.	2015	R169649 - Collembola (*Folsomia candida*) Reproduction Test in Soil Agrochemex Ltd., Aldhams research station, Manningtree, Essex, CO11 2NF, United Kingdom Report No. ENV-14-015 GLP, Unpublished Syngenta File No. CA3511_10011	N	SYN (ADAMA has LOA)
KCP 10.4.2.1	Friedrich, S.	2013	Mesotrione/Dicamba/Nicosulfuron WG (A18032E) plus Adigor (A12127R) – Effects on the Reproduction of the Collembolan *Folsomia candida* BioChem agrar, Labor für biologische und chemische Analytik GmbH, Kupferstraße 6, 04827 Gerichshain, Germany Report No. 12 10 48 090 S GLP, Unpublished Syngenta File No. A18032E_10011	N	SYN (ADAMA has LOA)

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 10.4.2.1	Ramsden, C.	2015	R169649 – Predatory Mite (*Hypoaspis (Geolaelaps) aculeifer*) Reproduction Test in Soil AgroChemex Environmental Ltd., Aldhams Farm Research Station, Dead Lane, Manningtree, Essex, CO11 2NF, United Kingdom Report No. ENV-14-012 GLP, Unpublished Syngenta File No. CA3511_10010	N	SYN (ADAMA has LOA)
KCP 10.4.2.1	Schulz, L.	2013	Mesotrione/Nicosulfuron/Dicamba WG (A18032E) plus Adigor (A12127R) – Effects on the Reproduction of the Predatory Mite *Hypoaspis aculeifer* BioChem agrar Labor für biologische und chemische Analytik GmbH, Kupfertraße 6, 04827 Gerichshain, Germany Report Number 12 10 48 148 S GLP, Unpublished Syngenta File No. A18032E_10012	N	SYN (ADAMA has LOA)
KCP 10.5	Schulz, L.	2012	Mesotrione/Nicosulfuron/Dicamba WG (A18032E) plus Adigor (A12127R) – Effects on the Activity of Soil Microflora (Nitrogen and Carbon Transformation Tests) BioChem agrar, Labor für biologische und chemische Analytik GmbH, Kupferstraβe 6, 04827 Gerichshain, Germany Report Number 12 10 48 048 C/N GLP, Unpublished Syngenta File No. A18032E_10004	N	SYN (ADAMA has LOA)
KCP 10.6.2	Bramby-Gunary, J.	2013	Mesotrione/dicamba/nicosulfuron WG (A18032E) plus A12127R (Adigor adjuvant) - Evaluation of the Phytotoxicity to Non Target Terrestrial Plant Vegetative Vigour Test AgroChemex Ltd, Aldhams Farm, Dead Lane, Manningtree, Essex, CO11 2NF, United Kingdom Report Number ACE-12-149 GLP, Unpublished Syngenta File No. A18032E_10025	N	SYN (ADAMA has LOA)
KCP 10.6.2	Bramby-Gunary, J	2013a	Mesotrione/dicamba/nicosulfuron WG (A18032E) plus A12127R (Adigor adjuvant) - Evaluation of the Phytotoxicity to Non Target Terrestrial Plant Seedling Emergence and Seedling Growth Test AgroChemex Ltd, Aldhams Farm, Dead Lane, Manningtree, Essex, CO11 2NF, United Kingdom Report No. ACE-12-148 GLP, Unpublished Syngenta File No. A18032E_10024	N	SYN (ADAMA has LOA)

*Syngenta requests data confidentiality for these data. Disclosure of the information might undermine Syngenta commercial interests by providing access to Syngenta specific know-how.

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 10.1.2.2	Funkenhaus, A. and Giessing, B.	2010	Exposure of mammals in maize fields in France - attractiveness of maize fields and relevant species RIFCON GmbH, Im Neuenheimer Feld 517. D-69120 Heidelberg, Germany Syngenta Report No. R09012-2. Study No. TK0003853 GLP, Unpublished Syngenta File No. NA_11991 (Data owned by Syngenta)	N	SYN (ADAMA has LOA)
KCP 10.1.2.2	xxxxxxxxxxx C.	2005	Generic field monitoring of birds and mammals on maize and beet fields in Austria. Bayer CropScience AG. Report No. WFC/FS 017 BCS reference: MO-05-001258 GLP, Unpublished Syngenta File No. N/1155 (Owner: Bayer Crop Science, Syngenta have access)	N	BCS (ADAMA has access (4 LOA))
KCP 10.1.2.2	xxxxxxxxxxxx	2013	Generic field study on small mammals focal species and wood mouse (*Apodemus sylvaticus*) PT in maize fields in Germany. Rifcon GmbH Oxon Report No.: P12225 GLP, Unpublished Syngenta File No. NA_13410 (Data owner: Oxon Italia, S.p.A., Syngenta access)	N	OXN (ADAMA has access (5 LOA))

zRMS comments:

Please note that majority of toxicity data for particular active compounds were taken from the EFSA conclusions and were thus evaluated at the EU level. For list of respective studies, please refer to Vol. 2 of the monograph for individual substances.

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	Reason for rejection
KCP 10.1.1 / 01	xxxxxxxxxx	2018	Mesotrione - An Acute Oral Toxicity Study with the Mallard Using a Sequential Testing Procedure Syngenta Crop Protection AG, Basel, Switzerland xxxxxxxxxxx GLP not published Syngenta File No ZA1296/10605	Y	SYN (ADAMA has access)	Not evaluated, new study with vertebrates, not required to finalise the risk assessment
KCP 10.1.2.2	Fülling, O. and Sainz-Elipe, S.	2015	Generic field study on the presence and abundance of common voles in maize fields in Northern France tier3 solutions GmbH Report No. B15009 GLP, Unpublished Syngenta File No. NA_13749 (Data owned by BASF, available to Syngenta by data agreement)	N	BASF (Study is only supplementary in Risk Assessment)	Not evaluated, not required for the risk assessment
KCP 10.1.2.2	Prescott, C.	2004	The assessment of Wood mouse acceptance/avoidance of different crop seeds when presented in free feeding conditions to individually caged animals in a six hour no-choice situation; and to monitor the incidence of de-husking for each seed type. University of Reading VPU Report. The Vertebrate Pests Unit School of Animal and Microbial Sciences, The University of Reading, Whiteknights, Reading. RG6 6AJ. UK. Syngenta study VPU/04/026 Not GLP, Unpublished Syngenta File No. N/1014	N	SYN (ADAMA has access)	Not evaluated, not required for the risk assessment
KCP 10.1.2.2	Voigt, U. and Zaccaroni, M.	2013	Generic field monitoring of hares in a mixed landscape in Germany University Of Veterinary Medicine Hannover, Bünteweg 2 30559 Hannover Germany Report Number BAR/FS069 GLP, Unpublished Syngenta File No. NA_13449 (Study owner BCS, Syngenta have access)	N	BCS	Not reliable

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	Reason for rejection
KCP 10.1.2.2	Voigt, U. and Zaccaroni, M.	2015	Generic field monitoring of hares in a mixed landscape in Germany – Jacobs Index University Of Veterinary Medicine Hannover, Bünteweg 2 30559 Hannover Germany Report Number BAR/FS069 Non-GLP, Unpublished Syngenta File No. NA_13997 (Study owner BCS, Syngenta have access)	N	BCS (Data protection not claimed)	Not reliable
KCP 10.1.2.2	Dittrich, R. and Benito, M.M.	2016	Occurrence and PT of Wood mice in pre- and post-emergence maize fields in France, southern zone. tier3 solutions GmbH, Kolberger Str. 61-63, 51381 Leverkusen, Germany Report Number B15064 GLP, Unpublished Syngenta File No. NA_14237 (Study owner BCS, Syngenta have access)	N	BCS (Study is only supplementary in Risk Assessment)	Not evaluated, as performed in the Southern Zone.
KCP 10.1.2.2	Späth V.	1989	Untersuchungen zur Populationsökologie des Feldhasen (*Lepus europaeus* Pallas) in der Oberrheinebene Translation: Studies on the Population Ecology of the Field Hare (*Lepus europaeus* PALLAS**)** in the Upper Rhine Plain PhD thesis, University of Freiburg, Freiburg im Breisgau. Not GLP published Syngenta File No. VV-243592	N	-	Not relevant for derivation of PT values.
KCP 10.2.1	Hengsberger, A. and Wydra, V.	2015a	Mesotrione Wet Paste (ZA1296) - Toxicity to the Aquatic Plant *Lemna gibba* in a Reciprocal Growth Inhibition Test. Ibacon GmbH Arheilger Weg 17 64380 Rossdorf Germany. Report No. 105731240 GLP, Unpublished Syngenta File No. ZA1296_10436	N	SYN (ADAMA has LOA)	Not evaluated; not required to finalise the aquatic risk assessment at the zonal level.
KCP 10.3.1.2/01	Ruhland, S.	2015	Dicamba SL (A7254B) - Chronic toxicity to the Honeybee *Apis mellifera* L. in a 10 Day Continuous Laboratory Feeding Study. BioChem agrar Labor für biologische und chemische Analytik GmbH Kupferstraße 6 04827 Gerichshain, Germany Report No. 14 10 48 057 B GLP, Unpublished Syngenta File No. A7254B_10378	N	SYN (ADAMA has LOA)	Not relevant for the risk assessment for A18032E (studies with the formulation in question must be performed)

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	Reason for rejection
KCP 10.3.1.2/02	xxxxxxxxxx	2018	Mesotrione - Honey Bee (Apis mellifera L.) Chronic Oral Toxicity Test 10 Day Feeding Test in the Laboratory, xxxxxxxxx Report Number S18-03658 GLP, Unpublished Syngenta file No. ZA1296_10608	Y	SYN (ADAMA has LOA)	Not relevant for the risk assessment for A18032E (studies with the formulation in question must be performed)
KCP 10.3.1.2/03 (report available from data owner)	Schmitt, H.	2014	Nicosulfuron (DPX-V9360) Technical: Assessment of Effects to the Honeybee, *Apis mellifera* L., in a 10 Days Chronic Feeding Test under Laboratory Conditions Eurofins Agroscience Services EcoChem GmbH, D-75223 Niefern-Öschelbronn, Germany Report No. S 14-00413 GLP, Unpublished	N	DuPont (ADAMA has access (1 LOA))	Not relevant for the risk assessment for A18032E (studies with the formulation in question must be performed)
KCP 10.3.1.3/01	Kleebaum, K.	2015	Dicamba SL (A7254B) – Chronic toxicity to the honeybee larvae *Apis mellifera* L. under laboratory conditions (in vitro) BioChem agrar, Labor für biologische und chemische Analytik GmbH, Kupferstraße 6, 04827 Gerichshain, Germany Report No. 14 10 48 072 B GLP, Unpublished Syngenta File No. A7254B_10377	N	SYN (ADAMA has LOA)	Not relevant for the risk assessment for A18032E (studies with the formulation in question must be performed)
KCP 10.3.1.3/02	xxxxxxxxxxxxx	2016	Mesotrione - Honey bee (*Apis mellifera* L.) Larval Toxicity Test (Repeated Exposure through to Adult Emergence), xxxxxxxxxxxxxx Report Number S16-00332 GLP, Unpublished Syngenta file No. ZA1296_10465	Y	SYN (ADAMA has LOA)	Not relevant for the risk assessment for A18032E (studies with the formulation in question must be performed)
KCP 10.3.1.3/03 (report available from data owner)	Klank, C.	2014	Nicosulfuron (DPX-V9360) Technical: Honey Bee (*Apis mellifera* L.) Larval Toxicity Test (Single Feeding Exposure) Eurofins Agroscience Services EcoChem GmbH, D-75223 Niefern-Öschelbronn, Germany Report No. S14-00341 GLP, Unpublished	N	DuPont (ADAMA has access (1 LOA)	Not relevant for the risk assessment for A18032E (studies with the formulation in question must be performed)

List of data relied on not submitted by the applicant but necessary for evaluation

Data point	Author(s)	Year	Title Source Company Report No. GEP status Published or Unpublished Syngenta File No.	Vertebrate study Y/N	Owner
There were no data not submitted by the Applicant and relied on.					

Appendix 2 Detailed evaluation of the new studies

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

Comments of zRMS:	The study summarised below was a new vertebrate study not required to finalise the risk assessment. No data gap in area of avian toxicity testing was identified in EFSA Journal 2016;14(3):4419. Taking this into account, the study was not evaluated and not considered in the risk assessment. The summary is thus struck through and shaded.

Reference:	10.1.1.1
Report	Hubbard PM, Davis RJ, Temple DL (2018). Mesotrione - An Acute Oral Toxicity Study with the Mallard Using a Sequential Testing Procedure. EAG, Inc. 8598 Commerce Drive Easton, MD 21601 USA Unpublished report number 528B-574. Experimental period June 28th 2018 to July 13th 2018 (Syngenta File No. ZA1296/10605)
Guideline(s):	OECD Draft Guideline 223
Deviations:	No
GLP:	Yes
Acceptability:	Not evaluated as submission of additional vertebrate study is not justified
Duplication (if vertebrate study)	No

~~**Materials and method**~~

~~**Test Material:** Mesotrione technical~~
~~**Description:** Brown powder~~
~~**Lot/Batch #:** 675385 (SMO0H028)~~
~~**Purity:** 84.6% w/w~~
~~**Stability of test compound:** Not stated, assumed stable for test period with re-analysis date of end February 2019~~
~~**Test concentrations:** Limit test: initial single dosing at 2000 mg/kg bodyweight~~
~~**Vehicle and/or positive control:** None~~
~~**Analysis of test concentrations:** No~~
~~**Test animals**~~

~~**Species:** Mallard (*Anas platyrhynchos*)~~
~~**Source:** Whistling Wings, Hanover, IL 61041, USA~~
~~**Acclimatisation period:** 17 weeks~~
~~**Treatment for disease:** None~~
~~**Weight and age of birds:** Initial weight: 801 – 1107 g~~
~~Age: 37 weeks~~
~~**Feeding:** Water and feed were provided ad libitum during acclimatisation and test, except for a period of 18 hour fasting prior to dose administration and one hour after dosing~~

~~**Environmental conditions**~~

~~**Test temperature:** Average 21.2–22.2°C; maximum = 24.6°C, minimum = 20.5°C~~
~~**Relative humidity:** Average 74-75%; maximum = 88%, minimum = 55%.~~

Photoperiod: 7 hours fluorescent light (324 lux) and 17 hours dark
Length of test: 14 days

Study Design and Methods
Experimental dates: June 28th 2018 to July 13th 2018
The objective of the study was to determine the acute oral toxicity of the test substance on the Mallard duck (*Anas platyrhynchus*). An initial single dosing was made at the limit dose (typically considered to be 2000 mg/kg bodyweight).
Birds of both sexes were assigned without regard to sex to a control group and a single test group that contained five birds each. Birds were acclimatized to the study facility and cages for 17 weeks prior to test initiation. The birds had not been fed for the 18 hours prior to treatment and were individually weighed just prior to treatment. The test substance was administered orally, by capsule, and was directly administered to the individual birds by oral gavage. Each bird was individually dosed (based on bodyweight) and received the same dose of test treatment per kilogram of bodyweight. After treatment the birds were provided with fresh water and feed *ad libitum* for the remainder of the test. Following treatment the birds were observed frequently on the day of treatment and twice daily for 14 day post-treatment observation period.

Results and Discussion
Environmental conditions: The average temperature recorded was 22.2°C from June 29, 2018 to July 9, 2018 and 21.2°C from July 10, 2018 to July 13, 2018 with a maximum of 24.6°C, a minimum of 20.5°C. The average relative humidity for this study was 74% from June 29, 2018 to July 9, 2018 and 75% from July 10, 2018 to July 13, 2018 with a maximum of 88%, a minimum of 55%. The photoperiod was approximately seven hours of light/17 hours of dark per day during acclimation and throughout the test. A photoperiod of seven hours instead of eight hours was maintained to deter the birds from coming into production and this difference from the photoperiod listed in the protocol is thought to have no impact on the study results.

Mortality and symptoms of toxicity:
There were no mortalities observed in the 2000 mg/kg bodyweight treatment, therefore the LD_{50} is > 2000 mg/kg bodyweight. All birds in the control group were normal in appearance and behaviour for the duration of the test. No regurgitation was noted after dosing birds in the control group or the 2000 mg a.i./kg dosage group. All birds in the 2000 mg a.i./kg dosage level were normal in appearance and behaviour for the duration of the study. There were no apparent treatment-related effects on body weight or on food consumption.

Conclusions
The acute oral LD_{50} for Mallard duck exposed to mesotrione is >2000 mg/kg bodyweight.

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

Please refer to Section B.6 (Toxicology) for study summary.

A 2.1.2.2 KCP 10.1.2.2 Higher tier data on mammals

The following studies are cited in ***Alvarez (2019)***, and full summaries are included in that report.

Comments of zRMS:	During the EU review it was already concluded that the common vole is not relevant focal species for maize at BBCH 12-18. Taking this into account, additional data supporting this conclusion were not necessary and the study by xxxxxxxxxxxx (2015) was not evaluated by the zRMS.

Reference:	10.1.2.2
Report	xxxxxxxxxxxx. (2015) Generic field study on the presence and abundance of common voles in maize fields in Northern France, unpubl. tier3 solutions GmbH Report No. B15009 Syngenta file No NA_13749 (Data owned by BASF, available to Syngenta by data agreement)
Guideline(s):	No guideline applicable. The study is consistent with guidance provided in EFSA Journal 2009; 7(12).
Deviations:	No
GLP:	Yes
Acceptability:	Not evaluated; focal species for maize at BBCH 12-18 were already confirmed at the EU level
Duplication (if vertebrate study)	No

~~**Conclusion:**~~
~~This is a study based on data of 11 maize fields with adjacent grasslands which were densely populated by common voles. The fact that in nine out of the eleven maize fields no common vole was ever caught leads to the conclusion that early stage maize fields are not attractive for this species.~~
~~In summary, only 1.62% of 2649 common vole captures (i.e. 1421 individuals) were made inside early stage maize fields, strongly suggesting that common voles do not inhabit early stage maize fields.~~

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Comments of zRMS:	Reason for submission of the study by Prescott (2004) is unclear to the zRMS as its results were not used in the risk refinement. The study just confirms that wood mouse may feed on seeds, prefer some seeds over others and de-husk part of the seeds. All this information is already known from literature. It should be also noted that the risk assessment for the wood mouse is performed with consideration of weed seeds representing part of the diet, while the weed seeds were not included in the study, so it cannot be concluded if the wood mouse would de-husk weed seeds. As results of the study were not used in the risk assessment, the study was not evaluated by the zRMS in detail. However, the summary has been completed by the zRMS and retained below so concerned Member States could see that results of the study do not provide any particularly useful or new information that could be implemented into the risk refinement.

Reference:	10.1.2.2
Report	Prescott C. (2004). The assessment of Wood mouse acceptance /avoidance of different crop seeds when presented in free feeding conditions to individually caged animals in a six hour no-choice situation; and to monitor the incidence of de-husking for each seed type. University of Reading VPU Report. The Vertebrate Pests Unit School of Animal and Microbial Sciences, The University of Reading, Whiteknights, Reading. RG6 6AJ.

	UK. Syngenta study VPU/04/026. Syngenta File No. N/1014 (Data owned by Syngenta)
Guideline(s):	No guidelines available, but following recommendations in the EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009) and its appendices
Deviations:	No
GLP:	No
Acceptability:	Not evaluated as not used in the risk assessment
Duplication (if vertebrate study)	No

~~**Conclusion:**~~

~~The individual consumption of crop seeds was quite variable. Mean consumption of crop seeds was below the mean daily consumption of laboratory diet (as determined during the 7 day conditioning period), with the exception of an individual female animal that consumed 5 g of cotton seed and 3 g of maize seed, although consumption of 2.6 g of wheat seed by a male mouse approached the mean daily consumption of laboratory diet. Mice appeared to prefer larger seeds like cucumber, soya and wheat and consume very little of the small seeds like carrot, tomato and lettuce. Pelleted sugar beet was the least consumed seed, presumably due to the thick clay coating around the seed. A full study summary is provided in ***Haaf and Alvarez (2017)***.~~

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Comments of zRMS:	The study by Voigt & Zaccaroni (2013) was already rejected as not reliable during zonal evaluation of the formulation Calaris, belonging to Syngenta due to following reasons: 1. When determining PT values, ideally the mammals should be visually observed. In this study the PT values were calculated based solely on GPS co-ordinates every 10 minutes and assumptions as to whether the hares are foraging. This adds major uncertainty to the study results. 2. None of the individuals tracked was still alive at the end of the study. Furthermore, due to technical issues, data was missing from eight individuals (33% of those initially tagged). This also reduces the reliability of the study. 3. Only three PT values above 0 could be obtained from the study. In addition to that, they originated from 2 individuals (one was tracked twice). It should be noted that data for minimum 4 individual crop consumers are required. 4. According to the study author maize was not attractive to brown hare as a food source. It cannot be, however, excluded that this was caused by the low proportion of maize in the study area (maximum of 16.5%). The low proportion of maize fields in the study site also adds uncertainty as to whether this study can be considered a reasonable 'worst-case' for assessing applications to maize in the central zone. These conclusions are also applicable for evaluation of the study in the context of authorisation of A18032E.

Reference:	10.1.2.2
Report	Voigt U., Zaccaroni M. (2013) Generic field monitoring of hares in a mixed landscape in Germany, Report Number BAR/FS069, University Of Veterinary Medicine Hannover, Bünteweg 2 30559 Hannover Germany. (Syngenta File No. NA_13449) Study owner BCS, Syngenta have access.
Guideline(s):	No guidelines available, but following recommendations in the EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009) and its appendices
Deviations:	No
GLP:	Yes
Acceptability:	Not reliable (see above for details).
Duplication (if vertebrate study)	No

~~**Conclusion:**~~

~~This study conducted, from March to June, highlighted the use of habitats in a population of hares tagged with GPS collars in an intensive agro-ecosystem of northern Germany, characterized by cereals, sugar beet, maize and oilseed rape. The feeding time of hares is nocturnal, and the results indicate that the population studied spent a high percentage of feeding time in crops during the whole monitoring period. Results show a strong selection for cereals in March and April, in May they were used in relation to the availability and in June cereals were clearly avoided. In contrast the use of sugar beet fields increased from March to June; noticeably these fields were avoided, in relation to the availability, in March and April and preferentially selected in May and June. Maize fields were avoided during the entire study period. Oilseed rape was used in relation to the availability in March and avoided from April to June. Other crops were under represented and are therefore not sufficiently representative. Bare soil was avoided; this habitat was used exclusively for social relations between individuals and as a resting habitat. Finally, when the availability of the off-crop areas is considered, these were less used during feeding times. This study highlights that the hare is very well integrated into an intensive agricultural system, and that most of the time devoted to foraging is spent in crops. The division in the study between PT_{night} and $PT_{foraging}$ showed only slight differences, leading to the same conclusions, confirming that hares are active almost entirely active at night. A full study summary is provided in ***Haaf and Alvarez (2017)***.~~

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Comments of zRMS:	Due to various deficiencies, the study by Voigt & Zaccaroni (2013) summarised above, is considered not suitable for the risk refinement purposes. Taking this into account, calculation of Jacobs Indices based on results of this study is also unreliable and was thus not evaluated by the zRMS.

Reference:	10.1.2.2
Report	Voigt U., Zaccaroni M. (2015) Generic field monitoring of hares in a mixed landscape in Germany – Jacobs Index; Report Number BAR/FS069, University of Veterinary Medicine Hannover, Bünteweg 2 30559 Hannover Germany. (Syngenta File No. NA_13997) Study owner BCS, Syngenta have access.
Guideline(s):	No guidelines available
Deviations:	No
GLP:	No
Acceptability:	Not reliable (see above for details).
Duplication (if vertebrate study)	No

Conclusion:

This report calculates Jacob's Indices for the data reported in Voigt & Zaccaroni (2013) (please see above).

The Jacobs Indices show a strong negative value, indicating avoidance, for maize (D = -0.91 to -1.0) and a positive preference for cereals up to early June (D = +0.56 up to +0.73). These findings are in close agreement with the review of Smith et al (2005).

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Comments of zRMS:	The study by Dittrich & Benito (2016) was performed in the Southern France (department of Ariège and HauteGaronne, region of Midi-Pyrénées) so it results are not representative for conditions of the Central Zone. Taking this into account the study was not evaluated by the zRMS and not considered for purposes of the risk refinement

Reference:	10.1.2.2
Report	Dittrich R. and Benito M.M. (2016) Occurrence and PT of Wood mice in pre- and post-emergence maize fields in France, southern zone. Report Number B15064. tier3 solutions GmbH, Kolberger Str. 61-63, 51381 Leverkusen, Germany Study owner Bayer – Syngenta has access (Syngenta File No. NA_14235 ~~14237~~)
Guideline(s):	No guidelines available
Deviations:	No
GLP:	Yes
Acceptability:	Not evaluated as not representative for the Central Zone (study performed in the Southern France).
Duplication (if vertebrate study)	No

~~**Conclusion:**~~

~~This study monitored the use of maize fields in southern France between drilling and BBCH 17 by Wood mice. Grids of live traps were established on study fields (including off-field habitat) to monitor use of maize fields by capture-mark-recapture techniques and also to catch animals for radio-tagging. Data was obtained from 63 full telemetry sessions, belonging to 28 individuals tracked between one and six times. The study demonstrated the presence of an abundant and stable population of Wood mice close to the study fields and the general avoidance of these fields by the animals. In a total of 4528 trap-nights, 768 captures of the focal species were made. The overall standardized trapping success for the Wood mouse was 16.1 times lower in the maize fields, with 3.2 captures per 100 trap-nights compared to 51.5 in the adjacent off-field habitat. The in-field to off-field ratio of the standardised trapping success was 0.02 and 0.08 for BBCH 00 – 09 and 10 – 17, respectively. A total of 28 potential consumers, which were trapped close to or inside the maize fields, were radio-tracked in order to measure their use of maize fields as feeding habitat. Only seven individuals entered, for a very short period of time, the study fields (in maximum 8.1% of the time potentially foraging was spent within maize fields).~~

~~Taking into account consumers for the pre-emergence period, the 90%ile of the PT value was 0.020 (maximum PT= 0.024) over all sessions. In the post-emergence period, repeated sessions of selected individuals were conducted, and the 90%ile of the PT value was 0.013 (maximum PT= 0.081) over all sessions (including repetitions) for consumers.~~

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Comments of zRMS:	The aim of the study by xxxxxxxxxxx (2019) was to determine the proportion of time the brown hares spent potentially foraging in early germinated maize fields (BBCH <20). The study was well performed and is considered acceptable by the zRMS. The full study summary has been provided by the zRMS below in order to facilitate concerned Member States independent review and submission of the comments. Initial site selection in the study was based on the presence of the European hare, high proportions of maize fields within the landscape and the suitability for performing radio-tracking. To increase representativeness and variability of landscape parameters among Central European maize growing areas, two different study areas in two different countries and five different study sites were chosen in areas of high proportions of maize within Central Europe. The region used for the study represented typical maize growing

region in Germany and Hungary and may be thus considered representative for conditions of the Central Zone.
At test sites the maize fields represented on average 36% of the landscape surface within the investigated hares home ranges and at some test sites their proportion in the total landscape exceeded 45 or even 50%.

The study was performed at early stages of maize and included BBCH from 00 to 19. However, as the aim of the study was to determine the time that brown hares potentially feed on maize shoots, results for fields with BBCH <09 were excluded from calculation of PT values.

For purposes of the radio-tracking, 23 individual adult brown hares were trapped and equipped with the radio tags. Trapping locations were chosen in areas with high number of maize/future maize fields and hares were captured either in such fields or nearby (e.g. in adjacent off-crop structures or neighbouring fields).
The total weight of the hare collar was about 40 g, representing approximately 1% of the bodyweight of the tagged animals. Due to the low weight of the tags (far below the recommended maximum of 5% of the total bodyweight) it was not expected that they would have influence the animals' behaviour. Visual observations confirmed normal behaviour of the animals. In order to give animal time to acclimatize, the radio-tracking started no earlier than 2 days after tagging with single check telemetry of the individuals. During the telemetry sessions each individual was tracked continuously for 24 hours, which is in line with recommendations of EFSA (2009). During this time all movements between different habitats and changes of the behaviour (e.g. foraging, resting) were recorded. In addition to that, animals were observed with binoculars, scopes and night observation devices.
During radio-tracking without visual contact, all instances of an active signal were interpreted as potential foraging behaviour and thus included in the calculation of PT values. However, based on the behaviour confirmed by visual contacts during the 24h telemetry, animals foraged for just 32.0% of their visually observed time and showed active behaviour other than foraging in 18.5% of the time. Therefore, the time spent potentially foraging in maize is rather overestimated for this habitat. This confirms that the PT values are conservative and rather overestimate the actual PT values for early maize (BBCH growth stages up to 20) than being a minimum value.

In general, results of the study indicate that brown hares do utilise early maize fields as the feeding habitat. During the 24-hours radio-tracking session most of 23 radio-tagged hares were observed in maize fields with individual PT values ranging from 0.02 to 0.94. One individual (or signal) could not be tracked after tagging and most probably the animal left the study site. One individual was found at the end of May far outside the study site. To increase the number of radio-tracking sessions and to cover wider range of BBCH stages, two individuals were radio-tracked twice, giving 23 radio-tracking sessions in total. One session was excluded from further calculations as being not "consumer session" (animal was never located in a maize field being active during the session, had no maize in the 24h home range and was not caught on a maize field).
Taking into account that 21 individuals (i.e. >20 recommended by EFSA, 2009) were observed potentially foraging during radio-tracking sessions (with one animal observed twice), in opinion of the zRMS the 90th percentile PT value is sufficiently reliable and may be used for purposes of the refinement of the risk for the brown hare. Nevertheless, according to the current national Polish requirements, individual with PT <0.1 should not be considered to be actual crop consumers and should be rejected from further calculations. Nevertheless, when data for individual with PT <0.1 are excluded, there are still 17 reliable PT values enabling consideration of 90th percentile PT for the risk refinement (>10 PT values greater than 0.1 are required).

It should be noted that PT values were derived for maize stages ranging from BBCH 09 to 19, while formulation A18032E is intended to applied at BBCH 12-14. Nevertheless, obtained results show that different BBCH growth stages up to <20 did not have an impact on the use of maize as foraging habitat by the brown hare.

	It is also noted that more radio-tracking sessions were performed at BBCH <15 comparing to BBCH >15 and for this reason there may be concern if calculation of the 90th percentile PT for the whole study period is relevant. First of all it should be pointed out that in EFSA (2009) the BBCH 10-19 is treated as a single period for early maize with no differentiation for single stages, which is captured in the study by Grimm and Katzschner (2019). Furthermore, in opinion of the zRMS, available results show that PT values for the whole period of study were comparable. For example, the maximum PT of 0.89 derived for BBCH 12-14 is close to maximum PT of 0.94, derived for BBCH 15-19. The next highest value of 0.56 for BBCH 10-11 (derived in both, Germany and in Hungary) is close to 0.63 at BBCH 15-19. The lowest PT of 0.23 for BBCH 15-16 is even lower than several PT values for BBCH 10-15. In addition to that, the mean PT values of 0.35 and 0.39 were calculated for BBCH <15 (16 sessions) and >15 (6 sessions), respectively, which demonstrates that derived PT values have not depended on the growth stage. Therefore, in opinion of the zRMS, PT values calculated for the whole period of study are comparable and may be merged in order to derive single 90th percentile PT. Overall, the 90th percentile PT of 0.743 could be calculated from individual PT values greater than 0.1 and this value is considered acceptable for purposes of refinement of the risk to the brown hare exposed after application of A18032E according to the intended use pattern. For the full study summary, please refer to the final Core Assessment (September 2020) for formulation Callisto (A12739A) owned by Syngenta, since according to the LoA, ADAMA may refer to results of the study, but does not have access to the full study, so the zRMS cannot include here parts of the report.

Reference:	KCP 10.1.2.2/07
Report	T Grimm and I Katzschner (2019) Generic monitoring of European hares to determine proportion of time spent foraging in early maize in Central Europe Syngenta Limited; unpubl. RIFCON GmbH report No. R1740045, March 2019. Syngenta File No. NA_14950
Guideline(s):	No official test guideline(s) available at present Conducted under consideration of the EFSA Guidance Document on Risk Assessment for Birds & Mammals (EFSA 2009).
Deviations:	Not relevant
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	No

Conclusion

The aim of this generic study was to investigate the use of maize fields as foraging habitat by Brown hare (*Lepus europaeus*) in the Central Europe. Focus was the determination of respective PT values (i.e. proportion of diet obtained in treated area, calculated as proportion of potentially foraging time spent in maize fields by hares) during the early growing period of maize via continuous 24-hour radio-tracking sessions of multiple individual hares.

In total, radio-tracking sessions of 21 individual hares at five study sites were performed during the early crop development of maize in Central Europe. Radio-tracking sessions were performed from late April until early June 2018. The number of conducted 24h telemetry sessions was 23 (17 in Germany, six in Hungary), since two individuals were radio tracked twice. One session had to be excluded from analysis, as this session was not considered as a 'consumer session' since the animal was never located being

'active' in a maize field during the session, had no maize in the 24h home range, and was not caught on a maize field.
Maize fields covered on average approximately 36% of the total landscape surface and 44% of the arable land surface within the 24h home ranges of hares in all study sites.
The calculated single PT values ranged from 0.02 to 0.94 resulting in an average of 0.36 (±0.26) and 90th percentile of 0.62. Calculated PT values did not differ substantially between different study sites; mean values were slightly higher in Germany (0.38) than in Hungary (0.31).

Foliage decline study used to refine the risk assessment:

Comments of zRMS:

Preliminary results of the study by North (2016) has been provided during the renewal process of mesotrione and initial assessment was performed by the RMS. It was concluded that the study is acceptable and detailed kinetic evaluation may provide reliable DT_{50} that may be used for purposes of the risk refinement. This was, however, not performed, as during the EU review trials in the Northern France were still ongoing.
Since then, the study was finalised and submitted in support of re-evaluation of A18032E at the zonal level.

The study is considered acceptable with following uncertainties noted:

- In the UK the distance between particular sites was in range 33-44 km, so DT_{50} values derived from UK sites cannot be considered to be fully independent when the minimum distance of 100 km is taken into account.
- 4 out of 5 trials were performed in the UK and only single trial was performed in the Northern France, which does not belong to the Central Zone. Although extrapolation from the Northern France to the Central Zone is possible, it is noted that temperature and radiation were clearly higher than at the UK sites. This could be due to different months (in UK studies were performed in June-July, while in France in August), but this difference adds some additional uncertainty.
- As no other country was involved in testing and majority of test sites was located in the UK, the zRMS has some concerns whether the variability in degradation between particular countries was sufficiently addressed.

The kinetic evaluation of the study was presented in Alvarez (2019) and was considered acceptable. It was, however, noted that kinetic evaluation was performed only using SFO, while other models could give improvement of fits in trials S15-02057-01 and S15-02057-03.
The Chi^2 error in trials S15-02057-01, S15-02057-03 and S15-02057-04 was >15%, however Chi^2 above 15% is not the reason for rejection of obtained results when the statistical analyses and visual fits are acceptable. As this is the case for trials mentioned and in general, SFO kinetics is preferred to derive DT_{50} values for residue decline trials in plants, consideration of only SFO is accepted by the zRMS.

Summary of the derived DT_{50} values is presented in table below:

Trial	DT_{50} [days]	DT_{90} [days]	Remarks
S15-02057-01 (UK)	**0.803**	2.67	Acceptable, acceptable visual fit, some potential for improvement
S15-02057-03 (UK)	0.512	1.7	Acceptable, acceptable visual fit but potential for improvement
S15-02057-04 (UK)	0.663	2.2	Acceptable, good visual fit, some potential for improvement
S15-02057-05 (N-FR)	0.636	2.11	Acceptable, good visual fit
S15-02057-06 (UK)	0.531	1.76	Acceptable, excellent visual

As results from 5 trials performed in only 2 countries are available (of which one is Northern France not belonging to the Central Zone, although conditions in Northern France are similar to the Central Europe) and due uncertainties listed above, it is proposed by the zRMS to use the worst case DT_{50} of 0.803 days for purposes of the risk refinement.

	The study summary below was provided by the Applicant for A18032E, however for the full summary of the study and kinetic evaluation, please refer to the Core Assessment for formulation Callisto (A12739A) owned by Syngenta, since according to the LoA, ADAMA may refer to results of the study, but does not have access to the full study, so the zRMS cannot include here parts of the reports.

Reference:	10.1.2.2
Report	North L (2016). Mesotrione – Foliage Decline Study with A12739A on Maize in Northern France and the United Kingdom in 2015. Report Number S15-02057. Eurofins Agroscience Services Ltd., Slade Lane, Wilson, Melbourne, Derbyshire, DE73 8AG, UK. Syngenta File No. A12739A_11065
Guideline(s):	Commission of the European Communities, General Recommendations for the Design, Preparation and Realization of Residue Trials; 7029/VI/95 (rev. 5, working document). OECD Guidelines for the Testing of Chemicals – Crop Field Trial, No. 509, OECD, Paris 2009. OECD Guidance Document on Crop Field Trials, Series on Pesticides No. 66 and Series on Testing and Assessment No. 164, ENV/JM/MONO(2011)50. OECD Guidance Document on Overview of Residue Chemistry Studies (as revised 2009), Series on Testing and Assessment (No. 64) and Series on Pesticides (No. 32), ENV/JM/MONO(2009)31. Guidelines and Criteria for the Preparation and Presentation of Complete Dossiers and of Summary Dossiers for the Inclusion of Active Substances in Regulations (EU) 283/2013 and 284/2013 implementing Regulation (EC) 1107/2009. OECD Guidance Document on Pesticide Residue Analytical Methods, ENV/JM/MONO(2007)17 (Unclassified, 13 Aug 2007).
Deviations:	No
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	No

Executive Summary

Five residue decline field trials on maize were successfully conducted in Northern France and the United Kingdom during 2015. Each trial consisted of a control and a treated plot, with the exception of trial S15-02057-05, where the control samples were taken from the treated plot immediately prior to application of the formulation.

To plot P1, mesotrione was applied to maize as A12739A, an emulsifiable concentrate (EC) formulation containing 100 g mesotrione per litre. One application was made at 150 g a.s./ha for mesotrione at BBCH 14-16.

Following the application, treated maize whole plant samples were collected at < 1 hour after application (HAA) , 4 HAA, 10 HAA, 24 HAA, 34 HAA, 48-51 HAA, 72-78 HAA and 96-99 HAA, with untreated maize whole plant samples being collected < 1 hour before application (HBA).

(Nominal sampling intervals for treated maize whole plant samples: < 1 HAA, 4 HAA, 10 HAA, 24 HAA, 34 HAA, 48 HAA, 72 HAA, and 96 HAA).

Samples were analysed for mesotrione and its metabolite MNBA.

The study design as detailed above was successfully carried out leading to the following conclusions.

Residues of mesotrione in treated maize whole plant samples taken at < 1 hour after application (HAA) were in the range 3.09 to 14.99 mg/kg, at 4 HAA were in the range 2.74 to 12.63 mg/kg, at 10 HAA were in the range 2.05 to 8.61 mg/kg, at 24 HAA were in the range 0.91 to 4.30 mg/kg, at 34 HAA were in the range 0.50 to 2.95 mg/kg, at 48-51 HAA were in the range 0.36 to 1.37 mg/kg, at 72-78 HAA were in the range below the limit of quantification (LOQ: 0.01 mg/kg) to 0.63 mg/kg, and at 96-99 HAA were in the range 0.06 to 0.13 mg/kg.

Residues of MNBA in treated maize whole plant samples taken at < 1 hour after application (HAA) were in the range below the limit of quantification (LOQ: 0.01 mg/kg) to 0.05 mg/kg, at 4 HAA were in

the range 0.04 to 0.25 mg/kg, at 10 HAA were in the range 0.07 to 0.36 mg/kg, at 24 HAA were in the range 0.07 to 0.37 mg/kg, at 34 HAA were in the range 0.05 to 0.37 mg/kg, at 48-51 HAA were in the range 0.06 to 0.35 mg/kg, at 72-78 HAA were in the range below the limit of quantification (LOQ: 0.01 mg/kg) to 0.17 mg/kg, and at 96-99 HAA were in the range 0.04 to 0.11 mg/kg.
No residues of mesotrione and MNBA were detected at or above the limit of quantification (LOQ: 0.01 mg/kg) in any of the untreated maize whole plant samples taken in this study.

Materials

Test system	The following test system is representative of the crop group required for product registration. Maize *(Zea Mays)* EPPO code ref. ZEAMX	
Test Item(s)	Formulation – Company Code	A12739A
	Formulation Content and Type	100 EC
	Batch No.	SAV5A15007
	Valid until:	Mar 2018
	Active ingredient	Mesotrione
	Nominal Content in Formulation (nominal)	100 g/L
	Actual Content in Formulation (actual)	99.3 g/L
	Stability	The test item is assumed to be stable for the period of use in the study, pending concurrent batch re-analysis

Study Design and Methods
Five residue field trials on maize were conducted in Northern France and the United Kingdom in 2015. Details of the application of mesotrione as formulation A12739A to maize in Trials S15-02057-01, S15-02057-03, S15-02057-04, S15-02057-05, S15-02057-06 are summarised in the table below.

Table A 1: Treatment details for Trial S15-02059-01, S15-02059-03, S15-02059-04, S15-02059-05, S15-02059-06

Trial. S15-02059-	Applications	Application date(s)	Formulation Code	Product rate (L/ha)	Actual spray volume (L/ha)	Growth stage at application (BBCH)	AI application rate (g mesotrione/ha)	
							Actual	Target
-01	1	09/07/2015	A12739A	1.45	193	15-16	145	150
-03	1	23/06.2015	A12739A	1.58	212	14-16	158	150
-04	1	09/07/2015	A12739A	1.48	197	16	148	150
-05	1	25/08/2015	A12739A	1.53	257	15	153	150
-06	1	14/07/2015	A12739A	1.47	196	15-16	147	150

There was no rainfall within 48 hours of the application being made at all trial sites, with the exception of trial S15-02059-04, which experienced 0.2 mm of rainfall on the day of application.

Selection of samples to be analysed and shipment:
Following the application, treated maize whole plant samples were collected at < 1 hour after application (HAA) , 4 HAA, 10 HAA, 24 HAA, 34 HAA, 48-51 HAA, 72-78 HAA and 96-99 HAA, with untreated maize whole plant samples being collected < 1 hour before application (HBA).
(Nominal sampling intervals for treated maize whole plant samples: < 1 HAA, 4 HAA, 10 HAA, 24 HAA, 34 HAA, 48 HAA, 72 HAA, and 96 HAA).
Specimens were kept deep frozen at or below -18°C during transport and storage prior to analysis.

Residue analysis
The analytical phase was conducted at the Eurofins Agroscience Services facility located in France, using method GRM007.11A. The Limit of Quantification (LOQ) required was 0.01mg/kg for mesotrione and its metabolite MNBA.

Results

Table A 2: Results of Analysis of Field Trial Samples for Mesotrione

Number and Nominal Rate of Application (g a.s./ha)	Sampling Interval (hours)	Crop Part	Mesotrione Residue (mg/kg)				
			Trial S15-02057-01	Trial S15-02057-03	Trial S15-02057-04	Trial S15-02057-05	Trial S15-02057-06
1 x 150	< 1 HAA	Whole plant	7.09	13.96	4.24	14.99	3.09
1 x 150	4 HAA	Whole plant	8.48	7.75	2.98	12.63	2.74
1 x 150	10 HAA	Whole plant	4.11	6.25	3.33	8.61	2.05
1 x 150	24 HAA	Whole plant	3.86	3.57	1.69	4.30	0.91
1 x 150	34 HAA	Whole plant	2.79	2.95	0.50	2.19	0.80
1 x 150	48-51 HAA	Whole plant	0.92	1.37	0.41	1.07	0.36
1 x 150	72-78 HAA	Whole plant	0.16	0.63	0.14	0.31	< 0.01
1 x 150	96-99 HAA	Whole plant	0.12	0.11	0.06	0.13	0.10
Control	< 1 HBA	Whole plant	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

HBA: hours before application, HAA: hours after application
No correction of results for either control residues or recovery values has been performed

Table A 3: Results of Analysis of Field Trial Samples for MNBA

Number and Nominal Rate of Application (g a.s./ha)	Sampling Interval (hours)	Crop Part	MNBA Residue (mg/kg)				
			Trial S15-02057-01	Trial S15-02057-03	Trial S15-02057-04	Trial S15-02057-05	Trial S15-02057-06
1 x 150	< 1 HAA	Whole plant	0.02	0.05	< 0.01	0.05	0.01
1 x 150	4 HAA	Whole plant	0.10	0.13	0.04	0.25	0.05
1 x 150	10 HAA	Whole plant	0.10	0.22	0.07	0.36	0.09
1 x 150	24 HAA	Whole plant	0.15	0.23	0.07	0.37	0.10
1 x 150	34 HAA	Whole plant	0.15	0.20	0.05	0.37	0.12
1 x 150	48-51 HAA	Whole plant	0.13	0.24	0.06	0.35	0.09
1 x 150	72-78 HAA	Whole plant	0.07	0.17	0.05	0.12	< 0.01
1 x 150	96-99 HAA	Whole plant	0.06	0.10	0.04	0.11	0.08
Control	< 1 HBA	Whole plant	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

HBA: hours before application, HAA: hours after application
No correction of results for either control residues or recovery values has been performed

Conclusions

Residues of mesotrione in treated maize whole plant samples taken at < 1 hour after application (HAA) were in the range 3.09 to 14.99 mg/kg, at 4 HAA were in the range 2.74 to 12.63 mg/kg, at 10 HAA were in the range 2.05 to 8.61 mg/kg, at 24 HAA were in the range 0.91 to 4.30 mg/kg, at 34 HAA were in the range 0.50 to 2.95 mg/kg, at 48-51 HAA were in the range 0.36 to 1.37 mg/kg, at 72-78 HAA were in the range below the limit of quantification (LOQ: 0.01 mg/kg) to 0.63 mg/kg, and at 96-99 HAA were in the range 0.06 to 0.13 mg/kg.

Residues of MNBA in treated maize whole plant samples taken at < 1 hour after application (HAA) were in the range below the limit of quantification (LOQ: 0.01 mg/kg) to 0.05 mg/kg, at 4 HAA were in the range 0.04 to 0.25 mg/kg, at 10 HAA were in the range 0.07 to 0.36 mg/kg, at 24 HAA were in the range 0.07 to 0.37 mg/kg, at 34 HAA were in the range 0.05 to 0.37 mg/kg, at 48-51 HAA were in the range 0.06 to 0.35 mg/kg, at 72-78 HAA were in the range below the limit of quantification (LOQ: 0.01 mg/kg) to 0.17 mg/kg, and at 96-99 HAA were in the range 0.04 to 0.11 mg/kg.

No residues of mesotrione and MNBA were detected at or above the limit of quantification (LOQ: 0.01 mg/kg) in any of the untreated maize whole plant samples taken in this study.

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Comments of zRMS:	The residue trials were performed in various Central Zone countries (UK, Hungary, Germany, Poland and Belgium) as well as in one Southern Zone country (Northern France). However, in opinion of the zRMS, environmental conditions in the Northern France are comparable with conditions of the Central Zone and for this reason results for this trial may be included in the overall analysis. The aim of the study was determination of the decline of the residues of mesotrione on clover, which may be considered as representative species for dicotyledonous weeds consumed by birds and mammals. The study was not performed with A18032E, but with another mesotrione SC formulation (A12738A) containing 480 g mesotrione/L. The application rate (150 g a.s./ha) was higher than the intended rate proposed for A18032E (60 g a.s./L), but in opinion of the zRMS this is not expected to have impact on the residue decline, while for the residue level it would represent worst case. In most of trials the application was made to early growth stages of clover (BBCH 15-18). In two trials the application was performed at BBCH 12-61 or 12-81 (it is not specified in the report at which stage exactly the product was applied). Nevertheless, the study does not need to simulate the growth stages of the target crop (maize), as at the time of application weeds may be at various growth stages. Furthermore, residues in trials performed at later BBCH stages were at level comparable with trials where the product was applied earlier, which gives additional reassurance that the residue decline on clover does not depend on the growth stage. Due to expected rapid decline of mesotrione, intensive sampling was performed during the first days after application, with two samplings performed on the day of application. The sampling schedule gave together 8 data points for each trial, which is sufficient to perform the reliable kinetic analysis. In some trials residues during first 24 hours were quite variable with slightly higher residue levels observed on later samplings. No explanation regarding this issue was provided in the study report. In some trials the variability of residues resulted with poor or unacceptable kinetic. Overall, the study is considered acceptable. The kinetic evaluation of the study was presented in Alvarez (2019) and was considered acceptable. It was noted that kinetic evaluation was performed only using SFO, while

other models could give improvement of fits in most of the trials (although in trial SRFR18-011-037HR potential for improvement is uncertain). Furthermore, improvement could be also possible with outliers removed or with consideration of residues analysed at 8 hours after application as initial residues (in most of trials maximum residues were observed at this sampling point and then decline of mesotrione was observed).

Kinetic fit for one trial performed in Germany (SRDE18-002-037HR) was, in opinion, of the zRMS, unacceptable, which is confirmed by very high Chi^2 (>40%). Nevertheless, there is potential for improvement using other models. Results for this trial were excluded from further considerations, as only unacceptable SFO fit was available.

The Chi^2 error in several trials was >15%, however Chi^2 above 15% is not the reason for rejection of obtained results when the statistical analyses and visual fits are acceptable. As this is the case for trials mentioned and in general, SFO kinetics is preferred to derive DT_{50} values for residue decline trials in plants, consideration of only SFO is accepted by the zRMS. Summary of the derived DT_{50} values is presented in table below:

Trial	DT_{50} [days]	DT_{90} [days]	Remarks
SRUK18-001-037HR	1.49	4.96	Acceptable, good visual fit
SRUK18-002-037HR	3.57	11.9	Acceptable, acceptable visual fit, DT_{90} overestimated by the model
SRHU18-053-037HR	1.99	6.61	Acceptable, acceptable visual fit
SRHU18-054-037HR	2.01	6.68	Acceptable, acceptable visual fit
SRFR18-010-037HR	1.55	5.13	Acceptable, good visual fit, but some potential for improvement
SRFR18-011-037HR	2.57	8.54	Acceptable but poor visual fit, improvement with bi-phasic models uncertain
SRDE18-001-037HR	1.77	5.89	Acceptable, but poor visual fit, clear potential for improvement using bi-phasic models
SRDE18-002-037HR	1.06	3.51	Unacceptable, unacceptable visual fit, Chi^2 >40%, but improvement possible using bi-phasic model
SRPL18-014-037HR	2.41	7.99	Acceptable but poor visual fit, improvement possible using bi-phasic model
SRPL18-015-037-HR	2.64	8.75	Acceptable but poor visual fit, improvement possible using bi-phasic model, DT_{50} overestimated by the model
G006-18H	2.65	8.8	Acceptable, good visual fit
Geometric mean	**2.19**	-	**Results of trial SRDE18-002-037HR excluded from the calculation**

As after exclusion of unacceptable fit results from 10 trials are available, it is proposed by the zRMS that mean DT_{50} value of 2.19 days may be used for purposes of the risk refinement.

The study summary below was provided by the Applicant for A18032E, however for the full summary of the study and kinetic evaluation, please refer to the Core Assessment for formulation Callisto (A12739A) owned by Syngenta, since according to the LoA, ADAMA may refer to results of the study, but does not have access to the full study, so the zRMS cannot include here parts of the reports.

Reference:	KCP 10.1.2.2/11
Report	Allen L. (2019). Mesotrione – Foliage Decline Study on Clover in Hungary, Germany, United Kingdom, Northern France and Belgium in 2018. Report Number CEMR-8397. CEMAS), Imperial House, Oaklands Park, Wokingham, Berkshire, RG41 2FD, UK. Syngenta File No. A12738A_10535
Guideline(s):	Commission of the European Communities, General Recommendations for the Design, Preparation and Realization of Residue Trials; 7029/VI/95 (rev. 5, working document). OECD Guidance Document on Crop Field Trials, Series on Pesticides No. 66 and Series on Testing and Assessment No. 164, ENV/JM/MONO(2011)50. OECD Guidance Document on Overview of Residue Chemistry Studies (as revised 2009), Series on Testing and Assessment (No. 64) and Series on Pesticides (No. 32), ENV/JM/MONO(2009)31. Guidelines and Criteria for the Preparation and Presentation of Complete Dossiers and of Summary Dossiers for the Inclusion of Active Substances in Regulations (EU) 283/2013 and 284/2013 implementing Regulation (EC) 1107/2009. OECD Guidelines for the Testing of Chemicals – Crop Field Trial, No. 509, OECD, Paris 2009. European Commission Guidance for Generating and Reporting Methods of Analysis in Support of Pre-registration Requirements for Annex II (Part A, Section 4) of Directive 91/414, SANCO/3029/99 revision 4 (11 Jul 2000). OECD Guidance Document on Pesticide Residue Analytical Methods, ENV/JM/MONO(2007)17 (Unclassified, 13 Aug 2007). The Application of the OECD Principles of GLP to the Organisation and Management of Multi-Site Studies, ENV/JM/MONO (2002) 9. OECD Series on Principles of GLP and Compliance Monitoring No. 1 (as revised in 1997) "OECD Principles on Good Laboratory Practice", Paris 1998. ENV/MC/CHEM(98)17 and respective national regulations. The national GLP requirements are based on the OECD Principles of Good Laboratory Practice, which are accepted by regulatory authorities throughout the European Community, the United States of America (FDA and EPA) and Japan (MHW, MAFF and METI) on the basis of intergovernmental agreements
Deviations:	No
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	No

Executive Summary

Twelve (10 + 2 contingency) foliar decline residue field trials on clover were planned, eleven (10 + 1 contingency) were successfully conducted in Northern France, Germany, Poland, Hungary, United Kingdom and Belgium during 2018. One trial was cancelled due to poor crop growth.
Mesotrione was applied to clover as A12738A, a suspension concentrate (SC) formulation containing nominal 480 g mesotrione per litre. One application, applied at BBCH 16-18 was made at a nominal rate of 150 g ai/ha for mesotrione, with the exception of trial SRDE18-001-037HR which was applied at BBCH 12-61 and trial SRDE18-002-037HR which was applied at BBCH 16-81. Untreated immature clover samples were taken from the plot at 0 DBA (days before application). Treated samples of immature clover were taken at 0, 8, 24, 32 and 48 HAA (hours after application) and at 3, 4 and 7 DAA (days after application).
Residue samples were shipped frozen to the analytical facility where they were analysed for mesotrione.
Residues of mesotrione in treated clover taken at < 1 hour after application (HAA) were in the range 3.63 to 11.97 mg/kg. At 8 HAA they were in the range 2.71 to 11.41 mg/kg, at 24 HAA in the range 2.28 to 11.02 mg/kg, at 32 HAA in the range 2.06 to 9.02 mg/kg, at 48 HAA they were in the range 1.78 to 7.14 mg/kg, at 3 days after application (DAA) they were in the range 0.22 – 6.06, 5 DAA in the range 0.08 – 4.37 and 7 DAA 0.05 – 1.70 mg/kg.
No residues of mesotrione and MNBA were detected at or above the limit of quantification (LOQ: 0.01 mg/kg) in any of the untreated maize whole plant samples taken in this study.

Materials

Test system	The following test system is representative of the crop group required for product registration. Clover (*Trifolium repens, Trifolium incarnatum, Trifolium alexandrinum)* EPPO Code: TRFRE, TRFIN, TRFAL	
Test Item(s)	Formulation – Company Code	A12738A
	Formulation Content and Type	480 SC
	Batch No.	SAV7B17001
	Valid until:	30 April 2020
	Active ingredient	Mesotrione
	Nominal Content in Formulation (nominal)	480 g/L
	Actual Content in Formulation (actual)	474 g/L
	Stability	The test item is assumed to be stable for the period of use in the study, pending concurrent batch re-analysis
Reference Item	Name	Mesotrione
	Batch No.	492970
	Valid until:	29 Feb 2020
	Purity	99.5%

Study Design and Methods

Eleven field trials on maize were conducted in Central Europe in 2018.
Details of the application of mesotrione as formulation A12738A to clover are summarised in the table below.

Table A 4: Treatment details for Clover Trials

Trial	Applications	Application date(s)	Formulation Code	Growth stage at application (BBCH)	AI application rate (g mesotrione/ha)	
					Actual	Target
SRFR18-010-037HR	1	24 May 2018	A12738A	18	155.2	150
SRFR18-011-037HR	1	9 Jul 2018	A12738A	16-18	148.1	150
SRHU18-053-037HR	1	18 Jun 2018	A12738A	16-18	154.6	150
SRHU18-054-037HR	1	18 Jun 2018	A12738A	16-18	155.1	150
SRUK18-001-037HR	1	9 May 2018	A12738A	16-17	157.5	150
SRUK18-002-037HR	1	6 Jul 2018	A12738A	15-17	139.5	150
SRPL18-014-037HR	1	7 May 2018	A12738A	16-18	154.4	150
SRPL18-015-037HR	1	8 May 2018	A12738A	16-18	151.3	150
SRDE18-001-037HR	1	25 Jun 2018	A12738A	12-61[a]	152.8	150
SRDE18-002-037HR	1	17 Jul 2018	A12738A	12-81[a]	155.5	150
G006-18H	1	18 Jun 2018	A12738A	16-17	149.5	150

[a] Growth stage at application was 12-61 or 12-81 rather than 16-18 as stated in the study plan due to hot, dry weather conditions causing the crop to have a large range of growth stages. Minimal impact was anticipated as the analytical results were comparable to the other trials

There was no rainfall within 48 hours of the application being made at all trial sites.

Selection of samples to be analysed and shipment:

Following the application, treated clover whole plant samples were collected at < 1 hour after application (HAA) , 8 HAA, 24 HAA, 32 HAA, 48 HAA, 3 days after application (DAA), 4 DAA and 7 DAA, with untreated clover whole plant samples being collected < 1 hour before application (HBA).
Specimens were kept deep frozen at or below -18°C during transport and storage prior to analysis.

Residue analysis

The analytical phase was conducted at the CEMAS facility located in the UK, using method GRM007.11A. The Limit of Quantification (LOQ) required was 0.01mg/kg for mesotrione.

Results

Table A 5: Results of Analysis of Field Trial Samples for Mesotrione

Time	Mesotrione residues (mg/kg)										
	SRUK18-001-037HR	**SRUK18-002-037HR**	**SRHU18-053-037HR**	**SRHU18-054-037HR**	**SRFR18-010-037HR**	**SRFR18-011-037HR**	**SRDE18-001-037HR**	**SRDE18-002-037HR**	**SRPL18-014-037HR**	**SRPL18-015-037HR**	**G006-18H**
0	6.10	3.63	11.97	11.69	11.51	8.75	4.46	9.11	6.15	6.50	8.58
8 HAA	6.03	4.20	11.41	8.99	8.78	9.98	5.66	2.71	4.34	4.58	8.48
24 HAA	4.58	3.39	11.02	8.76	9.86	8.73	4.59	2.59	2.28	4.72	8.17
32 HAA	2.69	4.09	9.02	8.80	6.72	4.77	3.98	3.29	2.06	6.37	5.65
48 HAA	2.73	2.61	7.14	6.89	5.47	4.66	4.21	2.54	1.78	5.78	5.54
72 HAA	1.95	2.17	6.06	5.51	2.00	4.77	0.22	2.61	3.82	1.8	3.26
96 HAA	0.58	2.76	0.14	0.12	0.58	4.37	0.08	0.43	1.67	1.66	3.43
168 HAA	0.27	0.19	0.11	0.07	0.09	0.70	0.09	0.05	1.23	0.27	1.70
0DBA	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

DBA: days before application, HAA: hours after application
No correction of results for either control residues or recovery values has been performed

Conclusions

Residues of mesotrione in treated clover taken at < 1 hour after application (HAA) were in the range 3.63 to 11.97 mg/kg. At 8 HAA they were in the range 2.71 to 11.41 mg/kg, at 24 HAA in the range 2.28 to 11.02 mg/kg, at 32 HAA in the range 2.06 to 9.02 mg/kg, at 48 HAA they were in the range 1.78 to 7.14 mg/kg, at 3 days after application (DAA) they were in the range 0.22 – 6.06, 5 DAA in the range 0.08 – 4.37 and 7 DAA 0.05 – 1.70 mg/kg.
No residues of mesotrione were detected at or above the limit of quantification (LOQ: 0.01 mg/kg) in any of the untreated clover whole plant samples taken in this study.

The below studies have already been evaluated and considered adequate during the EU AIR review of mesotrione:

Comments of zRMS:	The study has been already evaluated at the EU level and re-evaluation at the zonal level was deemed not necessary. For the study summary and respective evaluation, please refer to mesotrione RAR of 2015.

Reference:	10.1.2.2
Report	Funkenhaus A, Giessing B (2010) Exposure of mammals in maize fields in France - attractiveness of maize fields and relevant species. RIFCON GmbH, Im Neuenheimer Feld 517. D-69120 Heidelberg, Germany. Syngenta Unpublished Report No. R09012-2. Study No TK0003853 Syngenta file No NA_11991 (Data owned by Syngenta) – EU reviewed study
Guideline(s):	No guidelines available, but following recommendations in the EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009) and its appendices
Deviations:	No
GLP:	Yes
Acceptability:	Already evaluated at the EU level
Duplication (if vertebrate study)	No

~~**Conclusion:**~~

~~Three small mammal species occurred in off crop habitats adjacent to maize fields: the wood mouse (*Apodemus sylvaticus*), the common vole (*Microtus arvalis*) and the greater white-toothed shrew (*Crocidura russula*). Only the wood mouse was found inside maize fields and then only in very small numbers after emergence of maize. In addition to the wood mouse, the European brown hare (*Lepus europaeus*) and the European rabbit (*Oryctolagus cuniculus*) were also observed in maize fields. A full study summary is provided in **Haaf and Alvarez (2017).**~~

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Comments of zRMS:	The study has been already evaluated at the EU level and re-evaluation at the zonal level was deemed not necessary. For the study summary and respective evaluation, please refer to mesotrione RAR of 2015.

Reference:	10.1.2.2
Report	xxxxxxxxxxxxxxx (2005) Generic field monitoring of birds and mammals on maize and beet fields in Austria. Bayer CropScience AG. Unpublished Report No: WFC/FS 017, 20 January 2005. Study dates: 19 March 2004 – 14 December 2004. BCS reference: MO-05-001258 (Owner: Bayer Crop Science, Syngenta have access Syngenta file No: N/1155) – EU reviewed study
Guideline(s):	No guidelines available, but following recommendations in the EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009) and its appendices
Deviations:	No
GLP:	Yes
Acceptability:	Already evaluated at the EU level
Duplication (if vertebrate study)	No

~~**Conclusion:**~~

~~Trapping results confirmed that wood mouse is a focal species. The population densities were relatively low which is normal following winter and prior to the breeding season. The live trapping revealed that the uncultivated plain fields with last year's crop residues was more attractive than these same fields once cultivated and drilled with sugar beet and maize. Wood mice avoided maize fields after drilling and with germinating seedlings (low PT and negative Jacobs Indices). Sugar beet fields were less avoided with higher PT's and Jacobs Indices although the latter were still negative. During telemetry no indication was found that mammals excavated seeds of maize and sugar beet seeds.~~

~~According to the transect counts hares were most abundant in plain fields (0.14 hares/ha) followed by drilled maize fields (0.13/ha), germinated maize (0.12/ha) and sugar beet fields (0.03/ha). Roe Deer were only observed in plain fields and other crops but not maize and sugar beet. In 4 instances, hares were observed feeding on sugar beet seedling. Feeding on maize seedlings was not observed. Roe Deer neither fed on maize or sugar beet seedlings.~~

~~There was evidence of light grazing on germinating maize and sugar beet seedlings but the cause was not determined. The amounts grazed were negligible and neither maize, nor sugar beet seeds, nor their seedlings provided a significant food source for birds and mammals during this study. A full study summary is provided in **Haaf and Alvarez (2017).**~~

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Comments of zRMS:	The study has been already evaluated at the EU level and re-evaluation at the zonal level was deemed not necessary. For the study summary and respective evaluation, please refer to mesotrione RAR of 2015.

Reference:	10.1.2.2
Report	xxxxxxxxxxxxxxxx (2013) Generic field study on small mammals - focal species and wood mouse (*Apodemus sylvaticus*) PT in maize fields in Germany. Rifcon GmbH. Oxon unpublished Report No.: R12225. Syngenta File Number NA_13410 (Data owner: Oxon Italia, S.p.A., Syngenta access) – EU reviewed study
Guideline(s):	No guidelines available, but following recommendations in the EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009) and its appendices
Deviations:	No
GLP:	Yes
Acceptability:	Already evaluated at the EU level
Duplication (if vertebrate study)	No

~~**Conclusion:**~~
~~Three small mammal species occurred on and in the vicinities of maize fields: the wood mouse (*Apodemus sylvaticus*), the bank vole (*Clethrionys glareolus*) and the yellow-necked mouse (*Apodemus flavicollis*). Only the wood mouse was found inside maize fields and only in small numbers.~~
~~Radio tracking data support low portion of diet obtained from treated area for the wood mouse: mean PT ('consumers only' approach) was 0.04 (90th percentile 0.08). A full study summary is provided in ***Haaf and Alvarez (2017)***.~~

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

Comments of zRMS:	The study was conducted in line with OECD 201 with no deviations. All the validity criteria were met. It is noted that the product contains three active substances and in line with the requirements of the Central Zone the test concentrations of all active substances should be verified in the respective chemical analyses or at a minimum the least stable active substance should be analysed. In the present study only the concentration of mesotrione was measured and the analyses of nicosulfuron and dicamba were not carried out. No explanation or justification for the active substance selected for the chemical analysis was provided in the study report. However, information available in area of environmental fate and behaviour of particular active compounds indicates that with mean water DT_{50} of 5.6 days determined in the water/sediment studies, mesotrione is the least stable active substance (mean water DT_{50} of 41 and 65 days was determined for dicamba and nicosulfuron, respectively, in water/sediment systems). Dicamba was stable in EU agreed hydrolysis studies, while nicosulfuron was stable at pH 7 and 9. At pH 5 hydrolytic degradation was observed with DT_{50} determined to be 15 days. In order to further support conclusion on stability of dicamba and nicosulfuron, summaries of the aquatic toxicity studies of these two compounds were consulted in monographs. Dicamba was stable in all acute and chronic aquatic toxicity studies. Nicosulfuron was stable in all acute studies as well as chronic toxicity with algae and majority studies with *Lemna gibba* and single static *Myriophyllum aquaticum* study summarised in this report (Wenzel, 2010), where nicosulfuron was stable over 7 days of exposure. Concentrations of nicosulfuron dropped below 80% in two *Lemna* studies available in the course of EU review (for active compound and formulated product). However, following explanation has been provided by the RMS: *Data on the hydrolytic stability of the active substance, indicate that although stable at pH 7, at the test system pH of 5 significant hydrolysis of the active substance may be expected - this being likely to account for the recorded drop in a.s. concentration 2-3 days after medium renewal.* In the study on toxicity of A18032E to algae pH of the test solutions was >7 over the whole study period and it may be thus concluded that nicosulfuron was stable over the study period. Overall, performed chemical analyses confirmed that mesotrione was stable in study on toxicity of A18032E to algae, while dicamba and nicosulfuron may be concluded to be stable based on the data available in area of the fate and behaviour. Results of the study may be thus based on nominal concentrations of the test item. The study is considered acceptable with following endpoints relevant for the risk assessment: E_rC_{50} = 0.0728 mg/L (0.041 mg sum of a.s./L) E_yC_{50} = 0.0389 mg/L (0.022 mg sum of a.s./L) E_bC_{50} = 0.0374 mg/L (0.021 mg sum of a.s./L)

Reference:	10.2.1
Report	Falk S, 2012, Mesotrione/nicosulfuron/dicamba WG (A18032E) plus Adigor (A12127R) - Testing of Effects on the Single Cell Green Alga *Pseudokirchneriella subcapitata*, Report Number S12-02296, Eurofins Agroscience Services EcoChem GmbH, Eutinger Str.24, 75223 Niefern-Öschelbronn, Germany. (Syngenta File No. A18032E_10002)
Guideline(s):	OECD Guidelines for Testing of Chemicals, Section 2 - Effects on Biotic Systems, Method 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test (2006) Official Journal of the European Communities, Commission Regulation (EC) No 761/2009, Part C.3: Algal inhibition test (2009) JMAFF Agchem Test Guidelines 12 Nohsan No. 8147, Effects on Aquatic Organisms, 2-7-7: Algae growth inhibition studies (2008)
Deviations:	No
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	-

Materials

Test Material	A18032E Mesotrione/dicamba/nicosulfuron WG (15/31.25/10)
Lot/Batch #:	SMU2BP001
Actual content of active ingredients:	Mesotrione: 156 g/kg corresponding to 15.6 % w/w Nicosulfuron: 101 g/kg corresponding to 10.1 % w/w Dicamba: 313 g/kg corresponding to 31.3 % w/w
Description:	Beige granules
Stability of test compound:	Stable under standard conditions
Reanalysis/expiry date:	30 September 2014
Density:	Not applicable
Treatments	
Test concentrations:	Culture medium control and nominal concentrations of 0.0155, 0.0342, 0.0751, 0.165, 0.364 and 0.800 mg A18032E/L
Solvent:	None
Positive control:	Potassium dichromate
Adjutant:	Adigor (A12127R) (mixture of emulsified fatty acid esters on basis of oleic acid methylester). The ratio of A18032E to A12127R was 1: 2.5 (A18032E: A12127R). Results are expressed in terms of the test item containing the adjuvant in this ratio.
Analysis of test concentrations:	Yes, 0 and 96 hours (based on measurements of mesotrione) using HPLC-MS/MS
Test organism	
Species:	*Pseudokirchneriella subcapitata* Hindák, strain SAG 61.81
Source:	Laboratory culture, originally obtained from the Culture Collection of Algae (SAG), Albrecht-von-Haller-Institut, Nikolausberger Weg 18, 37073 Göttingen, Germany
Test design	
Test vessels:	500 mL Erlenmeyer flasks with aluminium caps and two baffles, containing approximately 150 – 200 mL of media
Test medium:	AAP algal medium
Replication:	Six vessels for the control and three vessels for each test concentration. Additionally one replicate per test concentration for measurements was run in parallel.
Starting cell density:	0.5×10^4 cells/mL
Exposure regime:	Static
Aeration:	None during test
Duration:	96 hours

Environmental conditions

Test temperature:	21.8 – 23.9 °C
pH:	test start: 7.33 to 7.45 test end: 7.27 to 8.67
Lighting:	Continuous illumination, 4400 to 5600 Lux

Study Design and Methods

Experimental dates: 16 July 2012 to 27 August 2012

A stock solution was prepared by directly weighing 100 mg of A18032E and 250 mg of A12127R into 1000 mL of test medium. The solutions were homogenised by shaking. The test concentrations were prepared by appropriate dilution of the stock solution. The control consisted of culture medium only.

The test was started by inoculation of 5,000 algal cells per mL of test medium. Test solutions were continuously shaken on a rotating shaker at 105 rpm, and were held under continuous illumination.

Small volumes of all test concentrations and the control were taken from all test flasks after 24, 48, 72 and 96 hours of exposure. The algal cell densities in these samples were determined by fluorescence detection. The morphological appearance of the algal cells was examined microscopically at the end of the test.

The pH was measured at the start and at the end of the test in each test concentration and the control. The water temperature and light intensity was measured daily.

The test concentrations were verified by chemical analysis of mesotrione at 0 and 96 hours, using HPLC-MS/MS.

Results and Discussion

At the start of the test, the analytically determined concentrations of A18032E (based on measurements of the active ingredient mesotrione) were in the range 86 to 118% of the nominal values and at the end of the test were in the range 89 to 107% (see table below). The limit of quantification in this study was 0.00978 mg A18032E/L (corresponding to 0.00153 mg mesotrione/L). As the initial test concentrations were >80%, nominal formulation concentrations were used for the calculation and reporting of results.

Table A 6: Analytical results

Nominal concentrations (mg A18032E/L)	% of nominal measured at 0 hours	% of nominal measured at 96 hours
Control	n.a.	n.a.
0.0155	118	107
0.0342	106	105
0.0751	109	103
0.165	102	100
0.364	86	89
0.800	95	94

n.a. = not applicable

The algal cell densities were measured at 24, 48, 72 and 96 hours and the mean biomass, growth rate and yield calculated. The 72-hour and 96-hour E_rC_{50}, E_bC_{50} and E_yC_{50} values (defined as the concentration resulting in 50% reduction of each parameter) were determined using Probit analysis following the normal, logistic distribution or Gompertz distribution. The NOEC (No Observed Effect Concentration) and LOEC (Lowest Observed Effect Concentration) were determined using a multiple comparison method (Jonckheere-Terpstra or Welch Bonferroni-Holms corrected).

There were no cell abnormalities, observed microscopically, in the control, with cells slightly increased in size up to the highest test item concentration.

Growth rates

The growth rate 0 to 72 hours and 0 to 96 hours were calculated for each replicate culture and the means are shown below, alongside the calculated EC_{50} values.

Table A 7: Mean values at each concentration of A18032E + A12127R for the growth rate at 72 and 96 hours for *Pseudokirchneriella subcapitata* and relevant endpoints

Nominal concentrations (mg A18032E/L)	Mean growth rate (1/day) 0 – 72 hrs	Percentage inhibition	Mean growth rate (1/day) 0 – 96 hrs	Percentage inhibition
Control	1.66906	n.a.	1.56235	n.a.
0.0155	1.75456	-5.1	1.60052	-2.4
0.0342	1.48195	11.2	1.51338	3.1
0.0751	0.79077	52.6	1.09332	30.0
0.165	-0.04005	102.4	0.47119	69.8
0.364	-0.55779	133.4	0.12158	92.2
0.80	n.c.	n.c.	-0.01021	100.7
ErC50 mg A18032E/L	0.0728[1]		0.117[2]	
(95% confidence limits)	0.0654 – 0.0836		0.104 – 0.131	
NOEC mg A18032E/L	0.0342[3]		0.0751[4]	
LOEC mg A18032E/L[4]	0.0751		0.165	

[1] probit procedure, Gompertz
[2] probit procedure, normal distribution
[3] Welch Bonferroni-Holms corrected
[4] Jonckheere-Terpstra
n.a. = not applicable
n.c.: not calculable due to negative cell numbers

Yield

The yield 0 to 72 hours and 0 to 96 hours were calculated for each replicate culture and the means are shown below, alongside the calculated EC_{50} values.

Table A 8: Mean values at each concentration of A18032E + A12127R for the yield at 72 and 96 hours for *Pseudokirchneriella subcapitata* and relevant endpoints

Nominal concentrations (mg A18032E/L)	Mean yield (x 104 cells/mL) 0 – 72 hrs	Percentage inhibition	Mean yield (x 104 cells/mL) 0 – 96 hrs	Percentage inhibition
Control	74.67	n.a.	258.76	n.a.
0.0155	98.30	-31.6	301.89	-16.7
0.0342	46.86	37.2	230.40	11.0
0.0751	4.87	93.5	39.48	84.7
0.165	-0.02	100.0	3.18	98.8
0.364	-0.45	100.6	0.45	99.8
0.80	-0.58	100.8	-0.41	100.2
EyC50 mg A18032E/L	0.0389[1]		0.0535[2]	
(95% confidence limits)	0.0354 – 0.0424		0.0491 – 0.0582	
NOEC mg A18032E/L[3]	0.0342		0.0751	
LOEC mg A18032E/L[3]	0.0751		0.165	

[1] probit procedure, logistic distribution
[2] probit procedure, normal distribution
[3] Jonckheere-Terpstra
n.a. = not applicable

Biomass (area under the growth curve)

The areas under the growth curve for 0 to 72 hours and 0 to 96 hours were calculated for each replicate culture and the means are shown below, alongside the calculated EC_{50} values.

Table A 9: Mean values at each concentration of A18032E + A12127R for the biomass integral (area under the growth curve) at 72 and 96 hours for *Pseudokirchneriella subcapitata* and relevant endpoints

Nominal concentrations (mg A18032E/L)	Mean biomass integral (area, 104*day) 0 – 72 hrs	Percentage inhibition	Mean biomass integral (area, 104*day) 0 – 96 hrs	Percentage inhibition
Control	54.46	n.a.	221.19	n.a.
0.0155	69.05	-26.8	269.15	-21.7
0.0342	32.15	41.0	170.78	22.8
0.0751	3.02	94.5	25.19	88.6
0.165	-0.50	100.9	1.09	99.5
0.364	-1.11	102.0	-1.11	100.5
0.80	-1.62	103.0	-2.11	101.0
EbC50 mg A18032E /L	0.0374[1]		0.0464[2]	
(95% confidence limits)	0.0339 – 0.0409		0.0424 – 0.0506	
NOEC mg A18032E/L[3]	0.0342		0.0342	
LOEC mg A18032E/L[3]	0.0751		0.0751	

[1] probit procedure, logistic distribution
[2] probit procedure, normal distribution
[3] Jonckheere-Terpstra
n.a. = not applicable

Validity criteria

The algal biomass in the control increased by a factor of 150.3 over 72 hours (must be least 16). The mean coefficient of variation of the daily growth rates during 72 and 96 hours in the control cultures were 20 and 22%, respectively (must be ≤ 35%). The coefficient of variation of average specific growth rates in the replicates of the control after 72 and 96 hours were 2.4 and 1.0 %, respectively, (must be <7%). Therefore, all validity criteria were met.

Conclusions

Based on nominal concentrations of A18032E, the 72-hour E_rC_{50} for toxicity to *Pseudokirchneriella subcapitata* was 0.0728 mg A18032E/L, the E_yC_{50} was 0.0389 mg A18032E/L and the E_bC_{50} was 0.0374 mg A18032E/L. The 96-hour E_rC_{50} was 0.117 mg A18032E/L, the E_yC_{50} was 0.0535 mg A18032E/L and the E_bC_{50} was 0.0464 mg A18032E/L.

The LOEC at 72 hours, based on growth rate, yield and biomass integral, was 0.0751 mg A18032E/L. The LOEC at 96 hours based on growth rate and yield was 0.165 mg A18032E/L and based on biomass integral was 0.0751 mg A18032E/L. The NOEC at 72 hours, based on growth rate, yield and biomass integral, was 0.0342 mg A18032E/L. The NOEC at 96 hours based on growth rate and yield, was 0.0751 mg A18032E/L and based on biomass integral was 0.0342 mg A18032E/L.

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Comments of zRMS:	<u>Pulsed-exposure part</u> It should be noted that most of the Central Zone Member States has concerns regarding reliability the modified exposure studies due to uncertainties related to the exposure profiles modelled using FOCUS. Extensive discussion regarding this issue took place during the Central Zone harmonisation meetings and it was concluded that results of Tier 2C studies should be considered only when no acceptable risk may be demonstrated using standard approach (i.e. standard toxicity endpoints and exposure calculated with consideration of the risk mitigation measures). For mesotrione applied as A18032E acceptable risk to aquatic organisms could be concluded using the endpoint required by EFSA, 2013 (i.e. E_rC_{50}) and applying standard

	risk mitigation measures. Taking this into account, the pulsed-exposure part of the summarised below study was not necessary to finalise the risk assessment at the zonal level and in consequence was not evaluated by the zRMS. Standard toxicity part With regard to the standard toxicity part, the design of the study in terms of test conditions and experimental treatment was in line with recommendations of OECD 239. No deviations regarding environmental conditions were observed. It was, however, noted that the number of shoots tested in control and test item groups was not in line with recommendations of OECD 239. According to the test guideline, 6 replicates per control and 4 replicates per test item group with 3 shoots each are recommended, resulting with 18 and 12 plants per control and test item group, respectively. In this study one shoot per replicate was used with 10 replicates per control and 5 replicates per test item group, resulting with 10 and 5 plants per control and test item group, respectively. In general, this deviation could reduce the statistical power of the study. Nevertheless, as clear dose-response relationship could be seen on all parameters measured, it was concluded by the zRMS that results of this study should not be rejected due to deviation mentioned, especially all validity criteria were met. The measured concentrations were analysed in fresh and aged medium at each renewal. Measured concentrations were within 80-120% of nominal, so the endpoints may be based on nominal concentrations. Overall, despite deviation indicated above, the study is considered acceptable with following endpoints corrected for the content of the pure active substance in the test item (84.6%, analysed) and relevant for the risk assessment: lowest 14-d E_rC_{50} = 0.0287 mg a.s./L lowest 14-d E_yC_{50} = 0.00255 mg a.s./L

Reference:	10.2.1
Report	Gonsior G, (2017), Mesotrione – Growth Inhibition of *Myriophyllum spicatum* in a Water/Sediment System. Report Number S16-06273. Eurofins Agroscience Services EcoChem GmbH / Eurofins, Agroscience Services Ecotox GmbH, Eutinger Strasse 24, 75223 Niefern-Öschelbronn, Germany. (Syngenta file no ZA1296_10504)
Guideline(s):	OECD Guidelines for Testing of Chemicals, Section 2 - Effects on Biotic Systems, Method 239: Water-Sediment *Myriophyllum spicatum* Toxicity Test (2014)
Deviations:	Minor (see the commenting box above)
GLP:	Yes
Acceptability:	Standard part of the test: acceptable Pulsed-exposure part: not evaluated as not required for finalisation of risk assessment performed in line with EFSA (2013)
Duplication (if vertebrate study)	No

Materials and methods

Test Material	Mesotrione Technical ZA1296
Lot/Batch #:	765385 SMO0H028
Purity:	84.6 % wt/wt
Description:	Brown powder
Stability of test compound:	Stable under standard conditions
Reanalysis/expiry date:	28 February 2019

Density:	Not applicable
Treatments	
Test concentrations:	Toxicity test: Dilution water control; nominal concentration of 4.77, 15.3, 48.8, 156 and 500 µg mesotrione tech./L ~~Pulse dose test 1: 120 µg mesotrione tech./L~~ ~~Pulse dose test 2: 70.0 µg mesotrione tech./L~~
Solvent:	None
Analysis of test concentrations:	Yes, analysis of mesotrione in overlying water at the start, day 4 (aged and fresh), day 8 (aged and fresh), day 11 (aged and fresh) and at day 14 (aged) in the toxicity test ~~and at test start, day 1 (aged and fresh) and day 4 (aged and fresh) in pulse dose tests~~, using HPLC-MS/MS
Test organisms	
Species:	*Myriophyllum spicatum* L.
Source:	In-house cultures, originally obtained from the Federal Environment Agency Berlin, Germany
Test design	
Test vessels:	300 mL glass vessels (9 cm diameter, 5 cm height) placed in 2 L glass-beakers (12 cm diameter, 24 cm height) containing approximately 350 g moist sediment and 1.5 L growth medium
Test medium:	SMART AND BARKO growth medium
Replication:	Toxicity test: five replicates for each test concentration and ten for the control ~~Pulse dose tests: ten replicates for each test concentration and ten for the control~~
Number of shoots per vessel:	1 rooted apical shoot
Exposure regime:	Semi-static
Duration:	14 days
Environmental conditions	
Temperature:	Toxicity test: 19.2 ± 1.0 °C (18.0 – 21.7 °C) ~~Pulse dose test 1: 19.7 ± 1.2 °C (18.0 – 21.9 °C)~~ ~~Pulse dose test 2: 19.5 ± 1.1 °C (18.0 – 21.6 °C)~~
pH:	Toxicity test: 7.93 ± 0.47 (7.48 – 9.84) ~~Pulse dose test 1: 8.25 ± 0.72 (7.55 – 9.83)~~ ~~Pulse dose test 2: 8.23 ± 0.71 (7.55 – 9.90)~~
Dissolved oxygen:	Toxicity test: 100 ± 14 % (78 – 156 %) ~~Pulse dose test 1: 116 ± 20 % (96 – 171 %)~~ ~~Pulse dose test 2: 114 ± 19 % (94 – 172 %)~~
Lighting:	16 hour day length, approximately 120 – 160 µE m^{-2} s^{-1}

Study Design and Methods

Experimental dates: 03 November 2016 to 02 December 2016

A semi-static toxicity test ~~and two single 24-hour pulse dose tests~~ was performed. A stock solution with a nominal concentration of 500 µg mesotrione tech./L was prepared by adding the required amount of the test item to a volumetric flask and adding test medium up to the benchmark. The solution was homogenised by shaking and ultrasonication for 4 hours. Appropriate volumes of the stock solution and preceding test solutions were diluted to give the test concentrations. The control consisted of test medium only.
Three days before the start of the test, approximately 350 g of moist sediment was transferred to the test vessels. The surface was overlaid with moist sediment without ammonium chloride and sodium phosphate and a thin layer of washed quartz sand to minimise displacement of the sediment when the medium was added. The test vessels were placed in 2 L glass beakers and filled carefully with 1.5 L of

growth medium to a depth of 14 cm. On the day of the test, one rooted apical shoot per vessel was planted carefully, ensuring the plant was rooted into the sediment. Only plants of the same size (e.g. ± 10 – 20 % of mean shoot length) were used for the test. The test item solution was then added and mixed in with gentle stirring. The test solution renewal was performed at day 4, 8 and 11 in the toxicity test ~~and at day 1 and 4 in the pulse dose tests~~. The test vessels were maintained in a controlled environment under the conditions indicated above.
Assessments of plant growth were made on days 0, 7 and 14. Plants were harvested for measurement of biomass (plant fresh weight and plant dry weight), shoot length and number and length of side shoots on day 14, and observations on shoot and root development (e.g. necrosis, deformation) were documented. The initial biomass (plant fresh weight and plant dry weight) and shoot length were determined using a sample of 15 additional plants, representative of those used in the test.
Water temperature, pH and dissolved oxygen saturation were recorded on days 0, 4 (aged and fresh), 8 (aged and fresh), 11 (aged and fresh) and 14 (aged) in the toxicity test and on days 0, 1 (aged and fresh), 4 (aged and fresh), 8 (aged and fresh), 11 (aged and fresh) and 14 (aged) in the pulse dose tests. Light intensity on the water surface was measured at test start.
The test concentrations were verified by analyses of mesotrione at all concentration levels by analysing the overlaying water at test start, day 4 (aged and fresh), 8 (aged and fresh), 11 (aged and fresh) and 14 (aged) in the toxicity test ~~and at test start, at day 1 (aged and fresh) and 4 (aged and fresh) in the pulse dose tests~~, using high performance liquid chromatography with tandem mass spectrometry.

Results and Discussion
The concentrations of the test item in the freshly prepared solutions were found to be in the range 83 to 110 % of the nominal values and in the aged solutions in the range 87 to 108 %.

~~At the start of the pulse dose tests, the concentrations of the test item in the freshly prepared solutions were found to be in the range 103 to 109 % of the nominal values and after 1 day were 98-99% of nominal; at day 4 of the test concentrations in the aged solution were 1 % of the nominal values (see table below).~~

The limit of quantification in this study was 0.4 µg mesotrione/L (in water) corresponding to 0.473 µg mesotrione tech./L. Since all concentrations were within 20% of nominal, the latter were used for the calculation and reporting of all results.

Table A 10: Analytical results

Test	Nominal concentrations		% of nominal mesotrione concentration measured in overlaying water									
	(µg mesotrione tech./L)	(µg mesotrione/L)	Day 0	Day 1		Day 4		Day 8		Day 11		Day 14
			fresh	aged	fresh	aged	fresh	aged	fresh	aged	fresh	aged
Toxicity test	Control	0	-	n.a.	n.a.	-	-	-	-	-	-	-
	4.77	4.04	102	n.a.	n.a.	108	91	101	83	87	84	91
	15.3	12.9	97	n.a.	n.a.	102	95	98	84	87	95	95
	48.8	41.3	99	n.a.	n.a.	100	93	95	86	88	94	96
	156	132	105	n.a.	n.a.	98	94	96	90	92	98	102
	500	423	108	n.a.	n.a.	101	96	100	98	99	110	105
Pulse dose test	Control	0	-	-	-	-	-	n.a.	n.a.	n.a.	n.a.	n.a.
	70.0	59.2	109	99	-	1	-	n.a.	n.a.	n.a.	n.a.	n.a.
	120	102	103	98	-	1	-	n.a.	n.a.	n.a.	n.a.	n.a.

- Not detectable
n.a. – not analysed

Biological Results 'Toxicity Test'

Data for total shoot length and biomass was used to calculate growth rates and yield for the control and each exposure concentration. Non-linear analysis was used to calculate the 14-day $E_rC_{10, 20, 50}$ and $E_yC_{10, 20, 50}$. For the No Observed Effect Concentration and Lowest Observed Effect Concentration, all data were subjected to ANOVA. Normality was tested using Shapiro-Wilk's test and homogeneity of variances across treatment groups were tested using a Bartlett's or Levene's test. Normally distributed and homogeneous data were analysed using a Dunnett's test and a Bonferroni-U Exact Test was used to analyse non-normal distribution data to determine significant differences from controls.

Mean total shoot length are presented below along with the growth rate, yield and respective inhibition values, alongside calculated $EC_{10, 20, 50}$ values:

Table A 11: Effect of mesotrione technical on growth rate and yield (mean total shoot length) of *Myriophyllum spicatum* in the 'toxicity test'

Test type	Nominal concentration (µg mesotrione tech./L)	Mean total shoot length (cm)		Based on mean total shoot length (0-14 days)			
		Day 0[1]	Day 14	Growth Rate (1/day)	Reduction of Growth Rate (%)	Yield (cm)	Reduction of Yield (%)
Toxicity test	Control	5.6	44.0	0.1466	-	38.44	-
	4.77	5.6	23.2	0.1013*	30.9*	17.6*	54.2*
	15.3	5.6	20.5	0.0915*	37.6*	14.9*	61.2*
	48.8	5.6	12.5	0.0562*	61.7*	6.9*	82.0*
	156	5.6	12.1	0.0540*	63.2*	6.5*	83.1*
	500	5.6	10.4	0.0429*	70.7*	4.8*	87.5*
EC_{10} µg mesotrione tech./L[2]				0.149		(-)	
95 % confidence limits				0.024 – 0.930		(-)	
EC_{20} µg mesotrione tech./L[2]				0.958		(-)	
95 % confidence limits				0.164 – 5.78		(-)	
EC_{50} µg mesotrione tech./L[2]				33.9		3.01	
95 % confidence limits				3.69 – 294		0.117 – 90.3	
NOEC				n.d.		n.d.	
LOEC				4.77		4.77	

[1] Based on 15 additional plants, representative of those used in the test
[2] Calculation based on 3-param. Normal CDF (cumulative distribution function)
(-) Values not reliable, control CV exceeded the effect level
* Significantly different reduction compared to the control
n.d.- not detectable; no NOEC could be determined

Mean fresh weights are presented below along with the growth rate, yield and respective inhibition values, alongside calculated $EC_{10, 20, 50}$ values:

Table A 12: Effect of mesotrione technical on growth rate and yield (mean fresh weight) of *Myriophyllum spicatum* in the 'toxicity test'

Test	Nominal concentration (µg mesotrione tech./L)	Mean fresh weight (g)		Based on mean fresh weight (0-14 days)			
		Day 0[1]	Day 14	Growth Rate (1/day)	Reduction of Growth Rate (%)	Yield (g)	Reduction of Yield (%)
Toxicity test	Control	0.1069	1.1573	0.1685	-	1.0504	-
	4.77	0.1069	0.7195	0.1357*	19.5*	0.6126*	41.7*
	15.3	0.1069	0.5317	0.1140*	32.3*	0.4248*	59.6*
	48.8	0.1069	0.3545	0.0847*	49.7*	0.2476*	76.4*
	156	0.1069	0.3261	0.0769*	54.4*	0.2192*	79.1*
	500	0.1069	0.2896	0.0709*	57.9*	0.1827*	82.6*
EC_{10} µg mesotrione tech./L[2]				0.300		(-)	
95 % confidence limits				0.044 – 2.03		(-)	
EC_{20} µg mesotrione tech./L[2]				2.26		(-)	
95 % confidence limits				0.341 – 15.3		(-)	
EC_{50} µg mesotrione tech./L[2]				108		6.90	
95 % confidence limits				8.97 - 1174		0.267 - 200	
NOEC				n.d.		n.d.	
LOEC				4.77		4.77	

[1] Based on 15 additional plants, representative of those used in the test
[2] Calculation based on 3-param. Normal CDF (cumulative distribution function)
(-) Values not reliable, control CV exceeded the effect level
* Significantly different reduction compared to the control
n.d.- not detectable; no NOEC could be determined

Mean dry weights are presented below along with the growth rate, yield and respective inhibition values, alongside calculated $EC_{10, 20, 50}$ values:

Table A 13: Effect of mesotrione technical on growth rate and yield (dry weight) of *Myriophyllum spicatum* in the toxicity test

Test	Nominal concentration (µg mesotrione tech./L)	Mean dry weight (g)		Based on mean dry weight (0-14 days)			
		Day 0[1]	Day 14	Growth Rate (1/day)	Reduction of Growth Rate (%)	Yield (g)	Reduction of Yield (%)
Toxicity test	Control	0.0116	0.0740	0.1311	-	0.0624*	-
	4.77	0.0116	0.0439	0.0943*	28.1*	0.0323*	48.2*
	15.3	0.0116	0.0415	0.0900*	31.4*	0.0299*	52.1*
	48.8	0.0116	0.0243	0.0509*	61.2*	0.0127*	79.6*
	156	0.0116	0.0278	0.0595*	54.6*	0.0162*	74.0*
	500	0.0116	0.0208	0.0412*	68.6*	0.0092*	85.3*
EC_{10} µg mesotrione tech./L[2]				(-)		(-)	
95 % confidence limits				(-)		(-)	
EC_{20} µg mesotrione tech./L[2]				1.42		(-)	
95 % confidence limits				0.124 – 17.1		(-)	
EC_{50} µg mesotrione tech./L[2]				53.3		5.81	
95 % confidence limits				2.37 - 1087		0.067 - 533	
NOEC				n.d.		n.d.	
LOEC				4.77		4.77	

[1] Based on 15 additional plants, representative of those used in the test
[2] Calculation based on 3-param. Normal CDF (cumulative distribution function)
(-) Values not reliable, control CV exceeded the effect level
* Significantly different reduction compared to the control

n.d - no NOEC could be determined

In the toxicity test, visible effects of the test material on shoot development were observed after 7 days at 48.8 µg mesotrione tech./L and 14 days at 15.3 µg mesotrione tech./L and above.

Biological Results 'Pulse Dose Tests'

Following exposure to Mesotrione Technical for 24 hours in a pulse dose design, no significant differences from the controls were seen in either the shoot length nor biomass (fresh weight and dry weight) results for either of the concentrations tested, as indicated in the tables below.

Table A 14: Effect of mesotrione technical on growth rate and yield (mean total shoot length and shoot fresh weight) of *Myriophyllum spicatum* in the 'pulsed dose test'

Nominal conc. for 24h pulse [µg/L]	Total shoot length after 14 days				Shoot fresh weight after 14 days			
	yield [g]	reduction in yield [%]	growth rate [1/day]	reduction in growth rate [%]	yield [g]	reduction in yield [%]	growth rate [1/day]	reduction in growth rate [%]
Control	42.6	-	0.1532	-	1.2139	-	0.1789	-
70.0	40.5	4.9 n.s.	0.1502	2.0 n.s.	1.1425	5.9 n.s.	0.1749	2.2 n.s.
120	41.4	2.8 n.s.	0.1517	1.0 n.s.	1.3112	-8 n.s.	0.1843	-3 n.s.

n.s. = not significantly different from control

Table A 15: Effect of mesotrione technical on growth rate and yield (mean total shoot dry weight) of *Myriophyllum spicatum* in the 'pulsed dose test'

Nominal conc. for 24h pulse [µg/L]	Shoot dry weight after 14 days			
	yield [g]	reduction in yield [%]	growth rate [1/day]	reduction in growth rate [%]
Control	0.0748	-	0.1429	-
70.0	0.0764	-2.1 n.s.	0.1440	-0.8 n.s.
120	0.0831	-11.1 n.s.	0.1497	-4.8 n.s.

n.s. = not significantly different from control

In the pulse dose tests, no visible effects of the test material on shoot development were apparent after 7 and 14 days.

Validity

Control plants had no visual symptoms of chlorosis and were visibly free from contamination by other organisms such as algae and/or bacterial films on the plants, at the surface of the sediment and in the aqueous growth medium. Since the coefficient of variations (CV) for fresh weight and shoot length yield were below 35 % (actual: 24.3 and 16.0 %, respectively, in the toxicity test and 15.5 and 14.1 %, respectively, in the pulse dose tests) and a doubling of shoot biomass and length was reached within the test duration (actual: > 6-fold), the mean control growth rates and variability were considered acceptable.

Conclusions

Based on nominal concentrations, the 14-day EC_{50} values for growth rate (E_rC_{50}) and yield (E_yC_{50}) for mesotrione technical to *Myriophyllum spicatum* were 33.9 and 3.01 µg mesotrione tech./L, respectively, based on total shoot length. The E_rC_{50} and E_yC_{50} values based on biomass (fresh weight) were 108 and 6.90 µg mesotrione tech./L, respectively, and were 53.3 and 5.81 µg mesotrione tech./L, respectively, based on biomass (dry weight). The 14-day NOEC for growth rate and yield based on total shoot length and biomass could not be determined. The 14-day LOEC for growth rate and yield was 4.77 µg mesotrione tech./L, based on total shoot length and biomass (fresh weight and dry weight).

~~No significant effects were observed due to a 24 hour pulse of exposure at rates up to and including 120.0 µg mesotrione tech./L.~~

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Comments of zRMS:	The study was conducted in line with OECD 221 with no deviations. The mean measured concentrations of mesotrione were maintained within 80-120% of nominal. All the validity criteria were met: - The doubling time of frond number in the control was < 2.5 days (observed 1.6 days) The recovery phase was not summarised by the Applicant below and was also not presented by the zRMS as not relevant for the risk assessment purposes. Overall, the study is considered acceptable with the following endpoints corrected for the content of the pure active substance in the test item (86.16%, analysed) and relevant for the risk assessment: lowest 7-d E_rC_{50} = 0.0241 mg a.s./L lowest 7-d E_yC_{50} = 0.0045 mg a.s./L

Reference:	10.2.1
Report	Hengsberger A. & Wydra V. (2015), Mesotrione Wet Paste (ZA1296) - Toxicity to the Aquatic Plant *Lemna gibba* in a Semi-Static Growth Inhibition Test with a Subsequent Recovery Period. Report Number 105732240. ibacon GmbH Arheilger Weg 17 64380 Rossdorf Germany. Syngenta file no ZA1296_10438).
Guideline(s):	OECD Guidelines 221: *Lemna* sp. Growth Inhibition Test (2006) US EPA Ecological Effects Test Guidelines, OPPTS 850.4400: Aquatic Plant Toxicity using *Lemna* spp., Tiers I and II, (1996)
Deviations:	No
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	-

Materials

Test Material	ZA1296 Mesotrione Wet Paste
Actual content of active ingredients:	631795 (SMO7F333)
Purity:	86.1% (wt/wt)
Description:	Brown solid
Stability of test compound:	Stable under standard conditions
Reanalysis/expiry date:	End of February 2016
Density:	n/a
Treatments	
Test concentrations:	Dilution water control; nominal concentration of 64, 32, 16, 8, 4 and 2 µg test item/L
Solvent:	None
Vehicle and/or positive control:	Potassium dichromate is used as a positive control at least twice a year.

Analysis of test concentrations:	Yes, analysis on days 0, 3 (fresh and aged), 5 (fresh and aged) and 7
Test organisms	
Species:	*Lemna gibba*
Source:	In-house cultures
Test design	
Test vessels:	250 mL glass flasks filled with 200 mL test medium covered with glass dishes
Test medium:	20X AAP-Growth Medium
Replication:	Three vessels for the control and each test concentration
Initial frond number:	4 fronds per plant, total 12 fronds per replicate
Exposure regime:	Semi-static (renewal at days 3 and 5)
Duration:	7 days
Environmental conditions	
Temperature:	23 - 24 °C
pH:	Fresh media:7.5 – 7.9 Aged media: 8.5 – 9.0
Lighting:	Continuous illumination, 7300 - 7770 Lux (mean 7467 Lux) (differences in light intensity over the test area did not exceed ± 15 %.) .

Study Design and Methods

Experimental dates: 21 August to 28 October 2015

Before test start and before the test medium renewal a concentrated stock solution was prepared by dissolving 10.2, 10.0 and 10.3 mg test item in 1020, 1000 and 1030 mL test water, respectively. The stock solution was intensively stirred for 40 minutes and short ultrasonic treatment was used for 15 minutes. Then, adequate volumes were mixed into test water to obtain the desired test concentrations. Appropriate volumes of the stock solution were diluted to give the test concentration series. The control consisted of culture medium only.

200 mL of the test solutions were transferred into 250 mL glass flasks and inoculated with *Lemna* plants. Cultures were then transferred to a temperature-controlled room where they were maintained under the conditions indicated above.

Assessments of frond number were made on days 0, 3, 5 and 7. Fronds were harvested for measurement of dry weight after 7 days, and the initial dry weight was determined using a sample of 12 fronds identical to that used to inoculate the test.

Temperature was measured continuously, light intensity was recorded once at test start and pH was recorded on days 0, 3, 5 and 7 days.

The test concentrations were verified by chemical analysis of ZA1296 at days 0 and 7, using high performance liquid chromatography with ultra violet-visible detection.

Results and Discussion

At the start of the test, the concentrations of the test item were found to be in the range 93 to 107 % of the nominal values and at the end of the test were in the range 87 to 122 % (see table below). At the start of the test and in the freshly prepared test media at renewal of the test media on day 3 and 5, 103% of the nominal test concentration was found (average of all test concentrations). After 72 and 48 hours test duration on day 3, 5 and 7 (test end), 98% of the nominal value was determined (average of all test concentrations) in the aged test media. During the test the *Lemna* were exposed to a mean of 101% of nominal. Nominal concentrations were used for the calculation and reporting of results.

Table A 16: Analytical results

Nominal concentrations (µg test item ~~a.s.~~/L)	% of nominal measured at 0 days	% of nominal measured at 3 days (aged)	% of nominal measured at 3 days (fresh)	% of nominal measured at 5 days (aged)	% of nominal measured at 5 days (fresh)	% of nominal measured at 7 days
2	107	100	110	101	110	103
4	119	108	110	109	113	122
8	97	97	113	108	102	103
16	97	92	99	94	100	87
32	93	88	97	93	95	94
64	101	91	102	95	98	91

Data for frond number and dry weight was used to calculate growth rates and yield for the control and each exposure concentration. Non-linear regression was used to calculate the 7-day ErC_{50} and EyC_{50}, based on percent inhibition relative to the control. For the No Observed Effect Concentration and Lowest Observed Effect Concentration, a Williams test was used to determine values significantly different to the control.
Mean frond numbers are presented below along with the growth rate, yield and respective inhibition values, alongside estimated EC_{50} values:

Table A 17: Effect of ZA1296 on growth rate and yield (frond number) of *Lemna gibba*

Nominal concentration (µg/L) [1)]	Mean No. fronds/replicate (day 7)	Based on Frond Number (0-7 days)			
		Growth Rate	Inhibition of Growth Rate (%)	Yield	Inhibition of Yield (%)
Control	250.0	0.434	-	238.0	-
2	246.3	0.431	0.6	234.3	1.5
4	138.7	0.349*	19.6	126.7*	46.8
8	107.0	0.312*	27.9	95.0*	60.1
16	66.0	0.243*	43.9	54.0*	77.3
32	49.0	0.200*	53.8	37.0*	84.5
64	43.7	0.184*	57.5~~7~~	31.7*	86.7
EC_{50} µg/L		28		6.0	
95% confidence limits		20 - 37		4.3 – 8.4	
NOEC		2.0		2.0	
LOEC		4.0		4.0	

Inoculum = 12 fronds
[1)] Given as the test item not corrected for purity
* mean value significantly different from the control (tested with Williams Test, $\alpha = 0.05$, one-sided)

Mean dry weights are presented below along with the growth rate, yield and respective inhibition values, alongside estimated EC_{50} values:

Table A 18: Effect of ZA1296 on growth rate and yield (dry weight) of *Lemna gibba*

Nominal concentration (µg/L) [1)]	Dry Weight (mg) (day 7)	Based on Dry Weight (0-7 days)			
		Growth Rate	Inhibition of Growth Rate (%)	Yield	Inhibition of Yield (%)
Control	32.8	0.440	-	31.3	-
2	32.5	0.439	0.3	31.0	0.7
4	16.9	0.345*	21.7	15.4*	50.9
8	11.2	0.287*	34.8	9.7*	69.0
16	8.0	0.238*	45.9	6.5*	79.2
32	7.5	0.230*	47.8	6.0*	80.8
64	5.6	0.188*	57.3	4.1*	86.9
EC_{50} µg/L		28		5.2	
95% confidence limits		19 - 42		3.5 – 7.7	
NOEC		2.0		2.0	
LOEC		4.0		4.0	

Inoculum = 1.5 mg dry weight per vessel
[1)] Given as the test item not corrected for purity
* mean value significantly different from the control (tested with Williams Test, $\alpha = 0.05$, one-sided)

Conclusions

For frond number, the 7-day EC_{50} for yield (EyC_{50}) and growth rate (ErC_{50}) for ZA1296 to *Lemna gibba* were 6.0 and 28 µg test item ~~a.s.~~/L respectively, based on nominal concentrations.
For dry weight, the 7-day EC_{50} for yield (EyC_{50}) and growth rate (ErC_{50}) for ZA1296 were 5.2 and 28 µg test item ~~a.s.~~/L respectively, based on nominal concentrations.
The 7-day NOEC was determined to be 2.0 µg/L and the 7-day LOEC was determined to be 4.0 µg test item/L

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Comments of zRMS:	It should be noted that most of the Central Zone Member States has concerns regarding reliability of the modified exposure studies due to uncertainties related to the exposure profiles modelled using FOCUS. Extensive discussion regarding this issue took place during the Central Zone harmonisation meetings and it was concluded that results of Tier 2C studies should be considered only when no acceptable risk may be demonstrated using standard approach (i.e. standard toxicity endpoints and exposure calculated with consideration of the risk mitigation measures). For mesotrione applied as A18032E acceptable risk to aquatic organisms could be concluded using the endpoint required by EFSA, 2013 (i.e. E_rC_{50}) and applying standard risk mitigation measures. Taking this into account, the summarised below Tier 2C study was not necessary to finalise the risk assessment at the zonal level and in consequence was not evaluated by the zRMS.

Reference:	10.2.1
Report	Hengsberger A. and Wydra V. (2015a), Mesotrione Wet Paste (ZA1296) - Toxicity to the Aquatic Plant *Lemna gibba* in a Reciprocal Growth Inhibition Test. Report Number 105731240. ibacon GmbH Arheilger Weg 17 64380 Rossdorf Germany. (Syngenta file no ZA1296_10436).
Guideline(s):	OECD Guidelines 221: *Lemna* sp. Growth Inhibition Test (2006) US EPA Ecological Effects Test Guidelines, OPPTS 850.4400: Aquatic Plant Toxicity using *Lemna* spp., Tiers I and II, (1996)
Deviations:	No
GLP:	Yes

Acceptability:	Not evaluated, not required for finalisation of risk assessment performed in line with EFSA (2013)
Duplication (if vertebrate study)	-

Materials

Test Material	ZA1296 Mesotrione Wet Paste
Batch no.:	631795 (SMO7F333)
Purity:	86.1 % (wt/wt)
Description:	Brown solid
Stability of test compound:	Stable under standard conditions
Reanalysis/expiry date:	End of February 2016
Density:	n/a
Treatments	
Test concentrations:	Dilution water control; nominal concentrations of 20, 30 and 60 µg a.s./L
Solvent:	None
Vehicle and/or positive control:	Potassium dichromate is used as a positive control at least twice a year.
Analysis of test concentrations:	Yes, analysis on days 0, 3 and 5
Test organisms	
Species:	*Lemna gibba*
Source:	In-house cultures
Test design	
Test vessels:	250 mL glass flasks with approximately 200 mL test medium, covered with glass dishes.
Test medium:	20X AAP Growth Medium
Replication:	Three vessels for the control and each test concentration
Initial frond number:	4 fronds per plant, total 12 fronds per replicate
Exposure regime:	Semi static: test water renewal on day 5
Duration:	7 days
Environmental conditions	
Temperature:	22 – 26 °C
pH:	7.4 – 7.6 at test initiation; 8.5 – 8.7 at test termination
Lighting:	Continuous illumination, 7020 – 7900 Lux (mean 7462 Lux).

Study Design and Methods

Experimental dates: 17 August to 29 October 2015

A stock solution was prepared by dissolving 20.2 mg test item into 1010 mL test water. Appropriate volumes of the stock solution were diluted to give the test concentration series. The control consisted of culture medium only.

200 mL of the test solutions were transferred into 250 mL glass flasks and inoculated with *Lemna* plants. Cultures were then transferred to a temperature-controlled room where they were maintained under the conditions indicated above.

Assessments of frond number were made on days 0, 1, 2, 3, 5 and 7. Fronds were harvested for measurement of dry weight after 7 days, and the initial dry weight was determined using a sample of 12 fronds identical to that used to inoculate the test.

Temperature was measured continuously, light intensity was recorded once at test start and pH was recorded on days 0, 1, 2, 3, 5 and 7 days.

The test concentrations were verified by chemical analysis on days 0, 1, 2, 3 and 5 where relevant, using LC-MS/MS.

Results and Discussion

The test concentrations were maintained within 20% of nominal for each of the three exposure periods. Nominal concentrations were used for the calculation and reporting of results.

Table A 19: Analytical results

Nominal concentrations (µg a.s./L)	% of nominal measured on day 0	% of nominal measured on day 1	% of nominal measured on day 2	% of nominal measured on day 3	% of nominal measured on day 5
20 x 72h	94	-	-	82	< LOD
30 x 48h	92	-	101	-	<LOD
60 x 24h	106	91	-	-	<LOD

For the determination of the LOE_rC and NOE_rC values significant differences at the test concentrations compared to the control values were tested by the Williams test (frond number and dry weight).For the determination of the LOEyC and NOEyC values, significant differences at the test concentrations compared to the control values were tested by the Williams test (frond number and dry weight).
The software used to perform the statistical analysis was ToxRat Professional, Version 3.2.1, ToxRat® Solutions GmbH.
Mean frond numbers are presented below along with the growth rate, yield and respective inhibition values, alongside estimated EC_{50} values:

Table A 20: Effect of ZA1296 on growth rate and yield (frond number) of *Lemna gibba*

Nominal concentration (µg/L)	Mean No. fronds/replicate (day 7)	Based on Frond Number (0-7 days)			
		Growth Rate	Inhibition of Growth Rate (%)	Yield	Inhibition of Yield (%)
Control	257.0	0.437	-	245.0	-
20 x 72h	120.0	0.329*	24.9	108.0*	55.9
30 x 48h	150.0	0.360*	17.7	138.0*	43.7
60 x 24h	185.3	0.391*	10.6	173.3*	29.3
NOEC		<20		<20	
LOEC		20		20	

Inoculum = 12 fronds
* mean value significantly different from the control (tested with Williams Test, α = 0.05, one-sided)

Mean dry weights are presented below along with the growth rate, yield and respective inhibition values, alongside estimated EC_{50} values:

Table A 21: Effect of ZA1296 on growth rate and yield (dry weight) of *Lemna gibba*

Nominal concentration (µg/L)	Dry Weight (mg) (day 7)	Based on Dry Weight (0-7 days)			
		Growth Rate	Inhibition of Growth Rate (%)	Yield	Inhibition of Yield (%)
Control	0.487	0.487	-	32.2	-
20 x 72h	0.345	0.345*	29.1	11.2*	65.1
30 x 48h	0.382	0.382*	21.6	14.9*	53.6
60 x 24h	0.418	0.418*	14.1	19.4*	39.8
NOEC		<20		<20	
LOEC		20		20	

* mean value significantly different from the control (tested with Williams Test, α = 0.05, one-sided)

~~**Conclusion**~~

~~The influence of Mesotrione Wet Paste (ZA1296) on the growth of the freshwater plant *Lemna gibba* was assessed in a reciprocal growth inhibition test.~~

~~All treatments had significant effects compared to the control for all endpoints. The effects increased with increasing exposure time and decreasing concentration.~~

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Comments of zRMS:	The study was conducted in line with OECD 202 with no deviations. All the validity criteria were met. It is noted that the product contains three active substances and in line with the requirements of the Central Zone the test concentrations of all active substances should be verified in the respective chemical analyses or at a minimum the least stable active substance should be analysed. In the present study only the concentration of mesotrione was measured and the analyses of nicosulfuron and dicamba were not carried out. No explanation or justification for the active substance selected for the chemical analysis was provided in the study report. However, information available in area of environmental fate and behaviour of particular active compounds indicates that with mean water DT_{50} of 5.6 days determined in the water/sediment studies, mesotrione is the least stable active substance (mean water DT_{50} of 41 and 65 days was determined for dicamba and nicosulfuron, respectively, in water/sediment systems). Dicamba was stable in EU agreed hydrolysis studies, while nicosulfuron was stable at pH 7 and 9. At pH 5 hydrolytic degradation was observed with DT_{50} determined to be 15 days. In order to further support conclusion on stability of dicamba and nicosulfuron, summaries of the aquatic toxicity studies of these two compounds were consulted in monographs. Dicamba was stable in all acute and chronic aquatic toxicity studies. Nicosulfuron was stable in all acute studies as well as chronic toxicity with algae and majority studies with *Lemna gibba* and single static *Myriophyllum aquaticum* study summarised in this report (Wenzel, 2010), where nicosulfuron was stable over 7 days of exposure. Concentrations of nicosulfuron dropped below 80% in two *Lemna* studies available in the course of EU review (for active compound and formulated product). However, following explanation has been provided by the RMS: *Data on the hydrolytic stability of the active substance, indicate that although stable at pH 7, at the test system pH of 5 significant hydrolysis of the active substance may be expected - this being likely to account for the recorded drop in a.s. concentration 2-3 days after medium renewal.* In the study on toxicity of A18032E to *Daphnia magna* pH of the test solutions was >7 over the whole study period and it may be thus concluded that nicosulfuron was stable over the study period. Overall, performed chemical analyses confirmed that mesotrione was stable in study on toxicity of A18032E to *Daphnia magna*, while dicamba and nicosulfuron may be concluded to be stable based on the data available in area of the fate and behaviour. Results of the study may be thus based on nominal concentrations of the test item. The study is considered acceptable with following endpoints relevant for the risk assessment: EC_{50} = 1.22 mg/L (0.69 mg sum of a.s./L)

Reference:	10.2.1
Report	Weber K, (2012). Mesotrione/nicosulfuron/dicamba WG (A18032E) plus Adigor (A12127R) – Assessment of Toxic Effects on *Daphnia magna* using the 48 h Acute Immobilisation Test. Report number S12-02294, Eurofins Agroscience Services EcoChem GmbH, Eutinger Strasse 24, 75223 Niefern-Öschelbronn, Germany. (Syngenta File No. A18032E_10008)
Guideline(s):	OECD Guidelines for Testing of Chemicals, Section 2 - Effects on Biotic Systems, Method 202: *Daphnia* sp., Acute Immobilisation Test (2004) Official Journal of the European Communities, Dir 92/69/EEC, O.J. L383A, Part C.2: Acute toxicity for Daphnia (1992) JMAFF Test Guidelines, 2-7-2-1, *Daphnia* acute immobilization studies. 12 Nousan No. 8147 (2000)
Deviations:	No
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	-

Materials

Test Material	A18032E Mesotrione/dicamba/nicosulfuron WG (15/31.25/10)
Lot/Batch #:	SMU2BP001
Actual content of active ingredients:	Mesotrione: 15.6% w/w corresponding to 156 g/kg Dicamba: 31.3% w/w corresponding to 313 g/kg Nicosulfuron: 10.1% w/w corresponding to 101 g/kg
Description:	Beige solid
Stability of test compound:	Stable under standard conditions
Reanalysis/Expiry date:	30 September 2014
Density:	Not applicable
Treatments	
Test concentrations:	Dilution water control and nominal concentrations of 0.188, 0.375, 0.750, 1.50 and 3.00 mg A18032E/L
Solvent:	None
Positive control:	Potassium dichromate at nominal concentrations of 1.0 and 2.0 mg $K_2Cr_2O_7$/L
Adjuvant:	Adigor (A12127R) (mixture of emulsified fatty acid esters on basis of oleic acid methylester). The ratio of A18032E to A12127R was 1: 2.5 (A18032E: A12127R). Results are expressed in terms of the test item containing the adjuvant in this ratio
Analysis of test concentrations:	Yes 0 and 48 hours (based on measurement of mesotrione) using HPLC-MS/MS analysis
Test organisms	
Species:	*Daphnia magna* Straus, Clone 5
Age:	6 - 24 hours
Source:	Continuous laboratory cultures, originally purchased from the Federal Environmental Agency, Berlin, Germany
Feeding:	None during test
Culture medium:	Water composed of dechlorinated drinking water and deionised water with a pH in the range of 6.0 – 9.0, dissolved oxygen above 60 % saturation and total hardness 140 – 268 mg/L (as $CaCO_3$)
Test design	
Test vessels:	100 mL glass beakers containing 50 mL of test medium
Test medium:	Water composed of dechlorinated drinking water and deionised water
Replication:	4 replicates of 5 daphnids

Exposure regime:	Static
Duration:	48 hours
Environmental conditions	
Test temperature:	21.1 to 22.0 °C
pH range:	Test start: 7.51 to 7.89 Test end: 8.22 to 8.29
Dissolved oxygen:	101 to 110 %
Total hardness of dilution water:	142.4 mg/L as $CaCO_3$
Lighting:	16 hours light and 8 hours dark

Study Design and Methods

Experimental dates: 24 July 2012 to 24 August 2012

A stock solution was prepared by dissolving 120 mg A18032E together with 300 mg A12127R in 1000 mL dilution water (dechlorinated drinking water and deionised water). The test concentrations were prepared by dilution of this stock solution. The control consisted of dilution water only. Test solutions were added to the test vessels and the *Daphnia* added without conscious bias.

The immobility of the daphnids was determined by visual observations after 24 and 48 hours of exposure. Organisms unable to swim within 15 seconds after gentle agitation of the test beaker were considered to be immobile.

The pH, temperature and dissolved oxygen were measured at the start, after 24 hours and at the end of the test in each test concentration and the control.

The test concentrations were verified by chemical analysis of mesotrione at 0 and 48 hours using HPLC-MS/MS analysis.

Results and Discussion

At the start of the test, the analytically determined concentrations of A18032E (based on measurements of the active ingredient mesotrione) were in the range 81 to 109% of the nominal values and at the end of the test were in the range 78 to 108% (see table below). The limit of quantification in this study was 0.1 mg A18032E/L plus A12127R (0.0156 mg mesotrione/L). Nominal concentrations were used for the calculation and reporting of results.

Table A 22: Analytical results

Nominal concentrations (mg A18032E/L)	% of nominal concentration measured at 0 hours	% of nominal concentration measured at 48 hours
Control	n.a.	n.a.
0.188	105	108
0.375	109	106
0.750	81	78
1.50	102	105
3.00	101	104

n.a. not applicable

The median effect concentration (EC_{50}) was defined as the concentration resulting in 50% immobilisation of the *Daphnia* in the time period specified and was calculated, together with the 95% confidence intervals, using Spearman-Kärber estimator. The NOEC (No Observed Effect Concentration) is defined as the highest tested concentration which did not produce an effect higher than the allowed control immobilisation and was determined by visual inspection of the data. Immobility data and estimated EC_{50} values are shown in the table below:

Table A 23: Effects of A18032E + A12127R on *Daphnia magna* following exposure for 48-hours in a static test

Nominal concentration (mg A18032E/L)	Immobilised daphnids after 24 hours		Immobilised daphnids after 48 hours	
	Number	%	Number	%
Control	0	0	0	0
0.188	0	0	0	0
0.375	1	5	0	0
0.750	9	45	10	50
1.50	15	75	9	45
3.00	19	95	17	85
EC_{50} mg A18032E/L	0.923		1.22	
95% Confidence limits mg A18032E/L	0.736 – 1.16		0.952 – 1.56	
NOEC mg A18032E/L	0.375		0.375	

Validity Criteria

The validity criteria for the test were met:

- in the control not more than 10 % of animals were immobilised (0 % observed)
- dissolved oxygen concentration at the end of the test was > 30 % of the air saturation value (measured: 107 to 110 %)

Additionally, the EC_{50} (48 h) of the reference item potassium dichromate was between 1.0 and 2.0 mg $K_2Cr_2O_7$/L. Since, the results fall within historical data generated, the daphnids were suitable for the determination of the toxicological effects of A18032E.

Conclusions

Based on nominal concentrations, the 48-hour EC_{50} for A18032E plus adjuvant A12127R to *Daphnia magna* was 1.22 mg A18032E/L, with 95% confidence interval of 0.952 – 1.56 mg A18032E/L. The corresponding 48-hour NOEC was 0.375 mg A18032E/L.

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Comments of zRMS:	The study was conducted in line with OECD 221 with no major deviations. It was noted that the pH of the control increased by more than 1.5 units (observed increase by 1.69 units); however, it does not invalidate the test since the validity criteria were met: - The doubling time of frond number in the control was < 2.5 days (observed 1.3 days) It is noted that the product contains three active substances and in line with the requirements of the Central Zone the test concentrations of all active substances should be verified in the respective chemical analyses or at a minimum the least stable active substance should be analysed. In the present study only the concentration of mesotrione was measured and the analyses of nicosulfuron and dicamba were not carried out. No explanation or justification for the active substance selected for the chemical analysis was provided in the study report. However, information available in area of environmental fate and behaviour of particular active compounds indicates that with mean water DT_{50} of 5.6 days determined in the water/sediment studies, mesotrione is the least stable active substance (mean water DT_{50} of 41 and 65 days was determined for dicamba and nicosulfuron, respectively, in water/sediment systems). Dicamba was stable in EU agreed hydrolysis studies, while nicosulfuron was stable at pH 7 and 9. At pH 5 hydrolytic degradation was observed with DT_{50} determined to be 15 days. In order to further support conclusion on stability of dicamba and nicosulfuron, summaries of the aquatic toxicity studies of these two compounds were consulted in monographs. Dicamba was stable in all acute and chronic aquatic toxicity studies. Nicosulfuron was stable in all acute studies as well as chronic toxicity with algae and majority studies with *Lemna gibba* and single static *Myriophyllum aquaticum* study summarised in this report (Wenzel, 2010), where nicosulfuron was stable over 7 days of

	exposure. Concentrations of nicosulfuron dropped below 80% in two *Lemna* studies available in the course of EU review (for active compound and formulated product). However, following explanation has been provided by the RMS: *Data on the hydrolytic stability of the active substance, indicate that although stable at pH 7, at the test system pH of 5 significant hydrolysis of the active substance may be expected - this being likely to account for the recorded drop in a.s. concentration 2-3 days after medium renewal.* In the study on toxicity of A18032E to *Lemna gibba* pH of the fresh and aged test solutions was >7 over the whole study period and it may be thus concluded that nicosulfuron was stable over the study period. Overall, performed chemical analyses confirmed that mesotrione was stable in study on toxicity of A18032E to *Lemna gibba*, while dicamba and nicosulfuron may be concluded to be stable based on the data available in area of the fate and behaviour. Results of the study may be thus based on nominal concentrations of the test item. The study is considered acceptable with following endpoints relevant for the risk assessment: E_rC_{50} = 0.0181 mg/L (0.0103 mg sum of a.s./L) E_yC_{50} = 0.0064 mg/L (0.0037 mg sum of a.s./L) E_bC_{50} = 0.0127 mg/L (0.0072 mg sum of a.s./L)

Reference:	10.2.1
Report	Weber K, (2012a), Mesotrione/nicosulfuron/dicamba WG (A18032E) plus Adigor (A12127R) – Assessment of Toxic Effects on the duckweed *Lemna gibba* in a Semi-Static Test. Report Number S12-02297. Eurofins Agroscience Services EcoChem GmbH, Eutinger Str. 24, 75223 Niefern-Öschelbronn, Germany. (Syngenta file no A18032E_10009).
Guideline(s):	OECD Guidelines for Testing of Chemicals, Section 2 - Effects on Biotic Systems, Method 221: *Lemna* sp. Growth Inhibition Test (2006)
Deviations:	Minor (see above)
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	-

Materials

Test Material	A18032E	
Parent:	Mesotrione/dicamba/nicosulfuron WG (15/31.25/10)	
Lot/Batch#	SMU2BP001	
Actual content of active ingredients:	Mesotrione:	15.6 % w/w corresponding to 156 g/kg
	Dicamba:	31.3 % w/w corresponding to 313 g/kg
	Nicosulfuron:	10.1 % w/w corresponding to 101 g/kg
Description:	Beige solid	
Stability of test compound:	Stable under standard conditions	
Reanalysis/expiry date:	30 September 2014	
Density:	Not applicable	
Treatments		
Test concentrations:	Dilution water control and nominal concentrations of 1.23, 3.70, 11.1, 33.3 and 100 µg A18032E/L	
Solvent:	None	

Vehicle and/or positive control:	None
Adjuvant:	Adigor (A12127R) (mixture of emulsified fatty acid esters on basis of oleic acid methylester). The ratio of A18032E to A12127R was 1: 2.5 (A18032E: A12127R). Results are expressed in terms of the test item containing the adjuvant in this ratio.
Analysis of test concentrations:	Yes, analysis of mesotrione in freshly prepared and aged test media on days 0, 3, 5 and 7 using HPLC-MS/MS
Test organisms	
Species:	*Lemna gibba* G3
Source:	In-house cultures, originally obtained from Dr. Janet Slovin, Horticulture Crops Quality Laboratory, U.S. Department of Agriculture, BARC-West, Bldg. 050 HH-4, Beltsville, MD 20705, U.S.A.
Test design	
Test vessels:	250-mL glass beakers filled with 150 mL of test medium on Day 0, and 400-mL glass beakers filled with 250 mL of test medium on Days 3 and 5
Test medium:	20X AAP-Growth Medium according to OECD test guideline
Replication:	Six vessels for the control and three for each test concentration
Initial frond number:	4 fronds per plant, total 12 fronds per replicate
Exposure regime:	Semi-static; test medium renewal on Day 3 and Day 5
Duration:	7 days
Environmental conditions	
Temperature:	22.0 – 23.7 °C
pH:	7.33 – 7.59 (new solutions); 8.57 – 9.25 (aged solutions)
Lighting:	Continuous illumination, 6500 - 7000 Lux

Study Design and Methods

Experimental dates: 10 August 2012 to 18 September 2012

A concentrated stock solution was prepared by dissolving 100 mg A18032E together with 250 mg A12127R in 1000 mL dilution water. The stock solution was diluted to prepare the test concentrations. The control consisted of culture medium only. The test media were prepared just before the start of the exposure, and before each test medium renewal.

150 mL of the test solutions were added to 250-mL glass beakers and inoculated with *Lemna* plants. Cultures were then transferred to an incubation chamber where they were maintained under the conditions indicated above. Colonies were transferred to fresh test solutions (in 400-mL glass beakers filled with 250 mL of test medium) on Day 3 and Day 5.

Assessments of frond number were made on days 0, 3, 5 and 7. Fronds were harvested for measurement of dry weight after 7 days. The initial dry weight was determined using six batches of 12 fronds identical to that used to inoculate the test.

Light intensity was recorded once at test start and temperature and pH were recorded in each treatment at the start and end of each test medium renewal period.

The test concentrations were verified by chemical analysis of mesotrione from the freshly prepared and the aged test media of all test concentrations and from the control at days 0, 3, 5 and 7 using HPLC-MS/MS.

Results and Discussion

The analytically determined concentrations of A18032E (based on the measurement of the active ingredient mesotrione) were between 86 to 106% of nominal values in the fresh solutions and between 84 to 112% of nominal values in aged solutions (see table below). The limit of quantification in this study was 1.0 µg A18032E/L (corresponding to 0.156 µg mesotrione/L). Nominal formulation concentrations were used for the calculation and reporting of results.

Table A 24: Analytical results

Nominal concentrations µg A18032E/L	% of nominal measured at 0 days, 0 hours	% of nominal measured at 3 days, 72 hours	% of nominal measured at 3 days, 0 hours	% of nominal measured at 5 days, 48 hours	% of nominal measured at 5 days, 0 hours	% of nominal measured at 7 days, 48 hours
Control	n.a.	n.a.	n.a	n.a.	n.a.	n.a.
1.23	86	99	91	84	94	88
3.70	91	105	91	99	95	91
11.1	106	110	92	108	101	91
33.3	100	112	99	99	95	97
100	104	110	94	103	94	92

The tabulated values represent rounded results obtained by calculation using the exact raw data
n.a.= not applicable

Data for frond number and dry weight were used to calculate growth and yield for the control and each exposure concentration. The 7-day EC_{50} values and their 95% confidence limits were determined by probit analysis following normal and logistic procedures. For the NOEC (No Observed Effect Concentration) and LOEC (Lowest Observed Effect Concentration), Dunnett's-t-test or Jonckheere-Terpstra were used to determine values significantly different to the control.
Mean frond numbers, growth rate, yield and respective inhibition values are presented below alongside estimated EC_{50} values:

Table A 25: Effect of A18032E + A12127R on frond number, growth rate and yield of *Lemna gibba*

Nominal concentration (µg A18032E/L)	Based on Frond Number (0-7 days)					
	Mean no. fronds/replicate (day 7)	Inhibition of frond no. (%)	Growth Rate	Inhibition of growth Rate (%)	Yield	Inhibition of yield (%)
Control	499	0.00	0.5322	0.00	487	0.00
1.23	487	2.40	0.5285	0.70	475	2.50
3.70	392*[1]	21.4	0.4968*[2]	6.70	380*[1]	22.0
11.1	107*[1]	78.6	0.3116*[2]	41.5	95*[1]	80.5
33.3	32*[1]	93.6	0.1401*[2]	73.7	20*[1]	95.9
100	20*[1]	96.0	0.0728*[2]	86.3	8*[1]	98.4
EC50 µg A18032E/L	6.95		18.1		6.43	
95% confidence limits µg A18032E/L	3.09 – 14.9		11.0 – 30.8		4.37 – 9.39	
NOEC µg A18032E/L	1.23		1.23		1.23	
LOEC µg A18032E/L	3.70		3.70		3.70	

Inoculum = 12 fronds
* Mean value statistically significantly lower than in the control ([1]Jonckheere-Terpstra, $p \leq 0.05$; [2]Dunnett's t-test $p \leq 0.05$)

Mean dry weights are presented below along with the yield and respective inhibition values, alongside estimated EC_{50} values:

Table A 26: Effect of A18032E + A12127R on dry weight and dry weight yield of *Lemna gibba*

Nominal concentration (µg A18032E/L)	Based on Dry Weight (0-7 days)			
	Mean dry weight (g) (day 7)	Inhibition of dry weight (%)	Dry weight yield (g)	Inhibition of dry weight yield (%)
Control	0.0752	0.0	0.0728	0.0
1.23	0.0774	-2.90	0.0750	-3.0
3.70	0.0698	7.20	0.0674	7.40
11.1	0.0268*	64.4	0.0244*	66.5
33.3	0.0111*	85.2	0.0087*	88.0
100	0.0101*	86.6	0.0077*	89.4
EC_{50} µg A18032E/L	12.7		11.6	
95% confidence limits µg A18032E/L	1.20 – 85.9		1.10 – 70.2	
NOEC µg A18032E/L	3.70		3.70	
LOEC µg A18032E/L	11.1		11.1	

Mean dry weight (day 0) = 0.0024 g (determined with 6 x 12 representative fronds from the culture)
* Mean value statistically significantly lower than in the control (Dunnett's t-test, $p \leq 0.05$)

Significant differences compared to the control were determined at concentrations ≥ 3.70 µg A18032E/L for frond number, frond number yield and growth rate. For dry weight and dry weight yield significant differences were determined at concentrations ≥ 11.1 µg A18032E/L.

Validity Criteria
The validity criterion for the study was fulfilled:
The doubling time (T_d) of frond number in the control must be < 2.5 days (observed: 1.3 days)

Conclusions
The 7-day EC_{50} for frond number, frond number yield and growth rate for A18032E plus adjuvant A12127R to *Lemna gibba*, were 6.95, 6.43 and 18.1 µg A18032E/L, respectively, based on nominal concentrations. The corresponding NOEC and LOEC for these parameters were determined to be 1.23 and 3.70 µg A18032E/L, respectively.
The 7-day EC_{50} for dry weight and dry weight yield were 12.7 and 11.6 µg A18032E/L, respectively, based on nominal concentrations. The corresponding NOEC and LOEC for these parameters were determined to be 3.70 and 11.1 µg A18032E/L, respectively.

The following *Myriophyllum aquaticum* growth study performed on nicosulfuron was provided in support of the assessment.

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Comments of zRMS:	The study was conducted in line with OECD 203 with no deviations. All the validity criteria were met. It is noted that the product contains three active substances and in line with the requirements of the Central Zone the test concentrations of all active substances should be verified in the respective chemical analyses or at a minimum the least stable active substance should be analysed. In the present study only the concentration of mesotrione was measured and the analyses of nicosulfuron and dicamba were not carried out. No explanation or justification for the active substance selected for the chemical analysis was provided in the study report. However, information available in area of environmental fate and behaviour of particular active compounds indicates that with mean water DT_{50} of 5.6 days determined in the water/sediment studies, mesotrione is the least stable active substance (mean water DT_{50} of 41 and 65 days was determined for dicamba and nicosulfuron, respectively, in water/sediment systems). Dicamba was stable in EU agreed

	hydrolysis studies, while nicosulfuron was stable at pH 7 and 9. At pH 5 hydrolytic degradation was observed with DT_{50} determined to be 15 days. In order to further support conclusion on stability of dicamba and nicosulfuron, summaries of the aquatic toxicity studies of these two compounds were consulted in monographs. Dicamba was stable in all acute and chronic aquatic toxicity studies. Nicosulfuron was stable in all acute studies as well as chronic toxicity with algae and majority studies with *Lemna gibba* and single static *Myriophyllum aquaticum* study summarised in this report (Wenzel, 2010), where nicosulfuron was stable over 7 days of exposure. Concentrations of nicosulfuron dropped below 80% in two *Lemna* studies available in the course of EU review (for active compound and formulated product). However, following explanation has been provided by the RMS: *Data on the hydrolytic stability of the active substance, indicate that although stable at pH 7, at the test system pH of 5 significant hydrolysis of the active substance may be expected - this being likely to account for the recorded drop in a.s. concentration 2-3 days after medium renewal.* In the study on toxicity of A18032E to fish pH of the test solutions was >7 over the whole study period and it may be thus concluded that nicosulfuron was stable over the study period. Overall, performed chemical analyses confirmed that mesotrione was stable in study on toxicity of A18032E to fish, while dicamba and nicosulfuron may be concluded to be stable based on the data available in area of the fate and behaviour. Results of the study may be thus based on nominal concentrations of the test item. The study is considered acceptable with following endpoints relevant for the risk assessment: LC_{50} = 3.44 mg/L (1.97 mg sum of a.s./L)

Reference:	10.2.1
Report	xxxxxxxxxx 2012, Mesotrione/nicosulfuron/dicamba WG (A18032E) plus Adigor (A12127R) – Acute Toxicity Testing in Rainbow Trout (*Oncorhynchus mykiss)* (Teleostei, Salmonidae). Report Number S12-02295, Eurofins Agroscience Services EcoChem GmbH, Eutinger Strasse 24, 75223 Niefern-Öschelbronn, Germany. (Syngenta File No. A18032E_10001)
Guideline(s):	OECD Guidelines for Testing of Chemicals, Section 2 - Effects on Biotic Systems, Method 203: Fish, Acute Toxicity Test (1992) Official Journal of the European Communities, Dir 92/69/EEC, O.J. L383A, Part C.1: Acute Toxicity for Fish (1992) JMAFF Test Guidelines, 2-7-1-1, Acute fish toxicity. 12 Nousan No. 8147, 2000
Deviations:	No
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	No

Materials

Test material	A18032E Mesotrione/nicosulfuron/dicamba WG (15/31.25/10)	
Lot/Batch #:	SMU2BP001	
Actual content of active ingredients:	Mesotrione:	15.6 % (w/w) corresponding to 156 g/kg
	Nicosulfuron:	10.1 % (w/w) corresponding to 101 g/kg
	Dicamba:	31.3 % (w/w) corresponding to 313 g/kg
Description:	Beige granules	

Stability of test compound:	Stable under standard conditions
Reanalysis/expiry date:	30 September 2014
Density:	Not applicable
Treatments	
Test concentrations:	Dilution water control and nominal concentrations of 0.342, 0.751, 1.65, 3.64 and 8.0 mg A18032E/L
Solvent:	None
Adjuvant:	Adigor (A12127R) (mixture of emulsified fatty acid esters on basis of oleic acid methylester). The ratio of A18032E to A12127R was 1:2.5 w/v)
Analysis of test concentrations:	Yes 0 and 96 hours (based on measurement of mesotrione) using HPLC-MS/MS analysis, except for the highest concentration of 8.0 mg A18032E/L where analysis was at 0 and 24 hours due to 100% fish mortality after 24 hours
Test organisms	
Species:	Rainbow trout *Oncorhynchus mykiss* Walbaum
Source:	Obtained from Forellenzucht am Kocherurpsrung, D-73447 Oberkochen, Germany
Acclimatisation period:	>12 days
Treatment for disease:	Not reported
Weight and length of control fish at end of exposure period:	Length: 49 ± 5 mm Weight: 1.04 ± 0.34 g
Feeding:	None during test
Test design	
Test vessels:	25 L glass aquaria, filled with 15 L of test medium
Test medium:	Dechlorinated drinking water and deionised water
Replication:	None
No of fish per tank:	7
Exposure regime:	Static
Duration:	96 hours
Environmental conditions	
Test temperature:	15.5 – 16.4 °C
pH:	At test start: 8.18 – 8.22 At test termination: 8.25 – 8.29
Dissolved oxygen:	At test start: 98 – 102% At test termination: 99 – 101% continuous aeration provided throughout the test
Hardness of dilution water:	143 mg/L as $CaCO_3$
Lighting:	16 hours light and 8 hours dark

Study Design and Methods

Experimental dates: 23 July 2012 to 22 August 2012

A concentrated stock solution was prepared by placing a weighed amount of A18032E together with the adjuvant A12127R in a volumetric flask and adding test medium up to the bench mark. The solution was homogenised by shaking, and the test solutions were prepared by appropriate dilution. The control consisted of dilution water only.

At the start of the test seven fish were randomly allocated to each of the test concentrations and the dilution water control. Observations for mortalities and symptoms of toxicity were made at 0, 3, 6, 24, 48, 72 and 96 hours.

Daily measurements of the test solutions were undertaken throughout the 96 hour period for pH, temperature and dissolved oxygen concentration.

The test concentrations were verified by chemical analysis of mesotrione at 0 and 96 hours using HPLC-MS/MS, except for the highest concentration of 8.0 mg A18032E/L where analysis was at 0 and 24

hours due to 100% fish mortality after 24 hours. Samples for analysis were taken from the centre of the test solutions.

Results and Discussion

At the start of the test, the analytically determined concentrations of A18032E (based on the measurement of the active ingredient mesotrione) were in the range 81 to 105 % of the nominal values and at the end of the test were in the range 79 to 106 % (see table below). The limit of quantification in this study was 0.1 mg A18032E/L (corresponding to 0.0156 mg mesotrione/L). Nominal concentrations were used for the calculation and reporting of results.

Table A 27: Analytical results

Nominal concentrations (mg A18032E/L)	Mean % of nominal measured at 0 hours	Mean % of nominal measured at 96 hours
Control	n.a.	n.a.
0.342	92	92
0.751	81	79
1.65	102	104
3.64	105	106
8.0	91	93*

n.a. = not applicable
* measured at 24 hours due to 100% fish mortality after 24 hours

The median lethal concentration (LC_{50}) was defined as the concentration resulting in 50% mortality of the fish in the time period. The 24-hour LC_{50} was calculated as a geometric mean between NOEC (mortality) and LC_{100}, and the 48-, 72- and 96-hour LC_{50} was calculated using Spearman-Kärber estimator. The NOEC (No Observed Effect Concentration) was defined as the highest tested concentration at which the mortality was not higher than the allowed control mortality.
Mortalities were observed at nominal concentrations of 3.64 and 8.0 mg A18032E/L. Symptoms of toxicity included reduced activity, orientation to the bottom or surface of the test vessels, dark pigmentation, difficulties in maintaining equilibrium, and upside down with gill movement as the only sign of life, and were observed at concentrations of ≥1.65 mg A18032E/L. No mortality or symptoms of toxicity were observed in the control. The mortality data and estimated LC_{50} values are shown in the table below:

Table A 28: Effects of A18032E + A12127R on the survival of *Oncorhynchus mykiss*

Nominal concentration (mg A18032E/L)	Mortality observed (n = 7)					
	3 hours	6 hours	24 hours	48 hours	72 hours	96 hours
Dilution water control	0	0	0	0	0	0
0.342	0	0	0	0	0	0
0.751	0	0	0	0	0	0
1.65	0	0	0	0	0	0
3.64	0	0	0	3	4	4
8.0	0	0	7	7	7	7
LC_{50} mg A18032E/L	>8.0	>8.0	5.40 [1]	3.85 [2]	3.44 [2]	3.44 [2]
95% confidence interval	n.d.	n.d.	n.d.	2.82 – 5.26	2.52 – 4.70	2.52 – 4.70
NOEC (mortality) mg A18032E/L	8.0	8.0	3.64	1.65	1.65	1.65

[1] calculated as a geometric mean
[2] calculated using Spearman-Kärber estimator
n.d. = could not be determined

Validity Criteria

The validity criteria were met since,

- the mortality in the control did not exceed one fish at the end of the test (observed: 0)

- the dissolved oxygen concentration was at least 60% of the air saturation value throughout the test (observed: ≥92%)

Conclusions

Based on the nominal concentrations, the 96-hour LC_{50} for A18032E plus adjuvant A12127R to rainbow trout (*Oncorhynchus mykiss*) was determined to be 3.44 mg A18032E/L, with 95% confidence interval of 2.52 – 4.70 mg A18032E. The 96-hour NOEC (mortality) was determined to be 1.65 mg A18032E/L.

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Comments of zRMS:	The study was performed in line with method described in the guidance from AMRAP workshop, before OECD TG 239 was developed. Nevertheless, as endpoints to be used in the risk assessment must be derived in studies performed in line with the current standards, the test design was checked against indications of OECD TG 239. The test conditions and general test design were comparable with current test guideline. Two major deviations were noted: 1. With 4 plants per replicates and 4 replicates per treatment group and 6 replicates per control the number of shoots per treatment (16 and 24 shoots for treatment and control, respectively) exceeded the number of plants recommended by the current test guideline (12 and 18 shoots for treatment and control, respectively). This deviation is expected to increase the statistical power of the study. 2. The study duration was 7 days, while according to OECD TG 239 the test duration should 14 days. Difference in the study duration may have significant effect on test results as it cannot be excluded that after 14 days of exposure more pronounced effects would be observed on tested plants. Taking this into account, the derived endpoints cannot be used for purposes of the risk assessment performed in line with EFSA (2013). Nevertheless, results of the study may serve as supportive information to compare sensitivity of *Myriophyllum aquaticum* and *Lemna gibba* in order to identify the more sensitive aquatic macrophyte species. Results obtained for the recovery period were not checked since in line with the approach relevant in the Central Zone and in Poland, recovery cannot be taken into account in the risk refinement at all Tiers of evaluation.

Reference:	10.2.1
Report	Wenzel, A. (2010). Macrophyte Growth Test with Nicosulfuron Technical. Test with a subsequent Recovery Period. Fraunhofer IME study No. CHE-009/4-80, Unpublished report No.: 185 NIS
Guideline(s):	Maltby L, Arnold D, Arts G, Davies J, Heimbach F, Pickl C, Poulsen V (eds) Aquatic Macrophyte Risk Assessment for Pesticides (AMRAP). Guidance from the AMRAP workshop in Wageningen (The Netherlands), January 2008. SETAC Press
Deviations:	No
GLP:	Yes
Acceptability:	Endpoints not relevant for the risk assessment due to too short study duration (7 days comparing to 14 days required by OECD TG 239). Nevertheless, study may be used as supportive information to identify more sensitive aquatic macrophyte species.
Duplication (if vertebrate study)	-

Executive Summary

The aquatic macrophyte '*Myriophyllum aquaticum'* was exposed to Nicosulfuron Technical in a 7 day static (without media renewal) toxicity test in defined standard media (water sediment and nutrients) at nominal exposure concentrations of 30, 94.3, 300, 943 and 3000 µg as./L. An assessment of the recovery potential in control medium following exposure was also performed.

Mean measured concentrations of Nicosulfuron Technical in samples taken at start and end of the 7 day exposure period were 30, 103, 350, 1084 and 3523 µg/L respectively (93 and 124 % of nominal) Endpoints were expressed in terms of the mean measured Nicosulfuron Technical concentrations.

There was a concentration dependent inhibition of plant growth, with shoot length found to be the most sensitive parameter. At the three lowest concentrations, no statistically significant inhibition of increase in shoot length and growth rate of length increase compared to controls during 7 day growth phase was detected. At the highest test concentration, inhibition of shoot length - increased and growth rates were 48.8 % and 40.9 % of the control respectively. Correspondingly, the EC_{50} for growth rate – length increase, fresh weight increase and dry weight were all > 3523 µg as./L. For shoot length (relative increase compared to the control)- the most sensitive parameter an EC_{50} of 3071 µg as./L was achieved.

Myriophyllum aquaticum plants exposed to 350, 1084 and 3523 µg test item/L were used for the assessment of the recovery potential, with fast recovery of the macrophytes after removal of the herbicidal active substance. Growth rate for length and length increase were not significantly different from controls during the 7 day growth period and consequently, the macrophytes were considered to have recovered. It was demonstrated that growth of *Myriophyllum aquaticum* plants was able to recover within 7 days, of a 7 day exposure period to Nicosulfuron Technical at concentrations up to 3523 µg/L.

Materials and methods

A. Materials:

1.Test material:	Nicosulfuron technical
Description:	Off-white solid
Batch No.:	10/07-08
Measured content:	95.7% w/w
2.Species:	*Myriophyllum aquaticum*, Haloragaceae, Dicotyledones
Source:	Institut für Gewässerschutz, MESOCOSM GmbH, Neu-Ulrichstein 5, 35315 Homberg (Ohm), Germany
Acclimatisation:	The plants are held immersed for at least 10 days prior to the test start in water and sediment of the same quality as used in the test.
3.Test system:	
Temperature:	20.5 – 21.0°C
Photoperiod:	16 hours
Light intensity:	3892 – 4147 Lux

B. Study design and methods

1. Test Design:

The test was conducted in 2-L glass beakers (approximately 24 cm high and 11 cm diameter). Small glass beakers (5.2 cm high, upper diameter 4.4 cm, bottom diameter 3.9 cm) were used as containers for potting the plants into artificial sediment (OECD 219), with one plant per small beaker with four small beakers per 2 L beaker.

2. Pre-culture:
Healthy shoot apices from the culture plants were clipped off at a length of 6 cm (± 0.5 cm). These shoot tips were maintained prior to the test for 3 days in culture vessels under test conditions with the lower 3 cm, including at least two nodes, in the sediment overlaid with nutrient-poor water to induce root development.

3. Test initiation:
Following pre-culture, healthy plants were removed from the pre-culture vessels and cleaned of sediment and surplus water;. The plants were weighed (to reduce variability, the weight of the shoot tips used in the study should not differ by more than 30 % from the mean), and then planted back into sediment and shoot lengths above sediment were measured. For a growth inhibition test, four plants (1 plant per plant pot) were used per test vessel with four replicates per treatment group (5 test concentrations arranged in a geometric series) plus 6 replicates for the control. Due to the inclusion of the assessment of the recovery potential of the macrophytes, double number of replicates were prepared as designated for a growth inhibition test for treatment levels where inhibition is expected.
A test media at 3000 µg as./L was prepared by direct addition of Nicosulfuron Technical to AAP growth media. The remaining concentrations were prepared by serial dilution of the 3000 µg as./L test medium. The pots with sediment and plants were placed into the glass beakers and carefully filled with 2 L of the appropriate test medium. Vessels were then randomly located within the growth chamber, to minimise spatial influences of light intensity and / or temperature.

2. Observations
Shoot lengths were measured on Day 0, , 5 and 7 of the test At the end of the 7 day exposure phase, all plant shoot lengths were measured and then plants from half of the replicates were harvested. Any symptoms (such as chlorosis or necrosis) or other observations were recorded. Fresh plant wet weight (after carefully blotting off remaining test medium) and then dry weights were determined. A visual assessment of the roots was made and unusual findings recorded. If side shoots are present, their numbers and length was measured.
At the three highest treatments (300, 943 and 3000 µg as./L) the remaining un-harvested plants were carefully transferred into new 2-L beakers and fresh AAP growth media was added. The plants were then incubated under test conditions for a further 7 days. Observations equivalent to the exposure phase were performed. The recovery phase was terminated after 7 days when the increase in shoot length was comparable to the controls.
Temperature was continuously monitored over the entire 14 day test period. Oxygen and pH measurements were performed at the start of the test, once during and then at the end of the exposure phase, in all replicate vessels. Light intensity at the water surface was measured at the start and end of the test.
Water losses (> 10%) through evaporation during the test were replenished to the start of test water height using distilled water.

3. Chemical analysis
Correct test concentrations were confirmed by chemical analysis of samples of test media taken at the start and again at the end of the test, from each exposure concentration and control. Following appropriate concentration, nicosulfuron was measured using an HPLC equipped with diode array detector (DAD) at 240 nm and an LOQ of 5 µg as./L.

4. Data evaluation
Growth rates per vessel were calculated for the wet weight and the increase in shoot length (including side shoots) were determined. Biomass (fresh and dry weight), the number of side shoots at test termination and general condition of roots and appearance of plant material were assessed and recorded. EC50, EC20 and EC10 endpoints were determined using ToxRat (ToxRat Solutions, Alsdorf, Germany). NOEC and LOEC were determined using ANOVA followed by Dunnetts or an equivalent statistical test as required.

Results and discussion

A. FINDINGS

Chemical analysis: The measured concentrations ranged between 93 and 118% of nominal at the start of the test, and between 107 and 124% of nominal at the end of the exposure phase. The overall mean (arithmetic) mean measured concentrations were 30, 103, 350, 1084 and 3525 µg /L.

Biology: - *Exposure phase*:

Shoot length was found to be the most sensitive endpoint parameter, with a concentration dependent inhibition of plant growth observed. At the three lowest test concentrations no statistically significant inhibition of increase in shoot length and growth rate of length increase compared to controls was observed during the 7 day exposure phase. At the highest test concentration, the inhibition of shoot length increase and growth rate were 48.8 % and 40.9 %.

There was a concentration dependent effect on the growth of *Myriophyllum aquaticum*, based on the increase in shoot length and growth rate of length increase. Growth promotion was observed at the 30 and 103 µg/L treatments. There was no clear concentration dependent effect on fresh and dry weight. The toxicity endpoints are summarised in the following table.

Increase of biomass (shoot length) in controls was > 50 %, continuous growth could be supported throughout the test duration, temperature was maintained constant (20 ± 2 °C) and pH did not increase by more than 1.5 units. Therefore the study was considered valid.

Table A 29: Endpoint data in *Myriophyllum aquaticum* exposed to Nicosulfuron Technical for 7 days (mean measured concentrations)

Parameter		EC_{50}	EC_{10}	LOEC	NOEC
Shoot length relative increase	Value [µg/L]	3071	362	1084	350
	95 %-cl	n.d.		n.d.	
Growth rate, length increase	Value [µg/L]	> 3523	446	1084	350
	95 %-cl	n.d.		n.d.	
Fresh weight increase	Value [µg/L]	> 3523	> 3523	> 3523	≥ 3523
	95 %-cl	n.d.		n.d.	
Dry weight	Value [µg/L]	> 3523	> 3523	> 3523	≥ 3523
	95 %-cl	n.d.		n.d.	

~~*Biology – Recovery phase*:~~

~~growth of *Myriophyllum aquaticum* plants transferred from the 350, 1084 and 3523 µg test item/L treatments rapidly recovered to control levels within 7 days of removal of the herbicidal active substance. It was therefore demonstrated that *Myriophyllum aquaticum* plants exposed to Nicosulfuron technical at concentrations up to 3523 µg/L for 7 days has the potential to recover within 7 days.~~

Conclusions

The toxicity of Nicosulfuron Technical to the macrophyte *Myriophyllum aquaticum* were evaluated over a 7 day exposure period with a subsequent 7 day recovery period. Exposure to nicosulfuron technical caused a concentration dependant inhibition of plant growth to *Myriophyllum aquaticum, achieving an* EC50 for the most sensitive biological endpoint (shoot length) of 3071 µg/L and a corresponding NOEC 350 µg/L.

~~Recovery of shoot length growth rates to control treatment levels was demonstrated within 7 days of the exposure period, at concentrations up to 3523 µg/L – the highest mean measured concentration tested.~~

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

Comments of zRMS:	The study was performed in line with OECD 213 and 214 with a minor deviation. It was noted that in the contact test a 2 µL droplet was chosen for application of the test item instead of 1 µL droplet since, according to the testing facility's experience, a higher volume ensures a more reliable dispersion of the test item and the experience has proven that higher volumes are suitable and no adverse effects on the outcome of the study are to be expected. In zRMS opinion this justification is acceptable. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: 48h oral LD_{50} > 227 µg product/bee 72h contact LD_{50} = 170 µg product/bee

Reference:	10.3.1.1.1 and 10.3.1.1.2
Report	Kling A, (2012), Mesotrione/nicosulfuron/dicamba WG (A18032E) plus Adigor (A12127R) – Acute Oral and Contact Toxicity to the Honeybee *Apis mellifera* L. in the Laboratory, Report Number S12-02293. Eurofins Agroscience Services EcoChem GmbH, Eutinger Str. 24, 75223 Niefern-Öschelbronn, Germany. (Syngenta file No. A18032E_10005)
Guideline(s):	OECD (1998a) guidelines for the testing of chemicals, 213. Honeybees, acute oral toxicity test OECD (1998b) guidelines for the testing of chemicals, 214. Honeybees, acute contact toxicity test
Deviations:	Minor (see the commenting box above)
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	-

Materials

Test Material	A18032E Mesotrione/dicamba/nicosulfuron WG (15/31.25/10)	
Lot/Batch #:	SMU2BP001	
Actual content of active ingredients:	Mesotrione: Dicamba: Nicosulfuron:	15.6 % w/w corresponding to 156 g/kg 31.3 % w/w corresponding to 313 g/kg 10.1 % w/w corresponding to 101 g/kg
Description:	Beige solid	
Stability of test compound:	Stable under standard conditions.	
Reanalysis/Expiry date:	30 September 2014	
Density:	Not applicable	
Treatments		
Test rates:	Oral and contact (nominal): 15.7, 31.3, 62.5, 125 and 250 µg A18032E/bee Oral (measured): 17.7, 35.5, 70.7, 136 and 227 µg A18032E/bee	

Control:	Contact: Tap Water Oral: 50% w/v aqueous sucrose solution
Adjuvant:	Adigor (A12127R) (mixture of emulsified fatty acid esters on basis of oleic acid methylester). The ratio of A18032E to A12127R was 1:2.5.
Toxic standard:	Perfekthion/ BAS 152 11 I (nominally 400.0 g dimethoate/L: measured 411.7 g dimethoate/L) Oral: Nominal: 0.06, 0.08, 0.11 and 0.15 µg a.s./bee Contact: Nominal: 0.10, 0.15, 0.23, and 0.34 µg a.s./bee
Administration:	Contact: cuticular absorption following the application of droplets dorsally to the thorax of each bee. Oral: ingestion in aqueous sucrose solution.
Test organisms	
Species:	*Apis mellifera* L. (Hymenoptera: Apidae)
Source:	Healthy colony of young adult worker bees descended from a breeding line of a beekeeper in Rheinland-Pfalz, Germany (Mr. Gerald Wolters, Im Bannen 38 – 54, 56727 Mayen, Germany)
Food:	50 % w/v aqueous sucrose solution
Test design	
Test cage description:	Stainless steel chambers (base: 8.2 x 4.0cm, height: 6.0 cm) with a transparent front and a perforated plate at the base which allows sufficient air supply in to the vessel. The cages were lined with filter paper.
Replication:	5
No. of bees/arena :	10
Duration of test:	Contact: 72 hours Oral: 48 hours
Environmental test conditions	
Temperature:	24.6 – 25.7 °C
Humidity:	61.5 – 68.9 % (RH)
Photoperiod:	Constant darkness

Study Design and Methods

Experimental dates: 12 June 2012 to 15 June 2012

Honeybees (*Apis mellifera*) were exposed to A18032E together with the adjuvant A12127R via two routes of administration: (1) contact, i.e. cuticular absorption following the application of a droplet dorsally to the thorax of each bee of a solution in tap water; after each application the applicator needle was cleaned with a mixture of water and water-wetting agent; and (2) oral ingestion in aqueous sucrose solution. To immobilise the bees during the course of treatment, they were anaesthetised using short bursts of CO_2.

Contact test procedures: Bees were treated with a 2 µL droplet of the test solution, the control or the toxic standard, applied to the dorsal surface of the thorax using a micro applicator. Droplets of 2 µL were chosen in deviation to the guideline recommendation of 1 µL, since a higher volume was considered to ensure a more reliable dispersion of the test item. No adverse effects on the outcome of the study were expected. The bees were returned to the test unit, allowed to recover and kept in the CE room with a continuous supply of 50 % w/v aqueous sucrose solution.

Oral test procedures: Bees were starved for 2 hours prior to treatment. Each group of bees was offered 250 µL (equivalent to 25 µL/bee) of the test material or toxic standard dispersed in aqueous sucrose solution. Treatments were calculated so that the target dose per bee was contained in 20 µL, however 25 µL was actually provided per bee. This was to ensure sufficient consumption of the test material so that the target dose was achieved. The doses were measured into the Eppendorf cups and the weights of these were recorded before the doses were made available to the bees. The bees were allowed to consume

the test solutions up to a maximum of six hours after which the Eppendorf cups were replaced and 50 % w/v aqueous sucrose solution provided *ad libitum*. All cups with test solutions were weighed after feeding in order to calculate actual mean consumption per bee for each treatment.

In both the contact and oral tests there were five replicates per treatment. Mortality and sub-lethal effects were assessed at 4, 24 and 48 hours for the test material, control and toxic standard for both oral and contact tests, and an additional assessment was carried out after 72 hours for the contact test.

The mortality [%] per treatment was calculated from the number of dead bees and the total number of introduced bees per treatment group. Since no mortality occurred in the control groups, no correction of the test and reference item mortality was necessary. The LD_{50} values with 95% confidence limits of the reference and test item treatments were calculated by means of a probit analysis. The oral LD_{50} values for the reference and test item treatments were calculated with the single consumption values per replicate.

Results and Discussion
Mortality data for the test material and toxic standard are summarised in the table below.

Table A 30: Summary of acute toxicity of A18032E + A12127R to the honeybee

Treatment	Exposure		LD_{50} values	95% confidence interval
	Route	Duration (hours)		
Test material* (µg A18032E/bee)	Contact	24	201	177 – 234
		48	170	153 – 189
		72	170	153 – 189
	Oral	24	>227 ~~691~~	n.d.
		48	>227 ~~691~~	n.d.
Toxic standard (µg dimethoate/bee)	Contact	24	0.15	0.13 – 0.16
	Oral	24	0.11	0.10 – 0.12

* The ratio of A18032E to A12127R was 1 : 2.5

In the water treated control group of the contact toxicity test no mortality occurred until the final assessment of the 72-hour observation period. At the highest tested dose level of 250 µg A18032E/bee a mortality of 90.0 % was observed 72 hours after application. In the contact test, sublethal effects (affected, apathetic and moribund bees) were observed at ≥ 62.5 µg A18032E/bee 4, 24 and 48 hours after application. At the final assessment, 72 hours after start of the test, no more remarkable sublethal effects were observed.

In the control group of the oral toxicity test no mortality occurred during the 48-hour observation period. At the highest nominal dose level of 250 µg A18032E /bee (actual tested dose: 227 µg A18032E/bee) 15.7 % mortality was observed after 48 hours. In the oral toxicity test sublethal effects (affected and apathetic bees) were observed at ≥125 µg A18032E/bee at the 4- and 24-hour assessments. No more remarkable sublethal effects were observed at the 48-hour assessment.

Validity of the test
The study is considered to be valid because:

- the mean mortality of the control in the oral and contact toxicity test was ≤ 10 % (observed 0% in both the oral and the contact test after 48 and 72 hours, respectively)
- The 24 h LD_{50} of the reference item in the oral toxicity test was within the range of 0.10 to 0.35 µg a.s./bee (measured 0.11 µg a.s./bee)
- The 24 h LD_{50} of the reference item in the contact toxicity test was within the range of 0.10 to 0.30 µg a.s./bee (measured 0.15 µg a.s./bee)

Conclusions
The 72-hour contact LD_{50} for the test material was 170 µg A18032E/bee, with 95% confidence limits of 153 – 189 µg A18032E/bee. Sublethal effects (affected, apathetic and moribund bees) were observed at

≥ 62.5 µg A18032E/bee 4, 24 and 48 hours after application. At the final assessment, 72 hours after start of the test, no more remarkable sublethal effects were observed.
The 48-hour oral LD_{50} for the test material was >227 µg A18032E/bee, the highest dose tested. Sublethal effects (affected and apathetic bees) were observed at ≥125 µg A18032E/bee at the 4- and 24-hour assessments. No more remarkable sublethal effects were observed at the 48-hour assessment.

A 2.3.1.1.2 KCP 10.3.1.1.2 Acute contact toxicity to bees

Please refer to A 1.3.1.1.1.

A 2.3.1.2 KCP 10.3.1.2. Chronic toxicity to bees

Comments of zRMS:	Study on chronic adult toxicity of dicamba solo-formulation (A7254B) was not validated by the zRMS since in case of A18032E, containing 3 active compounds, respective chronic toxicity study should be performed with the formulated product in order to fulfil data requirements, while active substance endpoints should be generated at the EU level.

Reference:	10.3.1.2/01
Report	Ruhland S, (2015), Dicamba SL (A7254B) - Chronic toxicity to the Honeybee *Apis mellifera* L. in a 10 Day Continuous Laboratory Feeding Study. Report Number 14 10 48 057 B. BioChem agrar Labor für biologische und chemische Analytik GmbH Kupferstraße 6 04827 Gerichshain, Germany. (Syngenta file No A7254B_10378)
Guideline(s):	Decourtye A, *et al*. Comparative sublethal toxicity of nine pesticides on olfactory learning performances of the honeybee *Apis mellifera*, 2005 Suchail S *et al.*: Discrepancy between acute and chronic toxicity induced by imidacloprid and its metabolites in *Apis mellifera*, 2001 AFPP Method No. 230: Evaluation of effects of plant protection products on *Apis mellifera* L. (French Association for Plant Protection: Guideline for chronic toxicity testing, 2012) EFSA Guidance Document on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees). EFSA Journal 11(7): 3295, 266 pp., 2013 AG-Bienenschutz, International ring test protocol: Adult honeybee (*Apis mellifera* L.), Chronic toxicity test (10 day feeding test in the laboratory) (Method validation), 2014
Deviations:	No
GLP:	Yes
Acceptability:	Not evaluated, not relevant for the zonal evaluation of A18032E (study with formulation in question should be performed to fulfil data requirements)
Duplication (if vertebrate study)	-

~~**Materials**~~

~~**Test Material**~~	~~Dicamba SL (480)~~ ~~A7254B~~ ~~SAN837 SL (480)~~
~~**Lot/Batch #:**~~	~~BSN4C1022~~
~~**Actual content of active ingredients:**~~	~~Dicamba: 41.7 % w/w corresponding to 487 g/L~~
~~**Description:**~~	~~Yellow liquid~~
~~**Stability of test compound:**~~	~~Stable under standard conditions~~
~~**Reanalysis/Expiry date:**~~	~~30 April 2018~~
~~**Density:**~~	~~1168 kg/m^3~~

~~**Treatments**~~

Test rates:	Nominal concentrations: 12.5, 25.0, 50.0, 100.1, 200.2 µg dicamba/bee (0.321, 0.643, 1.285, 2.570 and 5.140 g dicamba/kg food) Mean actual consumption: 15.326, 34.782, 72.058, 110.042 and 194.701 µg dicamba/bee
Control:	50 % (w/v) sucrose solution
Toxic standard:	Dimethoate EC 400 (nominal: 400.0 g dimethoate/L; measured: 400.9 g dimethoate/L)
Administration:	Ingestion in aqueous sucrose solution
Test organisms	
Species:	*Apis mellifera carnica* L. (Hymenoptera: Apidae) (young adult worker bees, 1 – 4 days old)
Source:	Derived from healthy and queen right colonies; source: Bienenfarm Kern GmbH, Rehbacher Anger 10, 04249 Leipzig, Germany
Food:	50 % w/v aqueous sucrose solution
Test design	
Test cage description:	Aluminium cages (20 x 15 x 10 cm) with two glass plates for observations and holes in the lateral walls for sufficient air supply and ventilation
Replication:	3
Duration of test:	10 days
Environmental test conditions	
Temperature:	32.6 – 33.2 °C
Humidity:	57.0 – 62.0 % (RH)
Photoperiod:	Constant darkness

Study Design and Methods

Experimental dates: 02 September 2014 to 12 September 2014

Four days prior to test initiation, brood combs containing capped cells were transferred into an incubator under controlled conditions. Brood combs were taken from outside hives and different colonies. One day prior to test start the newly hatched bees were transferred from combs to the test cages and kept under test conditions.

Feeding solutions were placed in plastic syringes and offered to the bees in each unit *ad libitum*. Bees in one replicate shared the feeding solution and thus received similar doses. Feeding solutions were replaced daily and the amount of feeding solution consumed was determined by weighing the syringe before and after feeding.

Mortality was recorded daily immediately prior to the next application of the treated diet for 10 days. The mortalities for each test item group were expressed as a percentage of the control population, according to the formula of Schneider-Orelli (1947):

$$M = \frac{t - c}{100 - c} \times 100$$

M: Corrected mortality (%)
c: Mortality in the control group (%)
t: Mortality in the test item/reference group (%)

The LC_{50} and LD_{50} values of the test item group were calculated by means of logit analysis using linear maximum likelihood regression. Fisher's Exact Binomial test with Bonferroni cCrrection (one-sided greater, $\alpha = 0.05$) was used to evaluate whether there was a difference between the mortality data of the test item and control groups and determine the NOEC and NOED. Statistical calculations were made using the statistical software ToxRat professional, Version 2.10.06.

Analysis of the test item in each test item and control solution was not reported.

Results and Discussion

Mortality data for the test material and control are summarised in the table below.

Table A 31: Summary of chronic toxicity of dicamba to honey bees (*Apis mellifera* L.)

Dose (g dicamba/kg food)	Mean cumulative mortality (%)									
	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10
Control	0.0	0.0	0.0	1.7	1.7	1.7	1.7	6.7	6.7	8.3
0.321	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.643	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1.285	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.7	1.7	1.7
2.570	0.0	1.7	1.7	3.3	5.0	5.0	5.0	5.0	6.7	6.7
5.140	0.0	0.0	0.0	1.7	1.7	1.7	3.3	5.0	8.3	11.7
Reference Item 0.152	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8.3	18.3	31.7
Reference Item 0.253	0.0	0.0	1.7	3.3	3.3	3.3	3.3	3.3	5.0	5.0
Reference Item 0.421	0.0	16.7	28.3	31.7	31.7	31.7	33.3	36.7	36.7	41.7
Reference Item 0.702	0.0	18.3	23.3	28.3	31.7	36.7	41.7	50.0	60.0	68.3
LC_{50}	> 5.140 g dicamba/kg food									
NOEC	5.140 g dicamba/kg food									
LD_{50}	> 194.7 µg consumed dicamba/bee/day									
NOED	194.7 µg consumed dicamba/bee/day									

Table A 32: Accumulated mean uptake of dicamba

Dose (g dicamba/kg food)	Accumulated mean uptake of test item (µg dicamba/bee/day)									
	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10
0.321	12.4	26.2	40.8	56.3	69.6	84.9	100.0	118.7	137.1	153.3
0.643	19.2	51.1	85.1	119.0	152.9	189.4	231.0	270.3	309.5	347.8
1.285	54.4	120.7	192.9	259.6	332.0	404.4	491.8	570.0	648.7	720.6
2.570	95.1	208.0	313.4	417.3	548.2	658.5	776.7	894.0	1002.5	1100.4
5.140	116.0	312.4	507.8	725.7	927.4	1147.2	1362.8	1559.6	1750.0	1947.0
Reference Item 0.152	6.1	12.8	19.1	23.8	28.9	34.3	39.4	43.1	46.5	50.3
Reference Item 0.253	6.5	18.6	29.6	40.3	48.5	56.0	64.1	70.9	76.5	83.5
Reference Item 0.421	17.1	30.2	39.9	51.6	65.8	81.1	91.4	104.6	118.5	132.0
Reference Item 0.702	12.3	33.1	57.2	74.7	93.3	115.3	139.1	149.3	158.5	175.2

Validity criteria

The validity criteria are listed below:

- The mortality in the control was 8.3 % (must be ≤ 15 %)

Conclusions

The LC_{50} was determined to be > 5.140 g dicamba/kg food and the NOEC was determined to be 5.140 g dicamba/kg food.

The LD_{50} was determined to be > 194.7 µg consumed dicamba/bee/day and the NOED was determined to be 194.7 µg consumed dicamba/bee/day.

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Comments of zRMS:	Study on chronic adult toxicity of mesotrione was not validated by the zRMS since in case of A18032E, containing 3 active compounds, respective chronic toxicity study should be performed with the formulated product in order to fulfil data requirements, while active substance endpoints should be generated at the EU level.

Reference:	KCP 10.3.1.2/02
Report	xxxxxxxxxxxxx., (2018), Mesotrione - Honey Bee (*Apis mellifera* L.) Chronic Oral Toxicity Test 10 Day Feeding Test in the Laboratory. Report Number S18-03658. Eurofins Agroscience Services Ecotox GmbH, Eutinger Str. 24, 75223 Niefern-Öschelbronn, Germany. (Syngenta file no ZA1296_10608)

Guideline(s):	OECD Guideline No. 245 (2017)
Deviations:	No
GLP:	Yes
Acceptability:	Not evaluated, not relevant for the zonal evaluation of A18032E (study with formulation in question should be performed to fulfil data requirements)
Duplication (if vertebrate study)	No

Materials and methods

Test Material	Mesotrione
Lot/Batch #:	SMO0H028
Purity:	84.6 % w/w
Description:	solid / brown
Stability of test compound:	Sufficient for the test purpose (at least 1h)
Reanalysis/Expiry date:	28 Feb 2019
Treatments	2 control groups 5 test item groups 1 reference item group
Test rates:	62.5, 125, 250, 500 and 1000 mg a.i./kg sucrose solution
Controls:	Control: 50 % aqueous sucrose solution Solvent control: 50 % aqueous sucrose solution containing 0.1 % Xanthan and 5 % acetone
Toxic standard:	0.9 mg dimethoate/kg feeding solution
Application method:	Continuous *ad libitum* feeding of test solutions
Analysis of test concentrations:	The actual mean concentrations of difenoconazole in all test item feeding solutions were in the range of 88 to 108 % of the nominal concentrations.
Test organisms	Young adult worker bees
Species:	*Apis mellifera* L. (Hymenoptera, Apidae)
Age:	1 to 2 days old
Source:	Stock beehives maintained by Eurofins Agroscience Services
Food:	50 % (w/v) aqueous sucrose solution
Test Design	Dose-response test
Test cage description:	Stainless steel cages (base: approx. 8 cm x 4 cm, height: approx. 6 cm). The front side of the cages was equipped with a transparent pane to enable observation. The bottom of the cages consisted of perforated steel, which guaranteed sufficient air supply. The cages were lined with filter paper.
Replication:	4 replicates per treatment group
No. of bees/replicate:	10 bees/replicate
Environmental test conditions	Climatic chamber
Temperature:	31.3 – 33.4°C
Humidity:	39.6 – 66.1 %
Photoperiod:	Constant darkness except during the exchange of feeding syringes and assessments
Duration of test:	10 days

Study Design and Methods

Experimental dates: 09 Jul 2018 to 22 Aug 2018

The target amount of 703 mg Mesotrione was weighed and dissolved in 25 mL acetone. Mesotrione stock solutions were prepared and diluted daily for each test item concentration. Acetone was used as the solvent.
The definitive feeding solutions for the test item treatments were freshly prepared every day by diluting the respective stock solution with 50 % (w/v) aqueous sucrose solution containing 0.1 % Xantahn. The final concentration of acetone in all test item feeding solutions was 5 %.
Bees were fed *ad libitum* with treated sucrose solutions presented with syringe feeders, which were renewed every day. Feeders were weighed before and after they were offered, so that the food consumed could be determined by comparison of the weight of the remaining solution with the initial weight. The individual daily consumption was corrected each day by the number of surviving bees at each assessment date as well as by the daily determined evaporation rate.
Direct treatment effects (mortality and other observed biological effects) were assessed at daily intervals during the 10-day exposure period by visual counting of honeybees.
Chi^2 test with Bonferroni Correction (one-sided greater, $\alpha = 0.05$) was used to evaluate whether there were significant differences between the mortality data of the control and the test item treatment group and to determine the NOEC and NOEDD based on mortality, respectively.

Results and Discussion
Analytical recoveries in the diets are presented below.

Table A 33: Analytical results

Feeding solution (mg a.i./kg)	Analytical Recovery in Diet (mg/kg)									
	D0	D1	D2	D3	D4	D5	D6	D7	D8	D9
Solvent Control	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
62.5	50.6	72.1	68.7	75.3	72.6	68.7	72.1	46.7	79.5	70.8
125	85.3	131	101	122	119	115	120	89.5	136	128
250	176	234	226	229	238	220	230	202	248	236
500	390	446	439	421	434	449	472	427	453	500
1000	834	832	889	908	886	840	894	872	953	889

LOD: 1.269 mg a.i./kg

Mortality data for the test material and reference item are summarised in the table below.

Table A 34: Summary of mortality of bees in the chronic toxicity test after 10 days

Nominal Concentration [mg a.i./kg sucrose solution]	Nominal Daily Consumed Dose [µg a.i./bee/day]	After 10 days	
		Mean mortality	
		absolute [%]	corrected [%]
Control		5.0	-
Solvent Control (5 % acetone 0.1 % Xanthan)		5.0	-
62.5	14.1	15.0	10.5
125	27.4	0.0	-5.3
250	59.2	2.5	-2.6
500	133	2.5	-2.6
1000	219	0.0	-5.3
Reference item (0.9 mg/kg)		100	100

* Statistically different from the pooled controls (Williams Multiple Comparison Test).

No unusual behavioural effects were observed.
Study endpoints are summarised in the table below.

Table A 35: Study endpoints at 10 days

Treatment	Endpoints	After 10 d
Test item doses	LDD_{10} [µg a.i./bee/day]	
	LDD_{20} [µg a.i./bee/day]	
	LDD_{50} [µg a.i./bee/day]	> 21.9
	NOED [µg a.i./bee/day]	21.9
Test item concentrations	LC_{10} [mg a.i./kg food]	
	LC_{20} [mg a.i./kg food]	
	LC_{50} [mg a.i./kg food]	> 1000
	NOEC [mg a.i./kg food]	1000

Validity Criteria

The test was considered valid:

- Average cumulative mortality was 5.0 % in the untreated sucrose solution control (must be < 15 %)
- Average cumulative mortality was 5.0 % in the acetone control (must be <15%)
- Mortality was 100% in the toxic reference (must be >50%)

Conclusions

The toxicity of Mesotrione to the honeybee *Apis mellifera* was determined in a 10-day continuous oral exposure study. Adult bees were exposed to nominal concentrations of 62.5, 125, 250, 500 and 1000 mg a.i./kg sucrose solution, alongside water and solvent controls.

The 10 day NOEC was determined to be 1000 mg a.i./kg sucrose solution. Based on actual consumption of the test solutions, the NOEDD was 21.9 µg a.i./bee/day. The LC_{50} was considered to be > 1000 mg a.i./sucrose solution corresponding to a LDD_{50} > 21.9 µg a.i./bee/day.

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Comments of zRMS:	Studies on chronic adult toxicity of nicosulfuron was not validated by the zRMS since in case of A18032E, containing 3 active compounds, respective chronic toxicity studies should be performed with the formulated product in order to fulfil data requirements, while active substance endpoints should be generated at the EU level.

Reference:	10.3.1.2/03
Report	Schmitt H, (2014), Nicosulfuron (DPX-V9360) Technical: Assessment of Effects to the Honeybee, *Apis mellifera* L., in a 10 Days Chronic Feeding Test under Laboratory Conditions. Report Number S 14-00413. Eurofins Agroscience Services EcoChem GmbH, Eutinger Str. 24, 75223 Niefern-Öschelbronn, Germany.
Guideline(s):	No specific guideline available
Deviations:	No
GLP:	Yes
Acceptability:	Not evaluated, not relevant for the zonal evaluation of A18032E (study with formulation in question should be performed to fulfil data requirements)
Duplication (if vertebrate study)	-

Executive Summary

The chronic oral toxicity of the honey bee exposed to the test item nicosulfuron for a period of ten days was determined in a laboratory study. The chronic oral feeding test was conducted by daily administration of the test item to bees in sugar solution at five concentrations of 280, 140, 70.0, 35.0 and 17.5 mg a.s./kg diet. These concentrations led to daily mean doses of 11.43, 5.23, 2.89, 1.41 and 0.67 µg a.s./bee/day after 10 days, respectively. 40 test newly hatched young adult worker bees (ten per replicate) were exposed to the test concentrations and the controls (50% aqueous sugar solution mixed with deionised water and 50% aqueous sugar solution containing 5% acetone). A treatment with a reference item (Perfektion containing 400 g/L dimethoate) was also included in this study.

Daily assessment of mortality and behavioural abnormalities was performed up to day ten.

The mean mortality after daily exposure of 280, 140, 70.0, 35.0 and 17.5 mg a.s./kg diet (corresponding to 11.43, 5.23, 2.89, 1.41 and 0.67 µg a.s./bee/day) ten days following the start of chronic exposure were 0, 2.4, 0, 0, and 2.5%, respectively. There was 2.5% and 7.5% mean mortality in control and solvent groups, respectively at test end, ten days following start of exposure. There were no remarkable sub-lethal effects recorded at all treatment levels during the entire observation period.

The 10-day LC_{50} value (10 days) for honey bees exposed to nicosulfuron was > 280 mg a.s./kg diet corresponding to a 10-day LDD_{50} value of > 11.43 µg a.s./bee/day. The NOEC and NOEDD (after 10 days) values were determined to be 280 mg a.s./kg diet and 11.43 µg a.s./bee/day, respectively.

Materials and methods

Test material: Nicosulfuron
Batch no.: MAY09MA215
Purity: 94.6% w/w
Description: Solid white
Vehicle and positive control

Vehicle: For the test item treatments acetone was used as the solvent. The definite feeding solutions contained 5 % acetone. For the toxic reference item tap water was used as solvent.

Reference: Perfekthion containing 400 g dimethoate/L

Test organism: *Apis mellifera carnica* L.
Stage: Young adult worker bees (newly hatched; 1 to 4 days old)
Source: Klaus Hampel, Mühlhausenerstr. 1/1, 75233 Tiefenbronn, Germany.
Feeding: During acclimatization: 50 % (w/v) aqueous sucrose solution *ad libitum*.
Test units: The test units were stainless steel cages 8 x 4 x 6 cm. The front side of the cage is equipped with a transparent pane to enable observation. The bottom of the cage consists of perforated steel, which guarantees sufficient air supply. The cages were lined with filter paper.

Environmental conditions
Temperature: 31.5 – 32.8°C
Humidity: 60.4 – 70.9%
Light: Darkness except during assessment (observations were made under neon light)

Study design and methods

In-life phase: Jun 11 to Jul 09, 2014

Test organism assignment and treatment

Four days prior to test start, brood combs containing capped cells which were expected to hatch on the same day were taken out of a honey bee colony and transferred into the climatically controlled chamber. To guarantee a sufficient number of bees for the test, brood combs from 2 hives were used. The brood

combs contained also some honey and pollen as food for the hatched bees. One day prior to test start the 0 - 3 days old bees were picked off the combs, transferred to the test cages and kept under test conditions until test start. Moribund bees were rejected and replaced by healthy bees before starting the test.
Nicosulfuron was tested at five concentrations of 476.36, 1306.12, 2285.71, 4000 and 7000 mg a.s./kg diet. Additionally, honeybees were treated with Perfekthion (400 g dimethoate/L) as the toxic reference at a concentration of 0.9 mg dimethoate/kg feeding solution and with 50% aqueous sugar solution mixed with deionised water and 50% aqueous sugar solution containing 5% acetone as a control. Each test group had 4 replicates, which consisted of 10 bees in one cage. The feeding solutions were offered to the test organisms of each test unit in feeders (plastic syringes, approx. 5 mL, tip removed). The feeding solution (about 3 – 4.00 mL/replicate) was offered *ad libitum* and was replaced daily by changing the feeders.

Dose preparation
Test item and reference item were measured using a balance.
The stock solution was prepared only once at the day of test start by using acetone (for the test item) and tap water (for the toxic reference item) as solvent on the day of test start. The stock solutions were stored tightly closed under cool conditions in the dark (refrigerator, ca. 6 ± 2°C).
The feeding solutions were prepared fresh every day. The highest concentrated feeding solution for the test and reference item were prepared from the stock solution with 50 % (w/v) aqueous sucrose feeding solution. All lower feeding solutions for the test item were prepared from the highest feeding solution with 50% (w/v) aqueous sucrose solution containing 5% acetone.
For the untreated control the 50% sucrose-water solution, mixed with an amount of water equivalent to the amount of solution added to the feeding solutions was administered to the honeybees. For the solvent control, the sucrose-water solution contained 5% acetone.

Measurements and observations
Mortality and sub-lethal effects were recorded 24 h (± 2h) after application (start of feeding). Sub-lethal effects or any abnormal behaviour in comparison to the control were recorded according to the following categories: moribund, affected, cramps and apathy.
The amount of feeding solution consumed was determined by weighing the feeders before and after feeding using calibrated equipment.
Analytical dose verification was performed in samples from the stock and all test item and control feeding solutions with a validated method.
Temperature and humidity were recorded continuously with appropriate, calibrated equipment.

Statistics
Calculations of the LC_{50} and LDD_{50} with 95 % confidence interval was not possible as mortality did not reach 50%. A statistical evaluation according to the Fisher's exact test was not conducted, as no increased mortality in the test item treatments compared to the control was observed.

Results and discussion

Food consumption and mortality
The mean mortality after daily exposure of bees to five concentrations of nicosulfuron is presented in the table below. There was 2.5% and 7.5% mean mortality in control and solvent groups, respectively at test end, ten days following start of exposure.
There were no remarkable sub-lethal effects recorded at all treatment levels during the entire observation period.
The reference item at a concentration of 0.9 mg dimethoate/kg feeding solution caused 100% mortality at day 9.

Table A 36: Food consumption and mortality of bees in a 10-day chronic oral toxicity test with nicosulfuron

Treatments (mg a.s./kg diet)	Mean consumption	Mean uptake of a.s.	Cumulative mortality	
	mg/bee/day	µg/bee/day	Mean ± SD %	Mean corrected %
Control (0)	47.5	0.00	2.5 ± 17.3	--
Acetone 5% control (0)	43.8	0.00	7.5 ± 10.0	--
17.5	38.3	0.67	2.5 ± 5.8	-5.4
35.0	40.2	1.41	0.0 ± 15.3	-8.1
70.0	41.4	2.89	0.0 ± 11.5	-8.1
140	37.3	5.23	2.4 ± 15.3	-5.5
280	40.8	11.43	0.0 ± 5.8	-8.1
Reference item (0.9 mg dimethoate/kg diet)	33.3	0.03	100.0	100.0

Validity criteria

The cumulative larval mortality for the controls was ≤15% across all replicates (actual 0.0 % in the control group and 7.1 % in the solvent control group). The cumulative larval mortality for the reference item (after correction) was ≥ 50 % at Day 7 across all replicates (actual 88.1 %). All validity criteria were met.

Endpoints

The LC_{50} and NOEC, based on nominal concentration, and the LDD_{50} and NOEDD, based on the mean uptake of test item per bee are presented in the following table.

Table A 37: Chronic oral toxicity to honey bees exposed to nicosulfuron – Summary of endpoints

LC_{50}	> 280 mg/kg diet
LDD_{50}	> 11.43 µg/bee/day
NOEC	280 mg/kg diet
NOEDD	11.43 µg/bee/day

NOEC = No Observed Effect Concentration based on mortality (A statistical evaluation according to the Fisher's exact test was not conducted, as no increased mortality in the test item treatments compared to the control was observed)
NOEDD = No Observed Effect Dietary Dose based on mortality (A statistical evaluation according to the Fisher's exact test was not conducted, as no increased mortality in the test item treatments compared to the control was observed)

Statistical analysis in the original report determined that there were no statistically significant (P>0.05) treatment related effects on all the parameters evaluated and the NOEC was determined to be >280 mg /kg, the highest concentration tested. As a result of these findings, it can be concluded that no reliable $EC_{10/20}$ values could be determined and no additional statistical analysis was performed on any of the parameters from the original study.

Conclusion

In a chronic oral toxicity test to honey bees with nicosulfuron, the10-day LC_{50} value (10 days) for honey bees exposed to nicosulfuron was > 280 mg a.s./kg diet corresponding to a 10-day LDD_{50} value of > 11.43 µg a.s./bee/day. The NOEC and NOEDD (after 10 days) values were determined to be 280 mg a.s./kg diet and 11.43 µg a.s./bee/day, respectively.

A 2.3.1.3 KCP 10.3.1.3 Effects on honeybee development and other honeybee life stages

Comments of zRMS:	Study on chronic larvae toxicity of dicamba solo-formulation (A7254B) was not validated by the zRMS since in case of A18032E, containing 3 active compounds, respective larvae toxicity study should be performed with the formulated product in order to fulfil data requirements, while active substance endpoints should be generated at the EU level.

Reference:	10.3.1.3/01
Report	Kleebaum K (2015) Dicamba SL (A7254B) – Chronic toxicity to the honeybee larvae *Apis mellifera* L. under laboratory conditions (*in vitro*), Report 14 10 48 072 B. BioChem agrar, Labor für biologische und chemische Analytik GmbH, Kupferstraße 6, 04827 Gerichshain, Germany. (Syngenta file No. A7254B_10377).
Guideline(s):	OECD DRAFT Guidance Document for testing chemicals: Honey bee (*Apis mellifera*) larval toxicity test, repeated exposure (February 2014) OECD 237 Guidelines for testing chemicals: Honey bee (*Apis mellifera*) larval toxicity test, single exposure (2013)
Deviations:	No
GLP:	Yes
Acceptability:	Not evaluated, not relevant for the zonal evaluation of A18032E (study with formulation in question should be performed to fulfil data requirements)
Duplication (if vertebrate study)	-

Materials

Test Material	Dicamba SL (480) A7254BSAN837 SL (480)
Lot/Batch #:	BSN4C1022
Actual content of active ingredients:	Dicamba: 41.7 % w/w corresponding to 487 g/L
Description:	Yellow liquid
Stability of test compound:	Stable under standard conditions
Reanalysis/Expiry date:	30 April 2018
Density:	1168 kg/m^3
Treatments	
Test rates:	Total product/larva: 76.1, 152.1, 304.3, 608.5 and 1217.0 µg A7254B Total a.s./larva: 31.3, 62.5, 125.0, 250.1 and 500.1 µg dicamba a.s./kg diet: 0.198, 0.395, 0.790, 1.581 and 3.161 g dicamba
Control:	Untreated artificial diet
Toxic standard:	Dimethoate tech. (BAS 152 I), purity 99.8 % (± 1.0 %)
Application method:	Oral application using a sterile pipette
Test organisms	
Species:	Worker honey bee larvae *Apis mellifera* L. subspecies *carnica* P. (Insecta, Hymenoptera, Apoidea)
Age:	First instar (L1) during grafting
Source:	Colonies purchased from Bienenfarm Kern GmbH, Rehbacher Anger 10, 04249 Leipzig, Germany
Food:	Aqueous sugar solution (comprising yeast, glucose, fructose and water) mixed with royal jelly at a 1:1 ratio w/w
Test Design	
Test cage description:	Crystal polystyrene grafting cells (e.g. CNE Nicoplast, internal diameter 9 mm) were placed in 48 well plates. The well plates were

	filled up to 1/3 with dental roll and the grafting cells were placed on wetted and disinfected dental rolls.
Replication:	3
No. of larvae/replicate:	12
Environmental test conditions	
Temperature*:	34.0 – 35.0 °C
Humidity*:	90 – 97 % (RH)
Photoperiod:	Constant darkness
Duration of test:	Pre-grafting (*in vivo*): Days -3 to 0 Grafting: Day 1 Pre-exposure (*in vitro*): Days 1 to 3 Application: Days 3 to 6 Post exposure (*in vitro*): Days 7 to 8

*Deviations < 2 hours were not reported.

Study Design and Methods

Experimental dates: 29 August 2014 to 05 September 2014

The test/reference item was mixed into sterile filtered aqueous sugar solution (stock A). Several dilutions were prepared by adding further sugar solution (stocks B and C) and royal jelly was added to each stock solution at a ratio of 1 : 1, based on (w/w), to reach the final test concentrations. Dosages were adjusted to reflect the target amounts of A7254B.

Honeybee larvae *Apis mellifera* L. were exposed to repeated oral applications of 31.3, 62.5, 125.0, 250.1 and 500.1 µg dicamba/larva (equivalent to 0.198, 0.395, 0.790, 1.581 and 3.161 g dicamba/kg diet) in an *in vitro* test. One control group was included in the test. The larvae of the control treatment were fed with untreated artificial diet, which served as a vehicle for the test item and reference item.

On Day 1 the combs containing the larvae were transported from the hive to an acclimated laboratory room. Larvae were transferred from the combs to the crystal polystyrene grafting cells using a suitable grafting tool (e.g. grafting needle Swiss type). During grafting the C-shaped larvae were placed on the surface of the artificial diet within the grafting cells. Cells were placed in 48 well plates filled up to 1/3 with a piece of dental roll. Each replicate unit consisted of 12 larvae, and there were 3 replicates per treatment and control. Each larva was fed the treated diet daily between Day 3 and Day 6 using a sterile pipette.

The number of dead larvae was recorded on Day 4to Day 8. The presence of unconsumed food was qualitatively described on Day 7 and Day 8. After the last assessment (Day 8) the culture plates with all organisms were placed in a freezer.

All observations were made in comparison to the control larvae. For each concentration, the corrected mortality was calculated according to ABBOTT (1925) modified by SCHNEIDER-ORELLI (1947).

The LD_{50} value was calculated using the Trimmed Spearman-Karber Procedure. The statistical significance of the mortality values and the NOEC and NOED were calculated using Fisher's Exact Binomial Test with Bonferroni Correction ($\alpha = 0.05$).

Results and Discussion

Mortality data and other observations for the test material and reference item are summarised in the table below.

Table A 38: Summary of chronic toxicity of dicamba to honeybee larvae

Item applied	Dosage[1] [µg dicamba/larva][3]	Concentration [g dicamba/kg diet]	Day 8		
			Mortality mean %		OO[3]
			Absolute	Correct.[2]	Mean %
Control	-	-	8.3	-	8.6
Test item	31.3	0.198	2.8	0.0	2.8
	62.5	0.395	13.9	6.1	0.0
	125.0	0.790	11.1	3.0	6.4
	250.1	1.581	33.3*	27.3	20.4
	500.1	3.161	97.2*	97.0	100.0
Reference item	6.2	0.039	72.2	69.7	33.3

Treatment	Endpoints	Day 8 (120 h after 1st application)
Test item doses	NOED [g dicamba/larva]	125.0
	LD_{50} [g dicamba/larva] (95 % CL/lower-upper)	301.7 (263.8 – 345.1)
Test item concentrations	NOEC [g dicamba/kg diet]	0.790

Results are averages based on 3 replicates, containing 12 larvae each
Calculations were performed with non-rounded values
* Statistically significant difference in pairwise comparison between treatment and untreated control (Fisher`s Exact Binominal Test with Bonferroni Correction; α = 0.05; one-sided greater)
[1] All test item doses were based on a sum of applications on days 3 to 6
[2] Corrected mortality (according to Schneider-Orelli 1947), negative values are set to "0"
[3] OO: Other observations (large quantities of remaining food, smaller body size of larva)
– Not applicable

Validity Criteria

All of the validity criteria were met:

- Control mortality should be ≤ 15 % for larvae across all control replicates at day 8 (actual value 8.3 %)
- Reference item mortality should be ≥ 50 % for larvae across all reference replicates at day 8 (actual value 72.2 %)
- Concentration of the active substance in analysed sample of test item stock solution A should be ± 20 % of the nominal concentration of A (actual value ranged between 96 and 98 %)

Conclusions

The 8-day NOEC was determined to be a food concentration of 0.790 g dicamba/kg diet. The 8 day NOED was determined to be a total dose of 125.0 µg dicamba/larva. The 8-day LD_{50} was estimated to be 301.7 µg dicamba/larva, based on total developmental period.

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Comments of zRMS:	Study on chronic larvae toxicity of solo formulaiton of dicmba was not validated by the zRMS since in case of A18032E, containing 3 active compounds, respective larvae toxicity study should be performed with the formulated product in order to fulfil data requirements, while active substance endpoints should be generated at the EU level.

Reference:	KCP 10.3.1.3/02
Report	xxxxxxxxxxx(2016), Mesotrione - Honey bee (*Apis mellifera* L.) Larval Toxicity Test (Repeated Exposure through to Adult Emergence), Report Number S16-00332. Eurofins Agroscience Services Ecotox GmbH, Eutinger Str. 24 75223 Niefern-Öschelbronn, Germany (Syngenta file No. ZA1296_10465).
Guideline(s):	OECD DRAFT Guidance Document on Honey bee (*Apis mellifera*) larval toxicity test, repeated exposure (version dated 20 July 2015)
Deviations:	No
GLP:	Yes

Acceptability:	Not evaluated, not relevant for the zonal evaluation of A18032E (study with formulation in question should be performed to fulfil data requirements)
Duplication (if vertebrate study)	No

Materials and methods

Test Material	Mesotrione technical
Lot/Batch #:	SMO0H028
Actual content of active ingredients:	Nominal: 79% w/w Analysed: 84.6% w/w
Description:	brown solid
Stability of test compound:	Stable in solution sufficient for test purpose
Reanalysis/Expiry date:	20 Jan 2017
Density:	not applicable
Treatments	
Test rates:	From day 3 until day 6 of the test, concentrations of 28.5, 51.3, 92.3, 166 and 299 mg mesotrione/kg diet were provided to larvae One single concentration of 48.0 mg dimethoate/kg diet was provided to the larvae of the reference item group
Control:	untreated artificial diet
Toxic standard:	Dimethoate tech. (BAS 152 I), purity 98.8%
Application method:	Oral application via sterile pipette
Test organisms	
Species:	Worker honey bee larvae *Apis mellifera* L. subspecies *carnica*
Age:	First instar (L1) during grafting
Source:	Eurofins Agroscience Services Ecotox GmbH Neulingen Göbrichen Field Station, Nordweg 10, 75245 Neulingen Göbrichen, Germany
Food:	Aqueous sugar solutions mixed with royal jelly and the test item (no test item in the control group) (Diets A, B and C from the test guidance)
Test Design	
Test cage description:	Crystal polystyrene grafting cells placed in 48 well plates.
Replication:	3
No. of larvae/replicate:	14
Environmental test conditions	
Temperature:	33.6 – 34.8 °C
Humidity:	95 ± 5 % (day 1-8), 80 ± 5 % (day 8-15) and 50 ± 10 % (day 15-22)
Photoperiod:	Constant darkness
Duration of test:	Pre-grafting (*in vivo*): D-3 to D0 Grafting: D1 Pre-exposure (*in vitro*): D1 to D3 Application: D3 to D6 Post exposure (*in vitro*): D7 to D8 Pupation phase: D8-D22

Study Design and Methods

Experimental dates: 27 June to 06 September 2016

On the first day of the dose response test, synchronised honey bee larvae (*Apis mellifera carnica* POLLMANN, first instar, L1) were transferred into well plates where they were fed a standardised amount of artificial diet. From day 3 until day 6 of the test, concentrations of 28.5, 51.3, 92.3, 166 and 299 mg mesotrione/kg diet were provided to larvae of the test item groups and one single concentration of 48.0 mg dimethoate/kg diet was provided to the larvae of the reference item group with diet B or C.

These concentrations are equivalent to cumulative doses of 4.39, 7.90, 14.2, 25.6 and 46.0 µg mesotrione/larva per developmental period and 7.39 µg dimethoate/larva per developmental period. The analysed purity was considered for calculation of the test item and reference item concentrations. The cumulative feeding volume from day 3 until day 6 of 140 µL diet per larva and the density of the diet (1.1 g/cm3) were considered for the calculation of the cumulative doses. The presence of uneaten diet was qualitatively recorded on day 8.
A control and a solvent control group were included in the test and exposed for the same period of time under identical exposure conditions to the untreated diet.
Each treatment group consisted of 42 larvae from three different colonies (each colony representing a replicate). Mortalities during the larval phase were assessed daily from day 4 until day 8. Mortalities during the pupation phase were assessed on day 15 and on day 22. The adult emergence rate was assessed on day 22. Other observations and adverse effects were recorded in comparison to the solvent control group.

Results and Discussion

The analytical dose verification of the test item treated larval diet for each test item group from day 3 until day 6 resulted in recoveries between 80 % and 101 % of nominal. From day 3 until day 8, the larval mortality in the control and solvent control group were both 0.0 %. Larval mortality in the reference item group was 97.6 %. Compared to the solvent control group larval mortality was not statistically significantly increased in any test item group on day 8 (Fisher's Exact Tests with Bonferroni Correction, one-sided greater, $\alpha = 0.05$). On day 8, the LC_{50}/LD_{50} value relating to larval mortality for mesotrione could not be calculated since the mortality was below 50 % in all test item groups, but can be regarded as > 299 mg mesotrione/kg diet or > 46.0 µg mesotrione/larva per developmental period, respectively. Uneaten food was observed in the control groups, the test item groups of 28.5, 92.3, 166 and 299 mg mesotrione/kg diet and in the reference item group.

Table A 39: The Effects of Mesotrione on the Larval Mortality of the Honey Bee, *Apis mellifera* at Day 8 after Repeated Exposure

Treatment Group	Concentration		Cumulative Dose		Larval Mortality [%]
Control	---	---	---	---	0.0
Solvent Control	---	---	---	---	0.0
Test Item (Mesotrione)	28.5	[mg mesotrione/ kg diet] [a]	4.39	[µg mesotrione/larva per developmental period] [a b]	0.0
	51.3		7.90		0.0
	92.3		14.2		4.8
	166		25.6		4.8
	299		46.0		4.8
Reference Item (Dimethoate)	48.0	[mg dimethoate/ kg diet] [a]	7.39	[µg dimethoate/larva per developmental period] [a b]	97.6
Endpoints for day 8					
NOEC	299 mg mesotrione/kg diet				
NOED	46.0 µg mesotrione/larva per developmental period				
LC_{50}	> 299 mg mesotrione/kg diet				
LD_{50}	> 46.0 µg mesotrione/larva per developmental period				

[a] Based on the analysed purity
[b] Based on the cumulative feeding volume from day 3 until day 6 of 140 µL diet/larva and a density of the diet of 1.1 g/cm^3

Table A 40: The Effects of Mesotrione on the Pupal Stage of the Honey Bee, *Apis mellifera* between Day 8 and Day 22 after Repeated Exposure

Treatment Group	Concentration		Cumulative Dose		Mortality on Day 15		Pupal Mortality on Day 22	
					[%]	Corrected [%]	[%]	Corrected [%]
Control	---	---	---	---	9.5	---	11.9	---
Solvent Control	---	---	---	---	16.7	---	19.0	---
Test Item (Mesotrione)	28.5	[mg mesotrione/ kg diet] [a]	4.39	[µg mesotrione/ larva per developmental period] [a b]	19.0	2.8	19.0	0.0
	51.3		7.90		19.0	2.8	21.4	3.0
	92.3		14.2		19.0	2.8	15.0	-4.9
	166		25.6		26.2	11.4	22.5	4.3
	299		46.0		16.7	0.0	15.0	-4.9

[a] Based on the analysed purity
[b] Based on the cumulative feeding volume from day 3 until day 6 of 140 µL diet/larva and a density of the diet of 1.1 g/cm^3

Analytical Verification

The analytical dose verification of the test item treated larval diet for each test item group from day 3 until day 6 resulted in recoveries between 80 % and 101 % of nominal, thus confirming correct preparation of the analysed test item stock solution.

Validity Criteria

All of the validity criteria were met:

- Control mortality should be ≤ 15% for larvae across all control replicates at day 8 (actual value 0%)
- Reference item mortality should be ≥ 50% for larvae across all reference replicates at day 8 (actual value 97.6%)
- On day 22 the adult emergence rates in the controls should be >70% across all replicates (actual values for the control and solvent control groups were 88.1 and 81.0 %, respectively).

Conclusions

On day 8, the NOEC relating to larval mortality for mesotrione was determined as 299 mg mesotrione/kg diet, equivalent to a NOED of 46.0 µg mesotrione/larva per developmental period.
On day 8, the LC_{50}/LD_{50} value relating to larval mortality could not be calculated but can be regarded as > 299 mg mesotrione/kg diet or > 46.0 µg mesotrione/larva per developmental period, respectively.
On day 22, the NOEC relating to adult emergence for mesotrione was determined as 299 mg mesotrione/kg diet, equivalent to a NOED of 46.0 µg mesotrione/larva per developmental period.
On day 22, the EC_{10}/ED_{10}, EC_{20}/ED_{20} and the EC_{50}/ED_{50} values relating to adult emergence could not be calculated but can be regarded as > 299 mg mesotrione/kg diet or > 46.0 µg mesotrione/larva per developmental period, respectively.

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Comments of zRMS:	Study on chronic larvae toxicity of nicosulfuron was not validated by the zRMS since in case of A18032E, containing 3 active compounds, respective larvae toxicity study should be performed with the formulated product in order to fulfil data requirements, while active substance endpoints should be generated at the EU level.

Reference:	10.3.1.3/03
Report	Klank C, (2014), Nicosulfuron (DPX-V9360) Technical: Honey Bee (*Apis mellifera* L.) Larval Toxicity Test (Single Feeding Exposure). Report Number S14-00341. Eurofins Agroscience Services EcoChem GmbH, Eutinger Str. 24, 75223 Niefern-Öschelbronn, Germany.
Guideline(s):	OECD (2013): Guideline for the testing of chemicals 237; Honey bee (*Apis mellifera*)

	Larval Toxicity Test, Single Exposure
Deviations:	No
GLP:	Yes
Acceptability:	Not evaluated, not relevant for the zonal evaluation of A18032E (study with formulation in question should be performed to fulfil data requirements)
Duplication (if vertebrate study)	-

Executive Summary

In a single exposure toxicity test, honeybee (*Apis mellifera* L.) 3 days old larvae were exposed to nicosulfuron at five test doses of 1.25, 2.5, 5.0, 10.0 and 20.0 µg/larva for 72 hours. First instar (L1) synchronised larvae were initially placed into 48 well plates and were fed with a standardised amount of untreated diet. On day 4, a single dose of the test item was administered to the larvae with the diet. There were three replicates of 14 honeybee larvae each for each treatment group. A reference item (dimethoate) was simultaneously tested at a single dose (8.80 µg dimethoate/larva). A control (untreated diet) and a solvent control (diet containing 5% acetone) were also tested in parallel.

Assessments on mortality were performed at 24, 48 and 72 h after feeding with treated diet.

The observed mean mortality after 72 hours at the administered doses of nicosulfuron 1.25, 2.5, 5.0, 10.0 and 20.0 µg/larva was 19.0, 19.0, 23.8, 9.5 and 14.3%, respectively and it was not statistically significantly different compared to the solvent control (7.1% mean mortality). Consequently, the 72-h LD_{50} was > 20.0 µg nicosulfuron/larva and the 72-h NOED higher or equal to 20.0 nicosulfuron/larva, the highest dose tested.

Materials and methods

1. **Test material:** Nicosulfuron
   Batch no.: MAY09MA215
   Purity: 94.6% w/w
   Description: Solid / white to grey
2. **Vehicle and positive control**
   Controls: For the test item treatments acetone was used as the solvent. The definite feeding solutions contained 5 % acetone.
   Reference item: BAS 152 I containing 99.8% dimethoate
3. **Test organism:** *Apis mellifera* L.
   Life stage: First instar larvae (L1)
   Source: Eurofins Agroscience Services, EcoChem GmbH, Eutinger Straße 24, D-75223 Niefern-Öschelbronn, Germany
   The hive(s) used for honey bee larvae collection for this test were adequately fed, healthy, as far as possible disease free and queen right. No chemical substances (such as antibiotics, anti varroa treatments, pesticides, etc.) had been used in the hive within 4 weeks preceding the start of the test.
   Feeding: The food was composed of three different diets (prepared freshly at each feeding day) adapted to the needs of larvae at different stages of development.
   Diet A provided on day 1: 50% weight of fresh royal jelly + 50% weight of an aqueous solution containing 2% weight of yeast extract, 12% weight glucose and 12% weight fructose.
   Diet B provided on day 3: 50% weight of fresh royal jelly + 50% weight of an aqueous solution containing 3% weight of yeast extract, 15% weight glucose and 15% weight fructose.
   Diet C provided on day 4: 50% weight of fresh royal jelly + 50% weight of an aqueous solution containing 4% weight of yeast extract, 18% weight glucose and 18% weight fructose.

Test units: Crystal polysterene grafting cells (internal diameter 9 mm). Each cell was placed into a well of a 48-well cellular culture plate.

4. Environmental conditions

Temperature: 32.4 – 34.4°C
Humidity: 51.9 – 100.00%
Light: Darkness

Study design and methods

1. In life phase: Jun 20 to Jul 10, 2014

2. Test organism assignment and treatment

To ensure the production of synchronized larvae of at least three replicate colonies, the queens of six colonies were confined in their own colony in an excluder cage containing a comb with empty cells four days prior to the test start. Within 30 hours after encaging, the queens were released from the cages. The combs containing eggs were left in the cages during the incubation stage and until hatching. On the day of test start three out of six colonies were selected containing the highest number of synchronized larvae. The corresponding combs were transferred from the hives to the laboratory using an insulated container in order to avoid temperature variation.

On day 1 the test was initiated with larvae in excess. Therefore two reserve plates were prepared containing larvae of the same replicate hives. Before application of the test item on day 4, it was assured that all larvae used were of similar size and alive. For each treatment group 3 to 6 non-suitable larvae were replaced with individuals from the reserve plates, using larvae from the same hive to maintain 14 larvae per replicate.

On day 1 (D1) the required amount of untreated diet A (20 µL/larva) was dropped into each cell of the well plate. Using a grafting tool, one larva was delicately transferred from the comb to each cell on the surface of the diet. When a plate was completed, it was placed into a hermetically sealed Plexiglas desiccator. On day 3 (D3) the larvae were fed with the required amount of untreated diet B (20 µL/larva). On day 4 the larvae were treated with 30 µL of the diet C containing the test solution at the suitable concentration (control: untreated diet C; solvent control: untreated diet C containing the same amount of solvent as the test item groups; treatments: diet C containing the application solutions i.e. part of the water to prepare diet C was replaced by the respective application solution).

3. Dose preparation

A stock solution was prepared in by adding 891 mg nicosulfuron in 49.1 g acetone. This stock was further diluted (four consecutive times with acetone (each time 1:1 dilution) in order to prepare four further test solutions. The reference item stock solution was prepared by mixing 88 mg of the test item in 29.9 g acetone. For each dose 0.36 g of each of these test solutions was added in diet C replacing the part of the water. A solvent control dose was also prepared by adding 0.36 g acetone in diet C replacing the part of the water. The mixing of the test solutions with the diet was performed just before administration on day 4.

4. Measurements and observations

Mortality was assessed after 24 h, 48 h and 72 h (± 45 min) after feeding with treated diet. A larva was recorded as dead if no respiration was observed. At each assessment date, dead larvae were removed for sanitary reasons.

On day 7 (D7) during the final assessment of mortality the presence of uneaten food was qualitatively recorded.

Analytical dose verification was performed in samples from the stock and all test item and control feeding solutions with a validated method. Samples of the test item stock solution, application solutions of each test item group and the control solutions were taken on day 4 directly after preparation and before application. Nicosulfuron was analysed in each test item and control solution by liquid chromatography and mass spectrometric detection (LC-MS/MS).

Temperature and humidity were recorded continuously with calibrated data loggers from day 1 to day 7.

5. Statistics

For day 7 (72h after feeding) the LD_{50} with 95 % confidence limits could not be calculated since the observed mortalities were below 50 % in all test item groups. Fisher's Exact Test with Bonferroni Correction (one-sided greater, $\alpha = 0.05$) was used to evaluate whether there was a significant difference between the mortality data of the test item groups and the solvent control group in order to determine the NOED (No-observed-effect-dose) for mortality on day 7 (72 h after feeding). Fisher's Exact Test (one-sided greater, $a = 0.05$) was used to evaluate whether there was a significant difference between the mortality data of the reference item group and the water control group.

For the statistical evaluation the statistics program ToxRat professional, Version 2.10 was used.

Results and discussion

A. Mortality

No mortality occurred in the control group on all assessment days over the whole test duration. In the solvent control group a mortality of 7.1 % across all replicates was observed at the final mortality assessment on day 7.

A summary of the mortality results over the test period 24 h, 48 h and 72 h after feeding with untreated and treated diet and the presence of uneaten food on day 7 are presented in the following table.

Table A 41: Honeybee larval toxicity test: mortality results

Treatments (µg a.s./ larva)	% cumulative mean mortality			% adjusted cumulative mean mortality			Alive larvae after 72 h with presence of uneaten food
	24 h	48 h	72 h	24 h	48 h	72 h	
Control (0)	0.0	0.0	0.0	–	–	–	5
Solvent control (0)	2.4	7.1	7.1	–	–	–	20
1.25	0.0	11.9	19.0	-2.5	5.2	12.8	18
2.5	2.4	19.0	19.0	0.0	12.8	12.8	20
5.0	2.4	21.4	23.8	0.0	15.4	18.0	28
10.0	0.0	7.1	9.5	-2.5	0.0	2.6	24
20.0	0.0	14.3	14.3	-2.5	7.8	7.8	29
Reference item 8.80	26.2*	85.7*	88.1*	26.2	85.7	88.1	5

* Significantly increased compared to the control (Fisher's Exact test, one-sided greater, $p<0.01$).

B. Validity Criteria

The cumulative larval mortality from day 4 to day 7 was < 15% across all replicates (actual 0.0 % in the control group and 7.1 % in the solvent control group). The reference item cumulative larval mortality (after correction) was > 50% on day 7 across all replicates (88.1%).

C. Analytical Verification

In the control groups no nicosulfuron was detectable, respectively the concentration of the test item was below the limit of detection of 0.3 mg nicosulfuron/L. The measured concentration of nicosulfuron in the stock solution was 112 % of nominal. The lower test item doses were prepared by serial dilution. The recovery rates of nicosulfuron in the test item solutions for all test item groups were between 110 % and 115 %. Thus, the concentration of the stock solution and any test item solution was confirmed.

D. Toxicity Endpoints

The NOED was determined to be 20 µg nicosulfuron/larva, the highest dose tested, based on mortality at the end of the test on D7 (72 hours after feeding with treated diet).

The 72 h LD_{50} could not be calculated since the observed mortalities were below 50 % in all test item groups tested but can be regarded as > 20 µg nicosulfuron/larva, the highest dose tested.

Statistical analysis in the original report determined that there were no statistically significant ($P>0.05$) treatment related effects on all the parameters evaluated and the NOEC was determined to be >20 µg nicosulfuron/larva, the highest concentration tested. As a result of these findings, it can be concluded that no reliable EC_x values could be determined and no additional statistical analysis was performed on any of the parameters from the original study.

~~**Conclusion**~~

~~In a single dose honeybee larval toxicity test the LD_{50} value at 72 hours after treatment was greater than 20.0 µg nicosulfuron/larva and the 72-h NOED was higher than or equal to 20.0 µg nicosulfuron/larva, the highest dose tested.~~

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 non-target arthropods other than bees

A 2.3.2.1 KCP 10.3.2.1 Standard laboratory testing for non-target arthropods

Comments of zRMS:	The study was performed in line with the respective guideline with no deviations. All the validity criteria were met and the study is considered acceptable with the following endpoint relevant for the risk assessment: LR_{50} = 1028.2 g product/ha

Reference:	10.3.2.1
Report	Fallowfield L, (2012), Mesotrione/Nicosulfuron/Dicamba WG (A18032E) plus A12127R (Adigor adjuvant) – A rate-response laboratory bioassay of the effects of fresh residues on the predatory mite, *Typhlodromus pyri* (Acari: Phytoseiidae). Report Number SYN-12-28. Mambo-Tox Ltd., 2 Venture Road, Chilworth Science Park, Southampton, SO16 7NP (Syngenta file No. A18032E_10003).
Guideline(s):	Blümel *et al*. (2000). Laboratory residual contact test with the predatory mite *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) for regulatory testing of plant protection products.
Deviations:	No
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	-

Materials

Test Material A18032E
Mesotrione/Dicamba/Nicosulfuron WG (15/31.25/10)

Lot/Batch #: SMU2BP001

Actual content of active ingredients:
Mesotrione: 15.6 % w/w corresponding to 156 g/kg
Dicamba: 31.3 % w/w corresponding to 313 g/kg
Nicosulfuron: 10.1 % w/w corresponding to 101 g/kg.

Description: Beige-coloured granules

Stability of test compound: Stable under standard conditions

Reanalysis/Expiry date: 30 September 2014

Treatments

Test rates: 75, 150, 300, 600 and 1200 g A18032E/ha

Control: Purified water

Toxic standard: BASF Perfekthion (nominally 400 g dimethoate/L, analysed 411.7 g dimethoate/L) was applied at a rate of 15 mL product per 200 L water/ha (6 g a.i./ha)

Adjuvant: Adigor (A12127R) (mixture of emulsified fatty acid esters on basis of oleic acid methylester). The ratio of A18032E to A12127R was 1: 2.5 (A18032E: A12127R). Results are expressed in terms of the test item containing the adjuvant in this ratio.

Spray volume rate: 200 L/ha

Application method: Schachtner track sprayer (3 bar pressure, 80° flat fan nozzle)

Test organisms

Species: *Typhlodromus pyri* (Acari: Phytoseiidae).

Age: Less than 24 h old protonymphs

Source:	In- house culture originally obtained from P.K. Nützlingszuchten, Welzheim, Germany in April 1995 and supplemented from the same source in 1996 and 1997.
Feeding:	1:1 v/v mixture of walnut (*Prunus* sp. Var. Butte) and apple (*Malus* sp. var. Red Delicious)
Test design	
Arenas:	2 glass plates (cover slides: 40 mm x 22 mm) placed on wet filter paper laid over a water-saturated synthetic foam block. A barrier of sticky material was drawn onto each plate to make an arena in which the mites were confined.
Replication:	3
No. of mites/arena :	20
Duration of test:	Mortality assessment: 0-7 days Fecundity assessment: 7-14 days
Environmental test conditions	
Temperature:	25 – 26 ºC
Humidity:	68 – 84 % relative humidity
Photoperiod:	16 h photoperiod (420 - 1000 lux).

Study Design and Methods

Experimental dates: 10 September 2012 to 2 October 2012

Treatments were applied to the glass plates and the bioassay initiated approximately 1.5 h later, once residues had dried. The glass plates were placed onto moistened tissue paper and a border of sticky material was applied to create arenas in which the mites were confined. Each test unit was then placed in a controlled-environment cabinet. The condition and survival of the mites was assessed after 24 hours and 7 days, by which time they were adult. Any dead, drowned and stuck mites were removed at the time of assessment.

The sex of the adult mites was then determined, to ensure a male to female ratio of 1:5 in each treatment, however, in rate 1200 g A18032E/ha treatment this ratio was not achieved due to the immaturity of the mites. They were then left in situ so that their reproduction could be assessed over a further 7 days. Any eggs that were produced prior to 7 DAT were removed and discarded. For 7 days, the total egg production (numbers of eggs plus live and dead juvenile stages) was recorded for each unit. Assessments of oviposition activities were carried out at 10, 13 and 14 DAT. Any eggs and nymphs present were recorded and then removed. In addition, the condition of the adult female and male mites in each arena was recorded on each date. These reproduction assessments were made for mites from all treatment rates of A18032E and from the control treatment.

The percentage mortality at each treatment rate was corrected for mortality in the control treatment using Abbott's formula (Abbott, 1925). The data for mortality at 7 day were analysed by Probit analysis, to determine the median lethal rate (LR_{50}) with the 95% confidence intervals determined by Chi square goodness of fit test ($\alpha = 0.05$). Prior to analysis, the dose rates were $\log_{10}$-transformed. The level of background mortality was estimated by the software taking account of all available data.

To determine the NOER, mortality in the individual test item treatments was compared to that in the control treatment using Fisher's Exact Test ($\alpha = 0.05$). The data for mite reproduction was analysed by one-way ANOVA and Dunnett's Test ($\alpha = 0.05$).

Results and Discussion

Mortality and fecundity are summarised in the table below.

Table A 42: Effects of A18032E + A12127R on mortality and fecundity of *Typhlodromus pyri*, when exposed under extended laboratory test conditions

Treatment (g A18032E/ha)	Mean % mortality at 7 DAT [a]	Mean corrected % mortality at 7 DAT [b]	Mean eggs/female from 7 to 14 DAT [c]	% Effect on reproduction compared to control [d]
Control	15	-	8.0	-
1200	62*	55	0.5**	93.6
600	42*	31	4.0**	49.6
300	30	18	4.7**	41.3
150	27	14	5.1**	35.6
75	12	0	4.7**	41.3
Toxic reference	95*	94	-	-

a Results for mortality in individual treatments at 7 DAT were compared to that in the control by Fisher's Exact Test (α = 0.05). Treatment means that differed significantly from the control are indicated with an asterisk (*)
b Calculated using Abbot's formula.
c Results for reproduction over the assessment period were compared by one-way ANOVA and Dunnett's test (α = 0.05). Treatment means differing significantly from the control are indicated with a double-asterisk (**)
d A positive value indicates a decrease.

Validity criteria

The validity criteria for the test were met:

- Mean mortality in the control < 20% on test day 7 (observed 15 %)
- Mean mortality in the toxic standard > 50% (observed 95 %)
- Mean cumulative number of eggs produced from 7 to 14 days ≥ 4.0 per female in the control treatment (8.0 observed)

Conclusions

The 7-day LR_{50} for effects of A18032E plus adjuvant A12127R on mortality of *Typhlodromus pyri* under laboratory test conditions was calculated to be 1028.2 g A18032E/ha with 95% confidence limits of 601.2 – 2488.9 g A18032E/ha.
The test item had adverse effects on the reproduction of the surviving mites at treatment rates ≥ 75 g A18032E/ha, the lowest concentration tested.
The no observed effect rate (NOER), defined as the highest rate tested that did not produce a statistically significant adverse effect relative to the control, based on mortality was 300 g A18032E/ha, and based on reproduction was < 75 g A18032E/ha, the lowest concentration tested.

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Comments of zRMS:	The study was performed in line with the respective guideline with a minor deviation. It was noted that during the fecundity assessment phase of the study, the temperature reached a maximum of 26.6ºC, with 21 consecutive hours of readings above the intended maximum threshold of 23ºC. The study report indicated that this deviation was due to an inadequate control being achieved in the test room. However, based on the performance of the insects in the control, it was considered that this deviation did not adversely affect the outcome or the integrity of the study. In zRMS opinion the justification for the deviation is acceptable. All the validity criteria were met and the study is considered acceptable with the following endpoint relevant for the risk assessment: LR_{50} = 23.3 g product/ha

Reference:	10.3.2.1
Report	Stevens J, (2012), Mesotrione/Nicosulfuron/Dicamba WG (A18032E) plus Adigor (A12127R) – A rate-response laboratory bioassay of the effects of fresh residues on the parasitic wasp *Aphidius rhopalosiphi* (Hymenoptera, Braconidae). Report Number SYN-12-29. Mambo-Tox Ltd., 2 Venture Road, Chilworth Science Park, Southampton SO16 7NP, United Kingdom. (Syngenta file No. A18032E_10000).
Guideline(s):	Mead-Briggs et al. (2000). A laboratory test for evaluating the effects of plant protection products on the parasitic wasp, *Aphidius rhopalosiphi* (DeStephani-Perez) (Hymenoptera, Braconidae) (Draft, 2001).
Deviations:	Minor (see the commenting box above)
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	-

Materials

Test Material A18032E
Mesotrione/dicamba/nicosulfuron WG (15/31.25/10)

Lot/Batch #: SMU2BP001

Actual content of active ingredients:
Mesotrione: 15.6 % w/w corresponding to 156 g/kg
Dicamba: 31.3 % w/w corresponding to 313 g/kg
Nicosulfuron: 10.1 % w/w corresponding to 101 g/kg

Description: Beige granules

Stability of test compound: Stable under standard conditions

Reanalysis/Expiry date: 30 September 2014

Density: Not applicable

Treatments

Test rates: 3.125, 6.25, 12.5, 25 and 50 g A18032E/ha

Control: Purified water

Toxic standard: Perfekthion BAS 152 11 I (nominally 400 g dimethoate/L, analysed 411.7 g dimethoate/L) in purified water, applied at a rate of 0.10 mL product per ha in 200 L water /ha (0.04 g a.i./ha)

Spray volume rate: 200 L spray solution/ha

Adjuvant: Adigor (A12127R) (mixture of emulsified fatty acid esters on basis of oleic acid methylester). The ratio of A18032E to A12127R was 1: 2.5 (A18032E: A12127R). Results are expressed in terms of the test item containing the adjuvant in this ratio.

Application method: Schachtner track sprayer (3 bar pressure, 80° flat fan nozzle)

Test organisms

Species: *Aphidius rhopalosiphi* De Stefani-Perez. (Hymenoptera: Braconidae).

Age: Adults; female

Source: Culture maintained at Test Facility on cereal aphids (*Metopolophium dirhodum* and *Rhopalosiphum padi*). Originally obtained from Katz Biotech AG, Baruth, Germany

Feeding: 1:3 v/v solution of honey and water

Test design – Mortality phase

Arenas: Treated glass plates fitted to a square frame (10 cm x 10 cm external dimensions) with three holes (10 mm diameter) covered with fine gauge stainless steel mesh, and one access hole sealed with cotton wool bung

Replication: 4

No. of wasps/arena :	10
Test design - Fecundity phase	
Arenas:	Clear acrylic cylinders (9cm diameter, 20 cm high, tops covered with nylon netting) were placed over pots containing 15 barley seedlings (*Hordeum vulgare* Westminster). The untreated barley had been infested eight days previously with host aphids (>100 adults and nymphs of *Metopolopium dirhodum* and *Rhopalosiphum padi*).
Replication:	15 female wasps/treatment
No. of wasps/arena :	1
Duration of test:	Mortality assessment: 48 hours Fecundity assessment: 24 hours Observation of mummies developing: 10 days after adult removal
Environmental test conditions	
Temperature:*	Mortality assessment phase: 20 - 21°C Fecundity assessment phase: 19 - 26.6°C
Humidity:	Mortality assessment phase: 72% - 77% RH.
Photoperiod:	Mortality assessment phase: 16 h photoperiod (864 lux) Fecundity assessment phase: 16 h photoperiod (5222 lux)

* The temperature in the fecundity phase reached a maximum of 26.6°C, with 21 consecutive hours of readings above the intended maximum of 22°C. Based on the performance of the insects in the control, this deviation was not considered to have adversely affected the outcome of the bioassay or the integrity of the study.

Study Design and Methods

Experimental dates: 25 July 2012 – 21 August 2012

Treatments were applied to glass plates and once dry were used to construct arenas. The wasps were introduced to these arenas and their behaviour and mortality were assessed 2, 24 and 48 h later.

To assess any sub-lethal effects, reproduction assessments were then carried out using surviving females from the control and from the test material treatment rates of 6.25, 12.5, and 25 g A18032E/ha. Wasps were confined individually over untreated aphid-infested barley plants for 24 hours, before being removed. The plants were left for a further 10 days before the number of aphid mummies that had developed on plants where wasps had been found alive after the 24-h oviposition period was recorded.

The percentage mortality, defined as the number of moribund and dead insects combined, was calculated over 48 hours. The corrected percentage mortality (taking into account any control treatment losses) was derived using Abbott's (1925) formula. The median lethal rate (LR_{50}) was determined by Probit analysis on the log-transformed dose rates. The 95% confidence intervals for the LR_{50} value were calculated and a Chi-square goodness of fit ($\alpha = 0.05$) performed on the Probit line. Where mortality was observed in the individual treatments this was compared to that in the control using Fisher's Exact Test ($\alpha = 0.05$).

The numbers of mummies produced per female found alive after the 24-h parasitism period were analysed by one-way ANOVA ($\alpha = 0.05$) of the square root-transformed data. The percentage change in numbers of mummies produced in individual test item treatments, relative to the control, was also calculated using the equation:

$$(1-R_t/R_c)*100\%$$

where R_t and R_c are the absolute values for reproduction observed in the treatment and control groups, respectively.

Results and Discussion

Mortality and fecundity are summarised in the table below.

Table A 43: Effects of fresh residues of A18032E + A12127R on mortality and fecundity of *Aphidius rhopalosiphi*, when exposed under laboratory test conditions

Treatment (g A18032E /ha)	Mean % mortality at 48 h [a]	Mean % corrected mortality at 48 h (M-value) [b]	Number females successfully assessed for reproductive capacity	Mean number mummies per surviving female [c]	% change in reproduction compared to control (R-value) [d]
Control	5.0	-	15	24.7	-
3.125	5.0	0.0	n.d.	n.d.	n.d.
6.25	15.0	10.5	12	24.1	2.6
12.5	32.5*	28.9	13	23.3	5.8
25	42.5*	39.5	15	23.7	4.0
50	87.5*	86.8	n.d.	n.d.	n.d.
Toxic reference	100*	100	n.d.	n.d.	n.d.

[a] The results for the test item and for the toxic reference treatment were compared to the control using Fisher's Exact Test ($\alpha = 0.05$).
[b] Derived using Abbott's formula
[c] The results for the test-item treatments were compared to the control by one-way ANOVA ($\alpha = 0.05$), but there were no significant differences..
[d] Percentage effect on reproduction, relative to the control. A positive value indicates a decrease relative to the control
* Significant differences from the control
n.d. Not determined

Validity criteria

The validity criteria for the control groups were met:

- Mean mortality in control ≤ 13% (observed: 5.0%)
- Mortality in toxic reference ≤ 25 % at 2 hours (observed: 0%), ≥ 50% at 48 hours (observed: 100%)
- Mean number of mummies per female in the control ≥ 5.0 with no more than two zero values (observed: 24.7, no zero values)

Conclusions

The 48-h LR_{50} for effects of A18032E plus adjuvant A12127R, on *Aphidius rhopalosiphi* under laboratory test conditions was determined to be 23.3 g A18032E/ha (95% confidence limits of 15.6 and 32.7 g A18032E/ha).

The test item did not have adverse effects on the reproduction of the surviving wasps at treatment rates of up to and including 25 g A18032E/ha.

The no observed effect rate (NOER) was defined as the highest rate tested that did not produce a statistically significant adverse effect relative to the control, and was determined to be 6.25 and 25 g A18032E/ha, based on mortality and reproduction, respectively.

A 2.3.2.2 KCP 10.3.2.2 Extended laboratory testing, aged residue studies with non-target arthropods

Comments of zRMS:	The study was performed in line with the respective guideline with a minor deviation. It was noted that during the mortality assessment phase of the study, the temperature reached a maximum of 22.6ºC, with two periods (one of 5 h and one of 9 h) being above the intended maximum threshold of 22 ºC. The study report indicated that these deviations were due to inadequate control being achieved by the room. Since all the treatments were exposed to similar conditions it was considered that these deviations did not adversely affect the outcome nor the integrity of the study. In zRMS opinion the justification for the deviation is acceptable. All the validity criteria were met and the study is considered acceptable with the following endpoint relevant for the risk assessment: LR_{50} > 1200 g product/ha

Reference:	10.3.2.2
Report	Stevens J, (2012a), Mesotrione/Nicosulfuron/Dicamba WG (A18032E) plus Adigor (A12127R) – A rate-response extended laboratory bioassay of the effects of fresh residues on the parasitic wasp *Aphidius rhopalosiphi* (Hymenoptera, Braconidae). Report Number SYN-12-45. Mambo-Tox Ltd., 2 Venture Road, Chilworth Science Park, Southampton SO16 7NP, United Kingdom. (Syngenta file No. A18032E_10010).
Guideline(s):	Mead-Briggs *et al.* (2009). An extended laboratory test for evaluating the effects of plant protection products on the parasitic wasp, *Aphidius rhopalosiphi* (Hymenoptera, Braconidae). *BioControl* (DOI 10.2007/s10526-009-92607). Published online 5 December 2009. Springer.
Deviations:	Minor (see the commenting box above)
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	-

Materials

Test Material	A18032E Mesotrione/dicamba/nicosulfuron WG (15/31.25/10)	
Lot/Batch #:	SMU2BP001	
Actual content of active ingredients:	Mesotrione: Dicamba: Nicosulfuron:	15.6 % w/w corresponding to 156 g/kg 31.3 % w/w corresponding to 313 g/kg 10.1 % w/w corresponding to 101 g/kg
Description:	Beige solid	
Stability of test compound:	Stable under standard conditions	
Reanalysis/Expiry date:	30 September 2014	
Density:	Not applicable	
Treatments		
Test rates:	150, 300, 600 and 1200 g A18032E/ha	
Control:	Purified water	
Toxic standard:	Perfekthion BAS 152 11 I (nominally 400 g dimethoate/L, analysed 411.7 g dimethoate/L) in purified water, applied at a rate of 10 mL product per ha in 400 L water /ha	
Spray volume rate:	400 L spray solution/ha	
Adjuvant:	Adigor (A12127R) (mixture of emulsified fatty acid esters on basis of oleic acid methylester). The ratio of A18032E to A12127R was 1: 2.5 (A18032E: A12127R). Results are expressed in terms of the test item containing the adjuvant in this ratio.	
Application method:	Schachtner track sprayer (3 bar pressure, 80° flat fan nozzle)	
Test organisms		
Species:	*Aphidius rhopalosiphi* De Stefani-Perez. (Hymenoptera: Braconidae)	
Age:	Adults; female	
Source:	Culture maintained at Test Facility on cereal aphids (*Metopolophium dirhodum* and *Rhopalosiphum padi*). Originally obtained from Katz Biotech AG, Baruth, Germany.	
Feeding:	1:3 v/v solution of honey and water	
Test design – Mortality phase		
Arenas:	Clear acrylic cylinders (8cm diameter, 20 cm high, tops covered with nylon netting) were placed over pots containing approximately 10 sprayed barley seedlings (*Hordeum vulgare* Westminster)	
Replication:	6	
No. of wasps/arena :	5	

Test design - Fecundity phase	
Arenas:	Clear acrylic cylinders (9cm diameter, 20 cm high, tops covered with nylon netting) were placed over pots containing 15 barley seedlings (*Hordeum vulgare* Westminster). The untreated barley had been infested eight days previously with host aphids (>100 adults and nymphs of *Metopolopium dirhodum* and *Rhopalosiphum padi*).
Replication:	15 female wasps/treatment
No. of wasps/arena :	1
Duration of test:	Mortality assessment: 48 hours Fecundity assessment: 24 hours Observation of mummies developing: 10 days after adult removal
Environmental test conditions	
Temperature:	Mortality assessment phase: 21 – 22.6 °C Fecundity assessment phase: 20 – 22.8 °C (temperature above 22°C for a period < 2h and not considered a deviation)
Humidity:	Mortality assessment phase: 63 – 72 % RH
Photoperiod:	Mortality assessment phase: 16 h photoperiod (1229 lux) Fecundity assessment phase: 16 h photoperiod (4401 lux)

Study Design and Methods

Experimental dates: 10 October 2012 – 03 December 2012

Treatments were applied to test plants (seedlings of barley - *Hordeum vulgare* var. Westminster) which, once dry, were placed within arenas. The wasps were introduced to these arenas and their behaviour and mortality were assessed 2, 24 and 48 h later.

To assess any sub-lethal effects, reproduction assessments were then carried out using surviving females from the control and from the test material treatment rates of 150, 300, 600, and 1200 g A18032E/ha. Wasps were confined individually over untreated aphid-infested barley plants for 24 hours, before being removed. The plants were left for a further 10 days before the number of aphid mummies that had developed on plants where wasps had been found alive after the 24-h oviposition period was recorded.

The percentage mortality, defined as the number of moribund and dead insects combined, was calculated over 48 hours. The corrected percentage mortality (taking into account any control treatment losses) was derived using Abbott's (1925) formula. Mortality in the individual test item treatments was compared to that in the control treatment using Fisher's Exact Test ($\alpha = 0.05$).

The numbers of mummies produced per female found alive after the 24-h parasitism period were analysed by one-way ANOVA and Dunnett's t-test ($\alpha = 0.05$) of the square root-transformed data. The percentage change in numbers of mummies produced in individual test item treatments, relative to the control, was also calculated using the equation:

$$\% \text{ change} = (1-[R_t/R_{c]})*100$$

where R_t and R_c are the absolute values for reproduction observed in the treatment and control groups, respectively.

Results and Discussion

Mortality and fecundity are summarised in the table below.

Table A 44: Effects of fresh residues of A18032E + A12127R on mortality and fecundity of *Aphidius rhopalosiphi*, when exposed under extended laboratory test conditions

Treatment (g A18032E/ha)	Mean % mortality at 48 h [a]	Mean % corrected mortality at 48 h (M-value) [b]	Number females successfully assessed for reproductive capacity	Mean number mummies per surviving female [c]	% Effect on reproduction compared to control (R-value) [d]
Control	0.0	-	15	20.8	-
150	0.0	0.0	n.d.	n.d.	n.d.
300	0.0	0.0	13	16.0	23.1
600	10.0	10.0	14	23.0	-10.6
1200	20.0*	20.0	15	23.0	-10.6
Toxic reference	100*	100	n.d.	n.d.	n.d.

[a] The results for the individual treatments were compared to the control using Fisher's Exact Test (α=0.05)
[b] Derived using Abbott's formula
[c] The results for the test item treatments were compared to the control by one-way ANOVA and Dunnett's t-test (α=0.05), but there were no significant differences
[d] Percentage effect on reproduction, relative to the control. A negative value indicates an increase relative to the control
* Significant differences from the control
n.d. Not determined

Validity criteria

The validity criteria for the control groups were met:

- Mean mortality in control ≤ 10 % (i.e. 3 wasps from 30) (observed 0%)
- Mortality in toxic reference ≤ 25 % at 2 hours (observed: 0%), ≥ 50% at 48 hours (observed 100%)
- Mean number of mummies per female in the control ≥ 5.0 with no more than two zero values (observed 20.8, no zero values)

Conclusions

The 48-h LR_{50} for effects of A18032E plus adjuvant A12127R on *Aphidius rhopalosiphi* under extended laboratory test conditions was determined to be >1200 g A18032E/ha, the maximum rate tested.
The test item did not have adverse effects on the reproduction of the surviving wasps at treatment rates of up to and including 1200 g A18032E/ha.
The no observed effect rate (NOER), defined as the highest rate tested that did not produce a statistically significant adverse effect relative to the control, based on mortality and reproduction, was 600 and 1200 g A18032E/ha, respectively.

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Comments of zRMS:	The study was performed in line with respective guideline with no deviations. All the validity criteria were met and the study is considered acceptable with the following endpoint relevant for the risk assessment: ER_{50} > 600 g product/ha

Reference:	10.3.2.2
Report	Tew, G (2013), Mesotrione/Nicosulfuron/Dicamba WG (A18032E) plus Adigor A12127R – A rate-response extended laboratory bioassay of the effects of fresh residues on the rove beetle, *Aleochara bilineata* (Coleoptera; Staphylinidae). Report Number SYN-12-46, Mambo-Tox Ltd., 2 Venture Road, Chilworth Science Park, Southampton SO16 7NP, United Kingdom. (Syngenta file No. A18032E_10015).
Guideline(s):	Grimm *et al.* 2000: A test for evaluating the chronic effects of plant protection products

	on the rove beetle *Aleochara bilineata* Gyll. (Coleoptera: Staphylinidae) under laboratory and extended laboratory conditions. IOBC Publiciation. ISBN 92-9067-129-7.
Deviations:	No
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	-

Materials

Test Material	A18032E Mesotrione/dicamba/nicosulfuron WG (15/31.25/10)	
Lot/Batch #:	SMU2BP001	
Actual content of active ingredients:	Mesotrione: Dicamba: Nicosulfuron:	15.6 % w/w corresponding to 156 g/kg 31.3 % w/w corresponding to 313 g/kg 10.1 % w/w corresponding to 101 g/kg
Description:	Beige solid	
Stability of test compound:	Stable under standard conditions	
Reanalysis/Expiry date:	30 September 2014	
Density:	Not applicable	
Treatments		
Test rates:	37.5, 75, 150, 300 and 600 g A18032E/ha	
Control:	Purified water	
Toxic standard:	Cyren (nominally 480 g chlorpyrifos/L) applied at a rate of 400 mL product /ha (192 g a.i./ha)	
Adjuvant:	Adigor (A12127R) (mixture of emulsified fatty acid esters on basis of oleic acid methylester). The ratio of A18032E to A12127R was 1: 2.5 (A18032E: A12127R). Results are expressed in terms of the test item containing the adjuvant in this ratio.	
Spray volume rate:	400 L/ha	
Application method:	Laboratory track-sprayer (Schachtner), 3 bar pressure, 80° flat-fan nozzle	
Test organisms		
Species:	*Aleochara bilineata*	
Age:	3 to 4 day old adults	
Source:	Obtained prior to test start as parasitised pupae of the onion fly, *Delia antiqua* Meig. (Diptera: Anthomyiidae) from De Groene Vlieg, Nieuwe Tonge, The Netherlands, and maintained in the Test Facility until test start	
Food:	Pellets of raw minced beef	
Host pupae for larvae to parasitize:	500 onion fly *Delia antiqua* (Meig.) pupae obtained from De Groene Vlieg, Nieuwe Tonge, The Netherlands were incorporated to the soil on days 7, 14 and 21	
Test design - Mortality phase		
Arenas:	Polystyrene boxes (17.1 cm x 11.3 cm x 6 cm) with close-fitting lids containing 4 to 5 holes covered with nylon netting. Each box was filled with approximately 977.18 g of a sandy soil to a depth of at least 4 cm.	
Substrate:	Sandy soil type LUFA 2.1 Organic carbon content: pH: WHC:	 0.62 ± 0.07% 5.1 ± 0.4 maintained at 35 ± 5 %
Replication:	4	
No. of beetles/arena :	20 (10 male + 10 female)	

Test design – Fecundity phase	
Arenas:	The soil from the mortality phase test vessels was transferred to two sizes of plastic pot placed one inside the other, measuring 9 cm diameter x 5 cm deep, and 9 cm diameter x 13 cm deep. Fine mesh (0.5 x 0.5 mm) nylon netting covered a hole in the lids of the smaller pots, and a coarser mesh (*ca*. 2.0 x 2.5 mm) covered a large hole in the base, allowing the emerging adults to fall through into the larger pot beneath.
Replication:	4
Duration of test:	70 days. Mortality phase: 0 – 28 days after treatment (DAT). Fecundity phase: 35 – 70 DAT
Environmental test conditions	
Temperature:	19.3 – 21.5 ºC (mortality phase) 19.6 – 21.2 ºC (fecundity phase)
Humidity:	67 – 75 % RH (mortality phase) 65 – 75 % RH (fecundity phases)
Photoperiod:	16 h photoperiod, 900 – 975 lux (mortality/fecundity phases)

Study Design and Methods

Experimental dates: 08 October 2012 to 09 January 2013

Treatments were applied to the test arenas and the adult beetles were introduced. At days 7, 14 and 21 during exposure, approximately 500 *Delia antiqua* pupae were incorporated beneath the soil. After 28 days all surviving adult beetles were removed from the substrate. The substrate containing the parasitized onion fly pupae was left to dry for one week. Thirty-five days after application the pupae were separated from the soil using a coarse sieve (*ca*. 1.5 mm mesh) and the pupae of each replicate were transferred to separate emergence pots and stored in a controlled environment room. Emerging beetles were counted and removed from the emergence containers every 2 – 3 days; emergence of the F_1 generation was monitored until the control treatment fell below a rate of two beetles per replicate per day (70 DAT).

Percentage mortalities were calculated, both before and after correction for control treatment losses using Abbott's formula. The 28-day survival assessment data were evaluated using Fisher's Exact Test ($\alpha = 0.05$).

The mean number of offspring produced per beetle and a measure of standard deviation was calculated for each treatment. The percentage effect on reproductive performance in the treated groups, compared to the control group, was calculated using the following equation:

% effect = $(1 – (Rt/Rc)) * 100$

Where Rt and Rc are the numbers of offspring observed in the treatment and control groups, respectively.

The numbers of progeny per replicate in the test item and control treatments were analysed by one-way analysis of variance (ANOVA). The data were not suitable for Probit analysis.

Results and Discussion

Mortality and fecundity are summarised in the table below.

Table A 45: Effects of A18032E + A12127R on survival and reproduction of *Aleochara bilineata*

Treatment Rate (g A18032E/ha)	% Mortality at 28 days [a]	Corrected % mortality at 28 days [b]	Mean number of F1 progeny (per replicate [c]	% Effect on reproduction (R value) [d]
Control	8.8	-	909.8	-
37.5	16.3	8	968.3	-6.43
75	7.5	0	928.3	-2.03
150	7.5	0	899.3	1.15
300	8.8	0	933.3	-2.58
600	10.0	1	881.3	3.13
Toxic reference	100.0*	100	0.0	100

[a] Mortality in individual treatments was compared to that in the control using Fisher's Exact Test ($\alpha = 0.05$). An asterisk indicates a significant increase in mortality relative to the control.

[b] Values corrected using Abbott's formula (Abbott, 1925). Negative values have been given as zero.

[c] Analysed by one-way ANOVA ($\alpha - 0.05$), but none of the test item treatments differed significantly from the control. Data from the toxic reference treatment were not analysed.

[d] Percentage change in numbers of F_1 progeny relative to the control, calculated using the formula: $R = (1-(Rt/Rc)) \times 100$, where Rt and Rc are the numbers of offspring observed in the treatment and control groups, respectively. A positive value indicates a decrease relative to the control and a negative value an increase.

Validity Criteria

The mean number of beetles emerging from fly pupae in the control should be > 400 per replicate (nominally 27% of those provided) (observed 909.8). The mean number of beetles emerging in the toxic reference treatment should be reduced by >50%, relative to the control (observed 100%). Both these criteria were met.

Conclusions

The reproduction capacity of the rove beetle *Aleochara bilineata* exposed to A18032E plus adjuvant A12127R, at rates equivalent to 37.5, 75, 150, 300 and 600 g A18032E/ha, was not statistically significantly reduced compared to the control. The ER_{50} was therefore determined to be > 600 g A18032E/ha, the highest rate tested, and the NOER was 600 g A18032E/ha.

A 2.3.2.3 KCP 10.3.2.3 Semi-field studies with non-target arthropods

A 2.3.2.4 KCP 10.3.2.4 Field studies with non-target arthropods

A 2.3.2.5 KCP 10.3.2.5 Other routes of exposure for non-target arthropods

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

Comments of zRMS:	The study was performed in line with OECD 222 with a minor deviation. It was noted that the minimum weight of worms used in this study was 281 mg while the guideline recommends a minimum of 300 mg. However, this deviation is considered to have no impact on the outcome of the study. The test design was relevant to derive both NOEC and ECx values (8 concentrations, 8 replicates for control, 4 replicates per treatment group). The reliability of the EC_{10} value was evaluated in line with recommendations of EFSA Supporting publication 2019:EN-1673: • NW (normalised width) of 0.54 was calculated, which results in rating "fair" in line with Table E9 in EFSA Supporting publication 2019:EN-1673, • median EC_{10} is lower than $EC_{20,low}$, • the dose-response curve is shallow with steepness of 0.3 (i.e. <0.33). Based on the above indications the calculated EC_{10} may be considered sufficiently reliable. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: 56d NOEC = 62.5 mg product/kg soil dw 56d EC_{50} = 163.2 mg product/kg soil dw 56d EC_{10} = 48.55 mg product/kg soil dw

Reference:	10.4.1.1
Report	Friedrich S, (2012). Mesotrione/Dicamba/Nicosulfuron WG (A18032E) plus Adigor (A12127R) – Sublethal Toxicity to the Earthworm *Eisenia fetida* in Artificial Soil, Report Number 12 10 48 147 S. BioChem agrar Labor für biologische und chemische Analytik GmbH, Kupferstraße 6 04827 Gerichshain, Germany. (Syngenta file No. A18032E_10007).
Guideline(s):	OECD Guideline for testing of chemicals No. 222 (adopted 13 April 2004): Earthworm Reproduction Test (*Eisenia fetida*/*Eisenia andrei*)
Deviations:	Minor (see the commenting box above)
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	-

Materials

Test Material	A18032E Mesotrione/dicamba/nicosulfuron WG (15/31.25/10)	
Lot/Batch #:	SMU2BP001	
Actual content of active ingredients:	Mesotrione:	15.6 % w/w corresponding to 156 g/kg
	Dicamba:	31.3 % w/w corresponding to 313 g/kg
	Nicosulfuron:	10.1 % w/w corresponding to 101 g/kg
Description:	Beige solid	

Stability of test compound:	Stable under standard conditions
Reanalysis/Expiry date:	30 September 2014
Density:	Not applicable
Treatments	
Test rates:	7.81, 15.63, 31.25, 62.5, 125, 250, 500 and 1000 mg A18032E/kg soil d.w.
Control:	Untreated substrate irrigated with deionised water
Toxic standard:	Nutdazim 50 FLOW (Carbendazim SC 500) was tested at concentrations of 5 and 10 mg product/kg soil dry weight (separate study - No.: R12 10 48 004 S dated 29 October 2012)
Adjuvant:	Adigor (A12127R) (mixture of emulsified fatty acid esters on basis of oleic acid methylester). The ratio of A18032E to A12127R was 1: 2.5 (A18032E: A12127R). Results are expressed in terms of the test item containing the adjuvant in this ratio.
Test organisms	
Species:	*Eisenia fetida* (Savigny, 1826) [subspecies *Eisenia andrei* (Bouché, 1972)]
Age and weight range at test start:	Adult worms (approximately 3 months old with clitellum); 281 – 458 mg/worm
Source:	Reared in the test facility (original breeding animals purchased from W. Neudorff GmbH KG, An der Mühle 3, 31860 Emmerthal, Germany)
Feeding:	Air-dried finely ground horse manure
Test design	
Vessels:	Plastic (Bellaplast) vessel (inner dimensions: 16.5 × 12 × 6 cm) with a lid pervious to air and light
Substrate:	Artificial soil comprising 10% sphagnum peat, 20% kaolin clay, 69.5% industrial quartz sand (> 50% of the particles between 0.05 mm and 0.2 mm) and 0.5% calcium carbonate. 810 g wet weight soil, corresponding to 600 g dry weight, of artificial soil was added to each test vessel.
Replication:	8 for control, 4 for treatment group
No. of worms/arena :	10
Duration of test:	8 weeks
Environmental test conditions	
Temperature:	18.2 – 21.4 °C
pH of soil*:	Test start: 6.02 – 6.11 Test end: 5.79 – 6.02
Water content of soil*:	Test start: 34.9 – 35.0 % (equivalent to 55.4 – 55.6 % of WHC) Test end: 34.1 – 35.0 % (equivalent to 54.1 – 55.6 % of WHC)
Photoperiod:	16 hours light:8 hours dark 650 Lux

* pooled replicates per treatment groups.

Study Design and Methods

Experimental dates: 09 October 2012 to 04 December 2012

Approximately 24 hours prior to test start, the artificial soil was prepared and deionised water was added to the dry components to obtain approximately 50 % of the final water content. The worms were acclimatised in a separate batch of the untreated artificial substrate for approximately 24 hours before test start. On the day of test start, the test item was introduced by dispersing the quantity of the test item required to obtain the desired test concentration in the volume of water required to hydrate the soil to 40-60 % of its WHC. The acclimatised test animals were washed, gently dried on a paper towel, weighed

and randomly placed onto the test substrate (10 animals per test vessel). After approximately 30 minutes the test vessels were covered with perforated transparent lids.
One day after application, 5 g dried and ground horse manure was scattered on the soil surface of each test vessel. This was sprinkled with 5 mL deionised water. The feeding interval was weekly during the first four weeks of the test.
After four weeks, the adult worms were removed from the test vessels, and mortality and the body weight of the surviving worms were determined. After all of the adult worms had been removed, the soil in each vessel was mixed with 5 g horse manure. Four weeks later, the number of surviving juveniles and any morphological alterations were recorded. Observations of behavioural and pathological symptoms were observed weekly.
The EC_{50} (number of juveniles) was calculated by linear maximum likelihood regression, and the 95% confidence limits were computed by normal approximation. Fisher`s Exact Binomial Test with Bonferroni Correction and the Williams-t-test were used to compare the control with the test item groups. For statistical evaluation of the biomass change, the changed mean fresh weight of surviving worms per replicate was used.

Results and Discussion
Mortality and fecundity are summarised in the table below.

Table A 46: Effect of A18032E + A12127R on mortality, growth and reproduction of *Eisenia fetida*

Endpoints	Treatment groups (mg A18032E/kg soil dry weight)								
	Control	**7.81**	**15.63**	**31.25**	**62.5**	**125**	**250**	**500**	**1000**
Mean adult mortality at 28 days (%)	2.5	5.0	0	5.0	0	2.5	10.0	80.0*[1]	100.0*[1]
Mean % biomass change of adults from 0-28 days	40.7	41.1	38.3	39.7	33.9	32.4	10.2*[2]	-6.3*[2]	n.d.
Mean number of juveniles after 8 weeks	107.8	105.0	111.3	98.8	87.5	71.5*[2]	34.0*[2]	11.0*[2]	0.0*[2]
Coefficient of variation for reproduction (cv %)	20.4	22.1	13.5	29.7	16.9	21.0	49.0	79.6	n.d.
% difference in reproduction relative to the control	-	2.6	-3.2	8.4	18.8	33.6	68.4	89.8	100.0
LC_{50}	351 mg A18032E/kg soil d.w. (95% confidence limits 180 to 684 mg A18032E/kg soil d.w.)								
EC_{10} (reproduction)	48.55 mg A18032E/kg soil d.w. (95 % confidence limits 34.9 to 61.3)								
EC_{20} (reproduction)	73.6mg A18032E/kg soil d.w. (95 % confidence limits 57.7 to 88.05)								
EC_{50} (reproduction)	163.2 mg A18032E/kg soil d.w. (95% confidence limits 142.7 to 186.6 mg A18032E/kg soil d.w.)								
NOEC (mortality)	250 mg A18032E/kg soil d.w.								
NOEC (biomass)	125 mg A18032E/kg soil d.w.								
NOEC (reproduction)	62.5 mg A18032E/kg soil d.w.								

*[1]statistically significant compared to control (Fisher's Exact Binomial test with Bonferroni Correction, $p \leq 0.05$, one-sided greater)
*[2]statistically significant compared to control (Williams-t-test, $p \leq 0.05$, one-sided smaller)
n.d.: not determined because all worms died at this concentration before the end of the test
negative values = increase relative to control
d.w.: dry weight (of artificial soil)

Validity criteria
Validity criteria for the control groups were met:

- Adult mortality after 4 weeks: ≤ 10% (being 2.5%)
- Number of juveniles per replicate: ≥ 30 (being ≥ 76)
- Coefficient of variation for reproduction: ≤ 30% (being 20.4%)

Conclusions
In a chronic toxicity test in which earthworms (*Eisenia fetida*) were exposed to A18032E plus Adigor A12127R, the NOEC was determined to be 250, 125 and 62.5 mg A18032E /kg soil dry weight for mortality, biomass and reproduction, respectively. The EC_{50} (reproduction) was determined to be 163.2 mg A18032E/kg soil dry weight, with 95 % confidence limits of 142.7 – 186.6 mg A18032E/kg soil dry weight.

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

Comments of zRMS:	The study was performed in line with OECD 232 with minor deviations. It was noted that the temperature of the test area fell slightly below the minimum (18°C) for approximately 12 hours; the lowest temperature was 16.4°C. The temperature was below the minimum for only a few hours and all the validity criteria (relating to adult survival and reproduction) were fulfilled. The mean temperature was 19.8°C which was in line with the guideline recommended temperature of 20 ± 1°C. Therefore, this deviation was not considered significant or to have affected the integrity of the study. During the extraction process it was noted that some of the test vessels contained more than the 10 adult Collembola stated in the study plan. This was most likely due to an addition error on Day 0. This is possible as the animals are very small (approximately 2 mm long) when added. The affected vessels (one vessel per specified treatment group) were at 30.9 mg R169649/kg dry soil weight (11 adults), 55.6 mg R169649/kg dry soil weight (13 adults), 100 mg R169649/kg dry soil weight (11 adults) and 180 mg R169649/kg dry soil weight (11 adults). The validity criteria (relating to adult survival and reproduction) were fulfilled and the study end point calculations were not affected by these additional animals. Therefore this deviation was not considered significant or to have affected the integrity of the study. There was a weight measurement error for the control soil moisture sample on day 0. As these data were not recorded correctly, it was not possible to calculate or report the day 0 percent soil moisture content or the percent difference at the end of the test. The day 28 moisture content for the control was acceptable and the moisture content of all vessels was maintained adequately during the study. Therefore this deviation was not considered significant or to have affected the integrity of the study. It is also noted that the CV value for the number of juveniles was 30%, while it should be less than 30%. Nevertheless, in opinion of the zRMS, CV calculated to be exactly the required limit should not invalidate the study. Up to 180 mg pm/kg dw soil, no clear dose-response relationship could be observed on reproduction and for this reason the lower confidence interval for EC_{10} could not be determined. Furthermore, the lower limit of EC_{50} (133 mg pm/kg dws) was slightly lower than the median EC_{10} (134 mg pm/kg dws). Moreover, at the concentration set as NOEC (180 mg pm/kg dws), 18% effect on reproduction was observed and the CV among replicates was 55.2% which along with the CV of 30 % in the control could prevent

	resulting in a statistically significant difference. Therefore the zRMS is of the opinion that the NOEC should be set to 100 mg pm/kg dws, where no effect on reproduction was observed, and use this value in the risk assessment for precautionary reasons. It is also noted that the Abbott correction for mortality in control was applied which is not appropriate for quantile data (see OECD GD No 54). Taking this into account the corrected mortality has been struck through in the Table A 40 below. Consideration of not corrected mortality has no impact on the test results, as according to OECD 232, the reproductive output is the main endpoint for *F. candida*. All the validity criteria were met and overall the study is considered acceptable with the following endpoints relevant for the risk assessment: NOEC (reproduction) = 100 mg test item/kg dw soil EC_{50} (reproduction) = 237 mg test item/kg dw soil

Reference:	10.4.2.1
Report	Dickinson RA, (2015) R169649 - Collembola (*Folsomia candida*) Reproduction Test in Soil, Report Number ENV-14-015. Agrochemex Ltd., Aldhams research station, Manningtree, Essex, CO11 2NF, United Kingdom. (Syngenta File No. CA3511_10011)
Guideline(s):	OECD Guideline for Testing of Chemicals, Method 232 (adopted 7 September 2009): Collembolan reproduction test in soil.
Deviations:	Minor (see the commenting box above)
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	-

Materials

Test Material R169649 (MNBA; CA3511)
- **Lot/Batch #:** 454319
- **Active ingredient:** 2-nitro-4-methylsulfonyl benzoic acid
- **Actual content of active ingredients:** 99.9%
- **Description:** Off-white powder
- **Stability of test compound:** Stable under standard conditions
- **Reanalysis/Expiry date:** 30 June 2017

Treatments
- **Test rates:** 17.2, 30.9, 55.6, 100, 180, 324, 583 and 1050 mg R169649/kg soil dry weight
- **Control:** Oven dried sand
- **Toxic standard:** Boric acid (Separate study – No.: ENV-13-051, date: February 2014)
- **Application method:** R169649 mixed in oven dried sand was mixed into artificial soil prior to introduction of collembolans

Test organisms
- **Species:** Collembolans *Folsomia candida* (Willem)
- **Age:** 9 to 12 days old
- **Source:** Bias Labs Ltd., UK
- **Feeding:** Approximately 10 mg ground baker's yeast at the start of the test and after 7, 14 and 21 days

Test design
- **Arenas:** Glass test vessels (60 mL capacity) with lids

Substrate	Artificial soil comprising 5% sphagnum peat, 20% kaolinite clay, 69.77% quartz sand (> 50% of the particles between 0.05 mm and 0.2 mm) and 0.23 % calcium carbonate. 30 g wet weight of artificial soil was added to each test vessel.
Replication:	Treated groups 4, control group 8, plus an additional vessel per treatment for measurement purposes
No./arena:	10*
Duration of test:	28 days
Environmental test conditions	
Temperature:	16.4 to 21.6 °C
pH of soil:	Test start: 5.96 to 6.50 Test end: 5.63 to 6.21
Water content of soil:	Test start: 15.86 to 16.37 % soil moisture content Test end: 13.86 to 15.05 % soil moisture content
Photoperiod:	16 hours light and 8 hours dark at 715 to 720 Lux

**During the extraction process it was noted that some of the test vessels contained more than 10 organisms. This was considered most likely due to an addition error on Day 0, and was not considered significant or to have affected the integrity of the study.*

Study Design and Methods

Experimental dates: 24 November 2014 to 22 December 2014

The highest test concentration was prepared by weighing 630.2 mg of the test item and making up to 30.0597 g with oven dried sand. This was mixed thoroughly and serially diluted with oven dried sand to prepare the lower test concentrations. Aliquots of the respective treated sand were thoroughly mixed with artificial soil at 25 % of the WHC, and distilled water was added to achieve a final nominal water content of 50 % of WHC. The control was treated with oven dried sand only.

Nominally ten juvenile collembolans were transferred after the application to the substrate surface of each test vessel using a pooter. Four and eight replicates were used for each test item treatment and control group, respectively (+ one replicate per treatment not loaded with collembolans for measurement purposes). The test organisms were fed four times during the experiment (at the start of the test and after 7, 14 and 21 days) with approximately 10 mg of ground baker's yeast per test vessel. Four weeks after introducing the test organisms, the surviving parental collembolans and offspring (juveniles) were counted.

All values presented throughout this report were calculated using the original raw data and were not based on rounded values.

The percentage mortality of the springtails was calculated for each treatment, both before and after correction for any control treatment losses using Abbott's formula (1925), modified by Schneider-Orelli (1947). The 28-day mortality data for the individual test-item treatments were compared to those for the control using Fisher Exact /Bonferroni-Holm Test ($\alpha = 0.05$). The LC_{50} was determined by nonlinear regression analysis. The percentage reduction in reproductive performance in the test item treatment groups, compared to the control group, was calculated.

For the fecundity assessment, the data from the test-item treatments were compared to the control data using Wilcoxon/Bonferroni Adjustment Test ($\alpha = 0.05$). The results were used to determine the NOEC with respect to reproduction. The median effect concentration (EC_{50}) and also values for the EC_{20} and EC_{10} were determined by nonlinear regression analysis.

Results and Discussion

Mortality and fecundity are summarised in the table below.

Table A 47: Effects of residues of R169649 on mortality and reproduction of *Folsomia candida*

Endpoint	Treatment group (mg R169649/kg soil d.w.)								
	Control	**17.2**	**30.9**	**55.6**	**100**	**180**	**324**	**583**	**1050**
% Mortality of parental collembolans after 4 weeks[a]	11	3	3	0	0	18	23	33*	40*
~~**% corrected mortality[b]**~~	~~-~~	~~10~~	~~10~~	~~13~~	~~13~~	~~7~~	~~13~~	~~24~~	~~32~~
Mean number of juveniles after 4 weeks[c]	323	267	400	505	466	264	86*	88*	59*
Standard deviation	96.8	54.3	189.1	61.9	117.7	145.9	9.1	24.8	18.5
CV (%)	30.0	20.4	47.3	12.3	25.2	55.2	10.7	28.1	31.2
% reduction compared to control[d]	-	17	-24	-56	-44	18	74	73	82
NOEC (mortality)	324								
NOEC (reproduction)	180								
LOEC (mortality)	583								
LOEC (reproduction)	324								
LC_{50}	> 1050								
EC_{10}	134 (95 % confidence limits: n.d. and 225)								
EC_{20}	163 (95 % confidence limits: n.d. and 274)								
EC_{50}	237 (95 % confidence limits: 133 and 423)								

[a] Mortality amongst springtails originally introduced. Individual treatments compared to the control data using Fisher Exact/Bonferroni-Holm Test ($\alpha = 0.05$), and an asterisk indicates where there was a significant difference.
[b] ~~Derived using Abbott's formula (Abbott, 1925), modified by Schneider-Orelli (Schneider-Orelli, 1947)~~
[c] Fecundity data were compared to the control data using Wilcoxon/Bonferroni Adjustment Test ($\alpha = 0.05$). Treatments marked with an asterisk (*) differed significantly from the control.
[d] A negative value indicates an increase in reproduction relative to the control and a positive value indicates a decrease
d.w.: dry weight
n.d.: could not be determined

Validity Criteria

The validity criteria for the control group were met:

- Control treatment mortality was 11 % (must be < 20 %)
- The mean number of juveniles recorded in the control treatment was 323 (must be > 100 per replicate)
- The coefficient of variation of reproduction in the control was 30 % (must not be > 30 %)

Conclusion

The toxicity of R169649 to the reproduction and the parental mortality of collembola species *Folsomia candida* were determined. The NOECs for survival and reproduction were determined to be 324 and 180 mg R169649/kg soil dry weight, respectively. The EC_{50} for number of juvenile collembolans was determined to be 237 mg R169649/kg soil dry weight.

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Comments of zRMS:	The study was performed in line with OECD 232 with no deviations. The test design was not relevant to derive both NOEC and ECx values (there were 5 concentrations, 8 replicates for control, 4 replicates per treatment group). Nevertheless, reliability of the EC_{10} value was evaluated in line with recommendations of EFSA Supporting publication 2019:EN-1673: • NW (normalised width) of 0.25 was calculated, which results in rating "good" in line with Table E9 in EFSA Supporting publication 2019:EN-1673, • median EC_{10} is lower than $EC_{20,low}$, • the dose-response curve is steep with steepness of 0.7 (i.e. >0.66). Based on the above indications the calculated EC_{10} is considered to be sufficiently reliable. It is noted that the Abbott correction for mortality in control was applied which is not appropriate for quantile data (see OECD GD No 54). Taking this into account the corrected mortality has been struck through in the Table A 41 below. Consideration of not corrected mortality has no impact on the test results, as according to OECD 232, the reproductive output is the main endpoint for *F. candida*. All the validity criteria were met and overall the study is considered acceptable with the following endpoints relevant for the risk assessment: NOEC (reproduction) = 100 mg product/kg dw soil EC_{50} (reproduction) = 161 mg product/kg dw soil EC_{10} (reproduction) = 112 mg product/kg dw soil

Reference:	10.4.2.1
Report	Friedrich S, (2013) Mesotrione/Dicamba/Nicosulfuron WG (A18032E) plus Adigor (A12127R) – Effects on the Reproduction of the Collembolan *Folsomia candida*, Report Number 12 10 48 090 S. BioChem agrar, Labor für biologische und chemische Analytik GmbH, Kupferstraße 6, 04827 Gerichshain, Germany. (Syngenta file No. A18032E_10011).
Guideline(s):	OECD Guideline for Testing of Chemicals, Section 2 – Effects on Biotic Systems, Method 232 (adopted 7 September 2009): Collembolan reproduction test in soil. ISO 11267 (1999): Soil quality – inhibition of reproduction of Collembola (*Folsomia candida*) by soil pollutants. International Standard, First edition 1999-04-01.
Deviations:	No
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	-

Materials

Test Material	A18032E Mesotrione/dicamba/nicosulfuron WG (15/31.25/10)	
Lot/Batch #:	SMU2BP001	
Actual content of active ingredients:	Mesotrione: Dicamba: Nicosulfuron:	15.6 % w/w, corresponding to 156 g/kg 31.3 % w/w, corresponding to 313 g/kg 10.1 % w/w, corresponding to 101 g/kg
Description:	Beige solid	
Stability of test compound:	Stable under standard conditions	
Reanalysis/Expiry date:	30 September 2014	
Density:	Not applicable	

Treatments	
Test rates:	50, 100, 200, 400 and 800 mg A18032E/kg soil d.w. (equivalent to 37.5, 75, 150, 300 and 600 g A18032E/ha soil d.w.)
Control:	Deionised water only
Toxic standard:	Boric acid at rates of 44, 67, 100, 150 and 225 mg/kg soil dry weight (separate study – BioChem project No: R 12 10 48 003 S, dated 24 May 2012)
Adjuvant:	Adigor (A12127R) (mixture of emulsified fatty acid esters on basis of oleic acid methylester). The ratio of A18032E to A12127R was 1: 2.5 (A18032E: A12127R). Results are expressed in terms of the test item containing the adjuvant in this ratio.
Application method:	Solutions of A18032E plus A12127R with deionised water were dispersed in pre-moistened artificial soil prior to introduction of collembolans
Test organisms	
Species:	Collembolans *Folsomia candida* (Willem)
Age:	Juvenile collembolans (9-12 days)
Source:	Culture maintained at Test Facility. Originally purchased from "Biologische Bundesanstalt (BBA)", Berlin-Dahlem in May 2000
Feeding:	2 mg granulated dry yeast per replicate at the start of the test and after 14 days
Test design	
Arenas:	Glass container (approximately 150 mL) covered with a glass lid
Replication:	Treated groups 4 (+ 2 replicates not loaded with springtails for measurement purposes) Control group 8 (+ 2 replicates not loaded with springtails for measurement purposes)
No./arena :	10
Duration of test:	28 days
Environmental test conditions	
Temperature:	18.0 – 20.8 °C
pH:	Test initiation: 6.15 – 6.21 Test completion: 5.96 – 5.98
Photoperiod:	Light : dark 16h : 8h (light intensity 690 lux)
Water content of soil:	Test start: 24.9 to 25.1 % soil moisture content Test end: 24.3 to 24.8 % soil moisture content

Study Design and Methods

Experimental dates: 22 October 2012 to 19 November 2012

Two days prior to test start, the dry artificial soil was pre-moistened with deionised water to adjust the water content to approximately half of the final water content. At test start the artificial soil was mixed with quantities of the test item and adjuvant necessary to achieve the required test concentration in the volume of deionised water required to hydrate the soil to 40 – 60 % water holding capacity (WHC). The control substrate contained the corresponding amount of deionised water only.

Ten juvenile collembolans were transferred after the application to the substrate surface of each test vessel using an exhauster. Four replicates (+ two replicates not loaded with collembolans for measurement purposes) were used per test concentration and eight replicates (+ two replicates not loaded with collembolans for measurement purposes) for the control. The test organisms were fed twice during the experiment (at the start of the test and after 14 days) with approximately 2 mg of granulated dry yeast per test vessel. Four weeks after introducing the test organisms, the surviving parental collembolans and offspring (juveniles) were counted.

The glass lids covering the test vessels were briefly opened twice a week for aeration. The water content was checked weekly by reweighing the two additional test vessels. Water loss was compensated in all vessels if exceeding 2% of the initial water content. The temperature was

18.0 – 20.8°C, the pH was 5.96 – 6.21, the water content of the artificial soil was 56.4 – 58.2% of WHC and there was a 16 hour light : 8 hour dark photoperiod (690 lux).

Calculation and Statistics

Fisher`s Exact Binomial Test with Bonferroni Correction and the Welch-t-test for Inhomogeneous Variances with Bonferroni-Holm Adjustment were used to compare the control with the independent test item groups. The EC_{50} was calculated by linear maximum likelihood regression and the 95% confidence limits of the EC50 value were computed by normal approximation. Mortality of adult collembolans was corrected using the formula by Abbott (1925).

Results and Discussion

Mortality and fecundity are summarised in the table below.

Table A 48: Effects of residues of A18032E + A12127R on mortality and reproduction of *Folsomia candida*

Endpoint	Treatment group (mg A18032E/kg soil d.w.)					
	Control	**50**	**100**	**200**	**400**	**800**
% Mortality of parental collembolans after 4 weeks	2.5	2.5	2.5	55.0*[1]	95.0*[1]	100.0*[1]
~~% Corrected mortality (Abbott)~~	~~-~~	~~0~~	~~0~~	~~54~~	~~95~~	~~100~~
Mean number of juveniles after 4 weeks	543	576	519	119*[2]	15*[2]	0*[2]
SD	45.4	100.1	33.7	18.7	5.0	0.0
CV %	8.4	17.4	6.5	15.7	33.1	-
% Reduction compared to control	-	-6	4	78	97	100
NOEC (mortality and reproduction)	**100 mg A18032E/kg soil d.w.**					
LC_{50}	**204 mg A18032E/kg soil d.w.** (95% confidence limits 182 – 230 mg A18032E/kg soil d.w.)					
EC_{10} (reproduction)	**112 mg A18032E/kg soil d.w.** (95% confidence limits 99 - 127 mg A18032E/kg soil d.w.)					
EC_{20} (reproduction)	**127 mg A18032E/kg soil d.w.** (95% confidence limits 115 - 140 mg A18032E/kg soil d.w.)					
EC_{50} (reproduction)	**161 mg A18032E/kg soil d.w.** (95% confidence limits 152 – 170 mg A18032E/kg soil d.w.)					

* Statistically significant compared to control ([1]Fisher's Exact Binomial Test with Bonferroni Correction, $p \leq 0.05$, one-sided greater; [2]Welch-t-test, $p \leq 0.05$, one-sided smaller)

~~Abbott's formula for corrected mortality (Abbott, 1925): M (%) = ((A-B)/A) * 100 %, where~~
~~A = mean number of surviving parental collembolans in the control group, and~~
~~B = mean number of surviving parental collembolans in the treated groups~~

d.w. = dry weight

Percent reduction: (1-Rt/Rc) * 100%, where
Rt = mean number of juveniles observed in the treated groups, and
Rc = mean number of juveniles observed in the control group

Negative values = increase, relative to control

Validity Criteria

The validity criteria for the control group were met:

- Mean adult mortality: ≤ 20 % (observed: 2.5%)
- Mean number of juveniles per test vessel: ≥100 (observed: average of 543/vessel)
- Coefficient of variation for the mean number of juveniles: ≤ 30 % (observed: 8.4%)

The requirement of the ISO guideline concerning the precision of the counting method (average error <10%) was fulfilled, the determined overall error of counting amounted to 4.3%.

Conclusions
The toxicity of A18032E plus adjuvant A12127R to the reproduction and parental mortality of collembola species *Folsomia candida* were determined. The NOEC for both parental mortality and reproduction was determined to be 100 mg A18032E/kg soil dry weight (d.w.). The LC_{50} was determined to be 204 mg A18032E/kg soil d.w. with 95% confidence limits of 182 – 230 mg A18032E/kg soil d.w. The EC_{50} (based on reproduction) was determined to be 161 mg A18032E/kg soil d.w. with 95% confidence limits of 152 – 170 mg A18032E/kg soil d.w.

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Comments of zRMS:	The study was performed in line with OECD 226 with a minor deviation. It was noted that during the exposure phase the temperature dropped to 17.4°C which was below the guideline recommended minimum of 18°C. However, the mean temperature during the exposure was 19.1°C. This deviation is considered to have no impact on the outcome of the study since all the validity criteria were met. The test design was relevant to derive both NOEC and ECx values (8 concentrations, 8 replicates for control, 4 replicates per treatment group). It is noted that the Abbott correction for mortality in control was applied which is not appropriate for quantile data (see OECD GD No 54). Taking this into account the corrected mortality has been struck through in the Table A 42 below. Consideration of not corrected mortality has no impact on the test results, as according to OECD 232, the reproductive output is the main endpoint for *H. aculeifer*. No dose-response relationship could be observed on mortality and reproduction and the NOEC from the study was determined to be 1050 mg pm/kg dw soil. This value is recommended for the risk assessment purposes. EC_{10} could not be calculated. Overall, the study is considered acceptable with the following endpoints relevant for the risk assessment: 14d NOEC (reproduction) = 1050 mg product/kg dw soil 14d EC_{50} (reproduction) > 1050 mg product/kg dw soil

Reference:	10.4.2.1
Report	Ramsden C, (2015), R169649 – Predatory Mite (*Hypoaspis (Geolaelaps) aculeifer*) Reproduction Test in Soil, Report Number ENV-14-012. AgroChemex Environmental Ltd., Aldhams Farm Research Station, Dead Lane, Manningtree, Essex, CO11 2NF, United Kingdom. (Syngenta file No. CA3511_10010)
Guideline(s):	OECD (2008). OECD Guideline for Testing of Chemicals, Section 2 – Effects on Biotic Systems, Method 226 (adopted 3 October 2008): Predatory mite (*Hypoaspis (Geolaelaps) aculeifer*) reproduction test in soil.
Deviations:	Minor (see the commenting box above)
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	-

Materials
Test Material R169649

	NMSBA
Lot/Batch #:	454319
Active ingredient:	2-nitro-4-methylsulfonyl benzoic acid
Purity:	99.9 % ± 0.5 % w/w
Description:	Off white powder
Stability of test compound:	Stable under test conditions
Reanalysis/Expiry date:	30 June 2017
Density:	Not applicable
Treatments	
Test rates:	17.2, 30.9, 55.6, 100, 180, 324, 583 and 1050 mg R169649/kg soil dry weight
Control:	Oven dried sand
Toxic standard:	Boric acid (separate study ENV-14-017; November 2014)
Test organisms	
Species	*Hypoaspis aculeifer*
Source:	Obtained from Bias Labs Ltd., UK
Food:	Cheese mites, *Tyrophagus putrescentiae* , three times per week, *ad libitum*
Age at test start:	28 – 35 days
Test design	
Vessels:	Glass test vessels (volume: 60 mL; inner diameter: 38 mm) fitted with a 53 µm plastic mesh, and screw tops with a hole approximately 10 mm in diameter. Each vessel was filled with approximately 20 g soil d.w.
Substrate:	Artificial soil comprising 5% sphagnum peat, 20% kaolinite clay, 74.77% quartz sand (with > 50% of particles between 50 – 200 µm) and 0.23% calcium carbonate
Replication:	Control group: 8 (+3 temperature surrogates) Treated group: 4 (+1 surrogate per concentration)
No. of mites/arena :	10
Duration of test:	14 days
Environmental test conditions	
Temperature:	17 to 21 °C (mean: 19 °C)*
pH:	Test start: 5.20 to 5.94 Test end: 5.40 to 6.03
Water content of soil:	Test start: 16.11 to 16.59 % wet weight of soil Test end: 13.51 to 15.54 % wet weight of soil
Photoperiod:	16 h light : 8 h dark, 480 lux

** The temperature briefly dropped below the intended minimum (i.e. the guideline range of 18 – 22 °C) but there was no apparent effect on control mites and no impact was identified on the outcome or validity of the study.*

Study Design and Methods

Experimental dates: 12 November 2014 to 29 November 2014

Adult females of the soil mite *Hypoaspis aculeifer* were exposed to concentrations of R169649 incorporated into the test soil. A 30 g aliquot of the highest test concentration was prepared using exactly weighed amounts of the test item and oven-dried sand in a 60-mL glass jar, which was shaken and inverted repeatedly until well mixed. The lower test item concentrations were prepared by serial dilution with sand, starting with an appropriate volume from the aliquot of the highest concentration. Appropriate amounts of the test concentrations were then mixed with pre-moistened soil, and distilled water added such that a final moisture content value of 50 % WHC was achieved. Adult females were transferred to the test vessels which contained untreated (control) or test item treated artificial soil. Ten adult females

were introduced to each test vessel. As a source of food, cheese mites (*Tyrophagus putrescentiae*) were added throughout the test. The test was carried out under controlled light-dark cycle. Fourteen days after introducing the test organisms, the surviving mites and the juveniles of *Hypoaspis aculeifer* were extracted by heat/light extraction. From these data the mortality of the adult females and the reproductive output were calculated.
The mean number of dead adult female mites for each treatment, the mean number of juvenile mites for each treatment, the NOEC, the LOEC, and the EC_{50} at day 14 were determined.
Mortality data were corrected for control mortality according to Abbott (1925) modified by Schneider-Orelli (1947), and statistically analysed using Fisher's Exact/Bonferroni-Holm test (p = 0.05). Reproduction data was statistically analysed using a Dunnett Multiple Comparison Test (p = 0.05).
The determination of the NOEC and LOEC values was based on the results of the statistical evaluation. The software used to perform the statistical analysis was CETIS™, Version 1.8.7.14. The LC_{50} and EC_{50} were not able to be determined by statistical analysis due to the outcome of the study.

Results and Discussion

Mortality and fecundity are summarised in the table below.

Table A 49: Effects of residues of R169649 on mortality and reproduction of *Hypoaspis aculeifer*

Endpoint	Treatment group (mg R169649/kg soil d.w.)								
	Control	**17.2**	**30.9**	**55.6**	**100**	**180**	**324**	**583**	**1050**
	Mortality of adult mites after 14 days								
% mortality[a]	5.0	10.0	17.5	17.5	2.5	15.0	5.0	7.5	15.0
~~**% corrected mortality**~~[b]	~~0.0~~	~~5.3~~	~~13.2~~	~~13.2~~	~~-2.6~~	~~10.5~~	~~0.0~~	~~2.6~~	~~10.5~~
	Number of juveniles after 14 days								
Mean no. progeny per replicate[c]	115	93	102	103	117	91	109	95	108
standard deviation	16.7	15.4	12.2	14.5	5.7	21.5	20.4	6.4	32.8
% reduction compared to control[d]	n.a.	19.2	11.2	10.3	-1.8	21.4	5.8	17.7	6.0

The results represent rounded values calculated from the exact raw data
[a] There were no statistically significant differences compared to the control for mortality (Fisher's Exact/Bonferroni Holm test)
[b] According to Abbott (1925) modified by Schneider-Orelli (1947)
[c] There were no statistically significant differences compared to the control for reproduction (Dunnett Multiple Comparison Test) [d] A positive value indicates a decrease and a negative an increase in reproduction, relative to the control
d.w.: dry weight

Validity Criteria

The validity criteria for the control group were met:

- Mean mortality of adult females: ≤ 20 % (observed: 5.0 %)
- Mean number of juveniles per replicate: ≥ 50 (calculated: 115)
- Coefficient of variation (mean number of juveniles per replicate): ≤ 30 % (calculated: 14.5 %)

Conclusion

The effects of R169649 on the mortality and reproductive output of the soil mite species *Hypoaspis aculeifer* were determined during a 14-day test.
The NOEC for mortality and reproduction was determined to be 1050 mg R169649/kg soil dry weight, and the 14-day EC_{50} and LC_{50} could not be determined but were considered to be > 1050 mg R169649/kg soil dry weight, the highest concentration tested.

*****

Comments of zRMS:	The study was performed in line with OECD 226 with no deviations. It is noted that in the test design there were 7 concentrations instead of 8 relevant to derive both NOEC and ECx values, 8 replicates for control and 4 replicates per treatment group. However, statistically significant effects > 10% (compared to the control) were observed and calculations of EC_{10} and EC_{20} were possible. The reliability of the EC_{10} value was evaluated in line with recommendations of EFSA Supporting publication 2019:EN-1673: • NW (normalised width) of 0.22 was calculated, which results in rating "good" in line with Table E9 in EFSA Supporting publication 2019:EN-1673, • median EC_{10} is lower than $EC_{20,low}$, • the dose-response curve is medium with steepness of 0.57 (i.e. >0.33 and <0.66). Based on the above indications the calculated EC_{10} is considered to be sufficiently reliable. It is noted that the Abbott correction for mortality in control was applied which is not appropriate for quantile data (see OECD GD No 54). Taking this into account the corrected mortality has been struck through in the Table A 43 below. Consideration of not corrected mortality has no impact on the test results, as according to OECD 232, the reproductive output is the main endpoint for *H. aculeifer*. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: 14d NOEC (reproduction) = 50 mg product/kg dw soil 14d EC_{50} (reproduction) = 129 mg product/kg dw soil 14d EC_{10} (reproduction) = 74 mg product/kg dw soil

Reference:	10.4.2.1
Report	Schulz L, (2013), Mesotrione/Nicosulfuron/Dicamba WG (A18032E) plus Adigor (A12127R) – Effects on the Reproduction of the Predatory Mite *Hypoaspis aculeifer*, Report Number 12 10 48 148 S, BioChem agrar Labor für biologische und chemische Analytik GmbH, Kupfertraße 6, 04827 Gerichshain, Germany. (Syngenta file No. A18032E_10012).
Guideline(s):	OECD (2008). OECD Guideline for Testing of Chemicals, Section 2 – Effects on Biotic Systems, Method 226 (adopted 3 October 2008): Predatory mite (*Hypoaspis (Geolaelaps) aculeifer*) reproduction test in soil.
Deviations:	No
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	-

Materials

Test Material	A18032E Mesotrione/Dicamba/Nicosulfuron WG (15/31.25/10)	
Lot/Batch #:	SMU2BP001	
Actual content of active ingredients:	Mesotrione:	15.6 % w/w corresponding to 156 g/kg
	Dicamba:	31.3 % w/w corresponding to 313 g/kg
	Nicosulfuron:	10.1 % w/w corresponding to 101 g/kg
Description:	Beige solid	
Stability of test compound:	Stable under standard conditions	
Reanalysis/Expiry date:	30 September 2014	

Density:	Not applicable
Treatments	
Test rates:	12.5, 25, 50, 100, 200, 400 and 800 mg A18032E/kg soil dry weight (corresponding to 9.375, 18.75, 37.5, 75, 150, 300 and 600 kg A18032E/ha)
Control:	Untreated substrate, i.e. deionised water only
Toxic standard:	Dimethoate EC 400) tested at 4.1, 5.12, 6.4, 8 and 10 mg a.i./kg soil d.w. (Separate study: BioChem project No. R 12 10 48 002 S, dated 05 March 2012)
Adjuvant:	Adigor (A12127R) (mixture of emulsified fatty acid esters on basis of oleic acid methylester). The ratio of A18032E to A12127R was 1: 2.5 (A18032E: A12127R). Results are expressed in terms of the test item containing the adjuvant in this ratio.
Application method:	Solutions of A18032E with deionised water were dispersed in pre-moistened artificial soil prior to introduction of mites
Test organisms	
Species	*Hypoaspis aculeifer* (Canestrini)
Source:	Originally purchased from Katz Biotech AG, An der Birkenpfuhlheide 10, 15837 Baruth, Germany. Cultured at Test Facility since 2005.
Food:	Every two days with *Tyrophagus putrescentiae* (Schrank) originally obtained from Bayer CropScience AG, Monheim, reared in the test facility since 2004
Age at test start:	Adults from a synchronised culture with an age difference of 3 days
Test design	
Arenas:	100 mL SCHOTT-bottles (4 cm diameter, 11 cm high) with screw tops
Replication:	Control group: 8 (+ 2 replicates for determination of water content and pH-value; not loaded with predatory mites) Treated group: 4 (+ 2 replicates for determination of water content and pH-value; not loaded with predatory mites)
No. of mites/arena :	10 females
Duration of test:	14 days
Environmental test conditions	
Temperature:	19.5 – 21.1 ° C
pH:	Test start: 6.3 – 6.5 Test end: 6.4 – 6.5
Water content of soil:	Test start: 48.28 – 49.40 % of WHC Test end: 46.72 – 49.78 % of WHC
Photoperiod:	16 h light : 8 h dark, 545 lux

Study Design and Methods
Experimental dates: 09 November 2012 to 26 November 2012

Adult females of the soil mite *Hypoaspis aculeifer* were exposed to concentrations of A18032E plus A12127R incorporated into the test soil. An exactly weighed amount of the test item was mixed with deionised water to make a stock, immediately before application. This stock solution was stepwise diluted with deionised water to prepare further test solutions (serial dilution) and mixed thoroughly with the artificial soil by means of a hand stirrer. Adult females from a synchronised culture were transferred to the test vessels which contained untreated (control) or test item treated artificial soil. Ten adult females were introduced to each test vessel together with the food mite *Tyrophagus putrescentiae*. The test was carried out under controlled light-dark cycle. The water content was maintained and food was added at regular intervals throughout the duration of the test. Fourteen days after introducing the test organisms, the surviving mites and the juveniles of *Hypoaspis aculeifer* were extracted by heat/light extraction. Any adult mites not found after extraction were recorded as dead. From these data the mortality of the adult females and the reproductive output were calculated.

The numbers of any missing mites were added to the number of dead mites found in each treatment to derive the overall "mortality". The percentage mortality at each treatment rate was corrected for mortality in the control treatment using Abbott's formula (Abbott, 1925). The LC_{50} and EC_{50} values were calculated using Probit analysis. Fisher's Exact Binomial Test with Bonferroni Correction and Williams' t-test were used to compare the control with the independent test item groups.
The reduction of reproductive output (R_r) for the treatment groups relative to the control was calculated using the formula:

$$R_r\ (\%) = [1-(R_t/R_c)] * 100\ \%$$

where R_t and R_c are the absolute values observed in the treatment and control groups respectively.

Results and Discussion
Mortality and fecundity are summarised in the table below.

Table A 50: Effects of residues of A18032E + A12127R on mortality and reproduction of *Hypoaspis aculeifer*

Endpoint	Treatment group (mg A18032E/kg soil d.w.)							
	Control	**12.5**	**25**	**50**	**100**	**200**	**400**	**800**
	Mortality of adult mites after 14 days							
% mortality	2.5	2.5	0.0	0.0	2.5	5.0	5.0	82.5*[1]
~~% corrected mortality (Abbott)[a]~~	~~-~~	~~0.0~~	~~-2.6~~	~~-2.6~~	~~0.0~~	~~2.6~~	~~2.6~~	~~82.1*[1]~~
	Number of juveniles after 14 days							
mean	326.9	329.3	323.0	328.3	234.5*[2]	50.3*[2]	15.8*[2]	0.0*[2]
standard deviation	31.3	26.6	19.7	26.2	59.5	12.1	10.9	0.0
coefficient of variation %	9.6	8.1	6.1	8.0	25.4	24.2	69.2	-
% reduction compared to controlb	-	-0.7	1.2	-0.4	28.3	84.6	95.2	100.0

* Statistically significant compared to the control ([1]Fisher's Exact Binomial with Bonferroni Correction for mortality, $p \leq$ 0.05, one-sided greater). ([2]Williams t-test for reproduction, $p \leq 0.05$, one-sided smaller)
[a] ~~Calculated using Abbott's formula for corrected mortality (Abbott, 1925): M (%) = (1-t/c)*100%~~
[b] A negative value indicates an increase relative to the control
Calculations were done using non-rounded values
Percent reduction: $(1-R_t/R_c)*100\%$, where Rt = mean number of juvenile mites observed in the treated group(s) and Rc = mean number of juvenile mites observed in the control group

Validity Criteria
The validity criteria for the test were met:

- Mean mortality of adult females: ≤ 20% (2.5 % observed)
- Mean number of juveniles per replicate: ≥ 50 (326.9 calculated)
- Coefficient of variation (mean number of juveniles per replicate): ≤ 30 % (9.6 % calculated)

Conclusions
The NOECs based on mortality and reproduction were determined to be 400 and 50 mg A18032E/kg soil dry weight, respectively. The LC_{50} was determined to be 579 mg A18032E/kg soil d.w. with 95% confidence limits of 409 – 883 mg A18032E/kg soil d.w. The EC_{50} for reproduction was determined to be 129 mg A18032E/kg soil d.w. with 95% confidence limits of 122 – 137 mg A18032E/kg soil d.w.

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 study was performed fully in line with OECD 216 with no deviations. All the validity criteria were met and the study is considered acceptable. It may be concluded that the effects of the test item on soil nitrogen formation rates were < 25% at the end of the study period (28 days) up to 4.00 mg A18032E/kg + 9.25 mg Adjuvant (A12127R)/kg dry soil.

Reference:	10.5
Report	Schulz L, (2012), Mesotrione/Nicosulfuron/Dicamba WG (A18032E) plus Adigor (A12127R) – Effects on the Activity of Soil Microflora (Nitrogen and Carbon Transformation Tests), Report Number 12 10 48 048 C/N. BioChem agrar, Labor für biologische und chemische Analytik GmbH, Kupferstraβe 6, 04827 Gerichshain, Germany (Syngenta file No. A18032E_10004).
Guideline(s):	OECD Guideline 216: Soil Microorganisms, Nitrogen Transformation Test, January 2000 ~~OECD Guidelines 217: Soil Microorganisms, Carbon Transformation Test, January 2000~~
Deviations:	No
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	-

Materials

Test Material	A18032E Mesotrione/Dicamba/Nicosulfuron WG (15/31.25/10)
Lot/Batch #:	SMU2BP001
Actual content of active ingredients:	Mesotrione: 15.6 % w/w corresponding to 156 g/kg Dicamba: 31.3 % w/w corresponding to 313 g/kg Nicosulfuron: 10.0 % w/w corresponding to 101 g/kg
Description:	Beige solid
Stability of test compound:	Stable under standard conditions
Reanalysis/Expiry date:	30 September 2014
Density:	Not applicable
Treatments	
Test rates:	0.80 and 4.0 mg A18032E/kg soil d.w. (equivalent to 0.6 and 3 kg A18032E/ha, respectively)
Control:	Deionised water only
Toxic standard:	Dinoterb (purity 98.0 ± 0.5%) at concentrations of 6.8, 16.0 and 27.0 mg Dinoterb/kg (Separate study – BioChem project No: R 12 10 48 001 C/N, date 13.01 to 11.02.2012)
Adjuvant:	Adigor (A12127R) (mixture of emulsified fatty acid esters on basis of oleic acid methyl ester) The ratio of A18032E to A12127R was 1: 2.3125 (A18032E : A12127R). Results are expressed in terms of the test item containing the adjuvant in this ratio.
Test design	
Soil:	Agricultural sandy loam soil, supplied by BioChem agrar GmbH
Soil type:	Sandy loam: 10.7 % clay (< 0.002 mm), 35.5 % silt (0.002 - 0.050 mm) and 53.9 % sand (0.050 – 2.0 mm) (USDA classification) Carbon content of microbial biomass [mg C/100 g soil d.w.]: 33.45 = 2.31% of Corg.; Corg [%]: 1.45

Test units:	Nitrogen transformation test: 200 g soil dry weight in 500 mL wide-mouthed glass flasks ~~Carbon transformation test: 1000 g soil dry weight in 4 L steel test vessels~~
Replication:	Nitrogen transformation test: 3 replicates per treatment rate and control ~~Carbon transformation test: 3 replicates per treatment rate and control~~
Sampling intervals :	Nitrogen transformation test: 3 hours, 7 days, 14 days and 28 days after application ~~Carbon transformation test: 3 hours, 7 days, 14 days and 28 days after application~~
Duration of test:	28 days
Environmental test conditions	
Temperature:	19.2 – 21.0 °C
pH of soil:	Nitrogen transformation test: 6.1 – 6.2 at test start, 6.1 – 6.2 at test end ~~Carbon transformation test: 6.1 – 6.2 at test start, 6.3 at test end~~
Soil moisture content:	Nitrogen transformation test: 13.51 – 14.21 g/100 g soil d.w. (equivalent to 40.38 – 42.47 % of WHC) ~~Carbon transformation test: 14.14 – 14.88 g/100 g soil d.w. (equivalent to 42.26 – 44.47 % of WHC)~~
Photoperiod:	Darkness

Study Design and Methods

Experimental dates: 30 August 2012 to 28 September 2012

Soil samples were treated with A18032E, together with the adjuvant A12127R, at two doses – 0.80 (low dose) and 4.0 mg A18032E/kg dry soil (high dose) relating to a soil depth of 5 cm and a soil density of 1.5 g/cm^3. The test item was mixed with deionised water, which was added to the soil samples and mixed thoroughly. The soil moisture content of all samples was adjusted to 45% of the WHC by adding deionised water and the samples incubated in the dark at a temperature of 19.2 – 21.0°C. The soil moisture content was checked weekly, and adjusted with purified water to maintain 40 – 50% of the soil WHC.

~~Respiration and~~ nitrification were determined for all treatments at 3 hours, 7, 14 and 28 days after treatment. ~~In order to measure the short-term respiration of soil microbes, 100 g soil d.w. were taken from each treatment at each sampling occasion. The samples were amended with glucose and the evolved CO_2 measured over a period of 12 hours.~~ To determine the nitrification, the soil samples were amended with Lucerne meal after application and 10 g soil d.w. per replicate were taken at each sampling point. The samples were extracted with KCl, and analysed for nitrite-nitrogen, nitrate-nitrogen and ammonium-nitrogen.

Data of nitrate formation ~~and O_2 consumption~~ were used to calculate the percentage deviation from the control on each sampling date which was then analysed statistically (2-sided Student-t-test at 5% significance level).

Results and Discussion

Table A 51: Effects on Nitrogen Transformation in Soil after Treatment with A18032E + A12127R

Days after application	Control			0.80 mg A18032E/kg soil dry weight + 1.85 mg adjuvant (A12127R) equivalent to 0.6 kg A18032E/ha				4.0 mg A18032E/kg soil dry weight + 9.25 mg adjuvant (A12127R) equivalent to 3.0 kg A18032E/ha			
	NO_3-N		CV [%]	NO_3-N		CV [%]	Deviation from control [%][1)]	NO_3-N		CV [%]	Deviation from control [%][1)]
	[mg/kg soil d.w.]	[mg/kg soil d.w./day]		[mg/kg soil d.w.]	[mg/kg soil d.w./day]			[mg/kg soil d.w.]	[mg/kg soil d.w./day]		
0	21.0	-	2.4	18.6	-	14.0	-11.1	20.6	-	1.6	-1.9
7	29.2	4.2	1.4	32.3*	4.6	3.7	+10.7	32.7	4.7	7.2	+12.2
14	33.8	2.4	5.6	37.6*	2.7	3.1	+11.3	39.6	2.8	11.7	+17.3
28	44.0	1.6	1.7	46.8*	1.7	0.9	+6.3	50.2*	1.8	4.0	+14.0

The calculations were performed with non-rounded values
CV [%] = Coefficient of Variation
1) based on NO_3-nitrogen production; - = inhibition; + = stimulation
* statistically significantly different to control (Student-t-test for homogeneous variances, 2-sided, $p \leq 0.05$)

No differences greater than 25% in the nitrogen transformation were found for the tested concentrations of the test item at the end of the 28-day incubation period in comparison to the respective control.
In a separate study the reference item Dinoterb caused a stimulation of nitrogen transformation of +40.4 %, +68.1% and +83.5% at 6.80 mg, 16.00 mg and 27.00 mg Dinoterb per kg soil dry weight, respectively, 28 days after application.

~~**Table A 52: Effects on Carbon Transformation in Soil after Treatment with A18032E + A12127R**~~

~~Days after application~~	~~Control~~		~~0.80 mg A18032E/kg soil dry weight + adjuvant (A12127R) equivalent to 0.6 kg A18032E/ha~~			~~4.0 mg A18032E/kg soil dry weight + adjuvant (A12127R) equivalent to 3.0 kg A18032E/ha~~		
	~~O_2 consumption [mg/kg soil d.w./h]~~	~~CV [%]~~	~~O_2 consumption [mg/kg soil d.w./h]~~	~~CV [%]~~	~~Deviation from control [%][1]~~	~~O_2 consumption [mg/kg soil d.w./h]~~	~~CV [%]~~	~~Deviation from control [%][1]~~
~~0~~	~~10.93~~	~~5.1~~	~~11.28~~	~~1.0~~	~~+3.2~~	~~10.42~~	~~3.3~~	~~-4.6~~
~~7~~	~~11.12~~	~~2.0~~	~~11.21~~	~~2.6~~	~~+0.9~~	~~10.46*~~	~~1.5~~	~~-5.9~~
~~14~~	~~10.79~~	~~0.8~~	~~10.95~~	~~0.7~~	~~+1.5~~	~~9.93*~~	~~1.7~~	~~-7.9~~
~~28~~	~~10.19~~	~~0.8~~	~~10.25~~	~~1.8~~	~~+0.5~~	~~9.45*~~	~~2.1~~	~~-7.3~~

~~The calculations were performed with non-rounded values~~
~~CV [%] = Coefficient of Variation~~
~~1) based on O_2 consumption; - = inhibition; + = stimulation~~
~~* statistically significantly different to control (Student-t-test for homogeneous variances, 2-sided, $p \leq 0.05$)~~

Validity Criteria

The validity criteria were fulfilled. The coefficients of variation in the control group in ~~both~~ the nitrogen ~~and carbon~~ transformation tests were ≤ 15% (maximum 5.6 ~~and 5.1~~ %, ~~respectively~~).

The results with the reference substance for ~~both~~ the nitrogen ~~and carbon~~ transformation tests demonstrated the sensitivity of the test system.

Conclusions

A18032E plus adjuvant A12127R was applied to the soil at concentrations of 0.80 mg A18032E/kg dry soil and at 4.0 mg A18032E/kg dry soil. No adverse effects are to be expected ~~on either short-term microbial respiration or~~ on the nitrification process and hence on soil fertility.

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

Comments of zRMS:	The study was performed in line with OECD 227 with no deviations. All the validity criteria were met. It is noted that the product contains three active substances and in line with the requirements of the Central Zone the test concentrations of all active substances should be verified in the respective chemical analyses or at a minimum the least stable active substance should be analysed. However, in the present study only the concentration of mesotrione was measured and the analyses of nicosulfuron and dicamba were not carried out. No explanation or justification for the active substance selected for the chemical analysis was provided in the study report, however from the data on fate and behaviour in water it may be concluded that of all three compounds, mesotrione is least stable and is thus expected to be least stable in the test solutions. Furthermore, under practical conditions of use the formulated product will be used and behaviour of particular active compounds will be the same as in the performed study. Taking this into account, confirmation of measured concentration of mesotrione is deemed sufficient, even if not ideal, since ideally concentration of all three compounds should be measured. It is noted that endpoints based on phytotoxic effects were not calculated although they are required in line with the agreements taken during the Central Zone harmonisation meetings. However, calculation of phytotoxicity endpoints is not required in Poland, since in line with indications of EFSA Supporting publication 2019:EN-1673, should be considered as an interim solution that will be reflected in the SANCO/10329/2002-rev.2 guidance document with its implementation considered further. However, the SANCO guidance document was not yet amended. Since Poland is the only cMS indicated in the GAP table, calculation of phytotoxicity endpoint is not required for this authorisation. The study is considered acceptable with following endpoint relevant for the risk assessment: lowest ER_{50} = 1.30 g product/ha (*Lycopersicon esculentum*)

Reference:	10.6.2
Report	Bramby-Gunary J (2013), Mesotrione/dicamba/nicosulfuron WG (A18032E) plus A12127R (Adigor adjuvant) - Evaluation of the Phytotoxicity to Non Target Terrestrial Plant Vegetative Vigour Test, Report Number ACE-12-149, AgroChemex Ltd, Aldhams Farm, Dead Lane, Manningtree, Essex, CO11 2NF, United Kingdom. (Syngenta file No. A18032E_10025)
Guideline(s):	OECD Guideline for the Testing of Chemicals, Volume 1, Number 2, April 1984, pp. 1 - 21 (21) Test No. 227: Terrestrial Plant Test: Vegetative Vigour Test
Deviations:	No
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	-

Materials

Test material	A18032E Mesotrione/dicamba/nicosulfuron WG (15/31.25/10)
Lot/Batch #:	SMU2BP001

Actual content of active ingredient:	Mesotrione:	15.6 % w/w corresponding to 156 g/kg
	Dicamba:	31.3 % w/w corresponding to 313 g/kg
	Nicosulfuron:	10.1 % w/w corresponding to 101 g/kg
Description:	Beige solid	
Stability of test compound:	Stable under standard conditions.	
Reanalysis/expiry date:	30 September 2014	
Density:	Not applicable	
Treatments		
Test concentrations:	24.7, 74.1, 222, 667, 2000 and 6000 g A18032E/ha (*A. sativa* and *L. perenne*) 0.914, 2.74, 8.23, 24.7, 74.1, 222 and 667g A18032E/ha (all other species)	
Control:	Water only	
Spray volume:	200L/ha ± 10%	
Adjuvant:	Adigor (A12127R) (mixture of emulsified fatty acid esters on basis of oleic acid methylester). The ratio of A18032E to A12127R was 1:2.5. Results are expressed in terms of the test item containing the adjuvant in this ratio	
Application method:	Mardrive cabinet track sprayer with 8003-E TeeJet flat fan nozzle	
Test organisms		
Species:	*Avena sativa* (oat) *Lolium perenne* (ryegrass) *Oryza sativa* (rice) *Beta vulgaris* (sugar beet) *Brassica napus* (oilseed rape) *Cucumis sativus* (cucumber) *Daucus carota* (carrot) *Lactuca sativa* (lettuce) *Lycopersicon esculentum* (tomato) *Raphanus sativus* (radish)	
Test soil:	Sandy loam mixed as follows: 20 litres of sterile loam + 10 litres of sand. The soil was determined to consist of 75% w/w sand (2.00 – 0.063mm), 8% silt w/w (0.063 – 0.002 mm), 17% w/w clay (<0.002 mm). The organic carbon content was 1.3% w/w. To obtain good plant health, 100g slow release fertiliser was incorporated into 30 litres of soil mix.	
Test design		
Test vessels:	Non-porous plastic pots (8 x 8 x 8 cm), placed in saucers filled with enough water to ensure that the pots were kept moist at all times	
Sampling interval:	Plants were assessed at 7, 14 and 21 days after application for mortality and visual phytotoxicity. Biomass and height were assessed at test termination	
Replication:	Five pots per treatments, 4 seedlings per pot	
Duration:	21 days after application of test substance	
Environmental conditions		
Test temperature:	Mean: 20.8 °C (Min: 15.2 °C, Max: 28.9 °C)	
Humidity:	Mean: 72.5 % (Min: 46.0 %, Max: 88.7 %)	
Soil pH:	7.4	
Lighting:	Ambient lighting was supplemented by sodium vapour lamps giving at least a 16 hour day. The mean ambient light intensity for the study period was 17.4 kilo lux (Kl), and the maximum intensity was 56.9 Kl	

Study Design and Methods

Experimental dates: 29 June 2012 to 28 August 2012

Young plants of three monocot species (*Avena sativa*, *Lolium perenne* and *Oryza sativa*) and seven dicot species (*Beta vulgaris*, *Brassica napus*, *Cucumis sativus*, *Daucus carota*, *Lactuca sativa*, *Lycopersicon esculentum* and *Raphanus sativus*) were sprayed with a series of at least six test concentrations of A18032E together with the adjuvant A12127R, with a fixed mixture ratio of 1:2.5 for the two respective components. Nominal test concentrations used in the definitive test ranged from 0.914 to 6000 g A18032E/ha. The number of surviving seedlings, seedling height and weight were determined at test termination.

All the species were germinated in seed trays of Levingtons F1 compost and transplanted shortly after emergence at BBCH Growth Stage 10 into plastic pots (8 cm diameter and 8 cm deep), four seedlings per pot. At the time of application seedlings had 2 to 4 open leaves.

Observations were made 7, 14, and 21 days after application (DAA) to document plant condition. Observations were made 21 DAA to document plant height. Plant condition was described by noting the presence or absence of possible signs of phytotoxicity such as stunting and chlorosis. Each plant was then assigned a numerical score that described the plant condition. This was a scale from 0 to 100% - a subjective or qualitative assessment that determines whether damage is absent (0%), slight (1 – 39%), moderate (40 – 69%), severe (70 – 99%) or all plants dead (100%). A score of 10 does not mean that 10% of the plant is showing the effect (e.g. chlorosis), merely that the severity of the effect (e.g. chlorosis) is very slight.

The growth of test plants was evaluated at the end of the test (21 DAA) by assessing height and dry weight (biomass). Plant biomass was estimated by measuring the total dry weight of the shoots within each replicate. Plant height was measured from the surface of the soil to the tip of the tallest leaf. Dead or non-emerged seedlings were assigned a height of 0 cm. Plants were then clipped at soil level, the shoots of all living plants within a replicate were placed in a labelled paper bag and dried to a constant weight. Mean height and total replicate biomass were determined for each treatment group containing living seedlings at test termination.

Results and Discussion

Statistical analyses were used to evaluate effects of test substance application on plant height and biomass. Least Significant Difference (LSD) was used to establish the NOER by determining which treatment groups differed significantly ($p \leq 0.05$) from the control group. Effect rates (i.e. ER_{50}) and their confidence limits were determined using simple probit - maximum likelihood estimation method. Statistical analysis was not conducted for plant condition and visual phytotoxicity because those data are qualitative.

The mean 21-day survival for each of the ten test species is presented in the table below:

Table A 53: Mean 21-Day Survival per Pot Expressed as %

Species	Rate: g A18032E/ha									
	Control	**0.914**	**2.74**	**8.23**	**24.7**	**74.1**	**222**	**667**	**2000**	**6000**
Monocots										
Avena sativa **(oat)**	100	n.a.	n.a.	n.a.	100	100	100	80	50	55
Lolium perenne **(ryegrass)**	100	n.a.	n.a.	n.a.	100	100	100	80	60	50
Oryza sativa **(rice)**	100	100	100	95	100	95	85	50	n.a.	n.a.
Dicots										
Beta vulgaris **(sugar beet)**	100	100	100	100	100	100	100	100	n.a.	n.a.
Brassica napus **(oilseed rape)**	100	100	100	100	100	100	95	45	n.a.	n.a.
Cucumis sativus **(cucumber)**	100	100	100	100	100	100	80	55	n.a.	n.a.
Daucus carota **(carrot)**	100	100	100	100	100	100	100	95	n.a.	n.a.
Lactuca sativa **(lettuce)**	100	100	100	100	100	100	90	30	n.a.	n.a.
Lycopersicon esculentum **(tomato)**	100	100	100	100	100	95	85	0	n.a.	n.a.
Raphanus sativus **(radish)**	100	100	100	100	100	80	25	15	n.a.	n.a.

n.a. = not applicable

The NOER and ER_{50} for each of the ten test species are presented in table below:

Table A 54: Effect Rates of A18032E + A12127R on 21-Day Biomass and Height

Species	Biomass (dry weight) (g A18032E/ha)			Height (g A18032E/ha)		
	NOER	**ER_{50}**	**95% Confidence limits**	**NOER**	**ER_{50}**	**95% Confidence limits**
Monocots						
Avena sativa **(oat)**	24.7	93.63	79.58 – 108.77	24.7	372.86	310.69 – 445.01
Lolium perenne **(ryegrass)**	<24.7	48.51	41.03 – 56.53	24.7	239.56	203.53 – 279.43
Oryza sativa **(rice)**	8.23	44.84	40.27 – 50.02	24.7	147.00	126.26 – 172.90
Dicots						
Beta vulgaris **(sugar beet)**	0.914	16.32	13.36 – 19.76	2.74	>667	n.a.
Brassica napus **(oilseed rape)**	8.23	23.52	21.16 – 26.14	8.23	125.20	110.94 – 142.09
Cucumis sativus **(cucumber)**	2.74	36.50	30.31 – 44.13	2.74	115.30	97.44 – 138.14
Daucus carota **(carrot)**	8.23	34.19	30.57 – 38.27	8.23	377.08	305.53 – 477.32
Lactuca sativa **(lettuce)**	<0.914	8.22	7.27 – 9.26	2.74	57.18	51.88 – 63.12
Lycopersicon esculentum **(tomato)**	<0.914	1.30	1.03 – 1.60	0.914	20.32	17.96 – 22.97
Raphanus sativus **(radish)**	2.74	18.76	17.10 – 20.57	8.23	34.81	31.54 – 38.47

n.a. = not applicable

Validity criteria

The validity criteria for the test were met:

- The control plants did not exhibit any phytotoxic effects
- There was more than 90% survival in the control plants
- The environmental conditions were identical for all the tested species

Conclusions

A single foliar application of A18032E plus A12127R, at rates up to 6000 g A18032E/ha, resulted in ER_{50} values ranging from 1.30 to 377.08 g A18032E/ha.
Lycopersicon esculentum (tomato) was the most sensitive species, with ER_{50} values based on dry weight and final height of 1.30 and 20.32 g A18032E/ha, respectively, and a NOER based on dry weight and final height of <0.914 and 0.914 g A18032E/ha, respectively.

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Comments of zRMS:	The study was performed in line with OECD 208 with no deviations. All the validity criteria were met. It is noted that the product contains three active substances and in line with the requirements of the Central Zone the test concentrations of all active substances should be verified in the respective chemical analyses or at a minimum the least stable active substance should be analysed. However, in the present study only the concentration of mesotrione was measured and the analyses of nicosulfuron and dicamba were not carried out. No explanation or justification for the active substance selected for the chemical analysis was provided in the study report, however from the data on fate and behaviour in water it may be concluded that of all three compounds, mesotrione is least stable and is thus expected to be least stable in the test solutions. Furthermore, under practical conditions of use the formulated product will be used and behaviour of particular active compounds will be the same as in the performed study. Taking this into account, confirmation of measured concentration of mesotrione is deemed sufficient, even if not ideal, since ideally concentration of all three compounds should be measured.

	It is noted that endpoints based on phytotoxic effects were not calculated although they are required in line with the agreements taken during the Central Zone harmonisation meetings. However, calculation of phytotoxicity endpoints is not required in Poland, since in line with indications of EFSA Supporting publication 2019:EN-1673, should be considered as an interim solution that will be reflected in the SANCO/10329/2002-rev.2 guidance document with its implementation considered further. However, the SANCO guidance document was not yet amended. Since Poland is the only cMS indicated in the GAP table, calculation of phytotoxicity endpoint is not required for this authorisation. The study is considered acceptable with following endpoint relevant for the risk assessment: lowest ER_{50} = 6.97 g product/ha (*Lactuca sative*)

Reference:	10.6.2
Report	Bramby-Gunary J (2013a), Mesotrione/dicamba/nicosulfuron WG (A18032E) plus A12127R (Adigor adjuvant) - Evaluation of the Phytotoxicity to Non Target Terrestrial Plant Seedling Emergence and Seedling Growth Test, Report Number ACE-12-148, AgroChemex Ltd, Aldhams Farm, Dead Lane, Manningtree, Essex, CO11 2NF, United Kingdom. (Syngenta file No.A18032E_10024)
Guideline(s):	OECD Guideline for the Testing of Chemicals. Guideline 208: Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test (July 2006)
Deviations:	No
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	-

Materials

Test material	A18032E Mesotrione/dicamba/nicosulfuron WG (15/31.25/10)	
Lot/Batch #:	SMU2BP001	
Actual content of active ingredients:	Mesotrione: Dicamba: Nicosulfuron:	15.6 % w/w corresponding to 156 g/kg 31.3 % w/w corresponding to 313 g/kg 10.1 % w/w corresponding to 101 g/kg
Description:	Beige solid	
Stability of test compound:	Stable under standard conditions	
Reanalysis/expiry date:	30 September 2014	
Treatments		
Test concentrations:	24.7, 74.1, 222, 667, 2000 and 6000 g A18032E/ha (*A. sativa* and *L. perenne* only) 0.914, 2.74, 8.23, 24.7, 74.1, 222 and 667g A18032E/ha (all other species)	
Control:	Water only	
Spray volume:	200L/ha ± 10%	
Adjuvant:	Adigor (A12127R) (mixture of emulsified fatty acid esters on basis of oleic acid methylester). The ratio of A18032E to A12127R was 1:2.5. Results are expressed in terms of the test item containing the adjuvant in this ratio.	
Application method:	Mardrive cabinet track sprayer with 8003-E TeeJet flat fan nozzle	
Test organisms		
Species:	*Avena sativa* (oat) *Lolium perenne* (ryegrass) *Oryza sativa* (rice) *Beta vulgaris* (sugar beet)	

	Brassica napus (oilseed rape) *Cucumis sativus* (cucumber) *Daucus carota* (carrot) *Lactuca sativa* (lettuce) *Lycopersicon esculentum* (tomato) *Raphanus sativus* (radish)
Test soil:	Sandy loam mixed as follows: 20 litres of sterile loam + 10 litres of sand. The soil was determined to consist of 75% w/w sand (2.00 – 0.063mm), 8% silt w/w (0.063 – 0.002 mm), 17% w/w clay (<0.002 mm). The organic carbon content was 1.3% w/w.
Test design	
Test vessels:	Non-porous plastic pots (8 x 8 x 8 cm), placed in saucers filled with enough water to ensure that the pots were kept moist at all times
Sampling interval:	Plants were assessed at 6 or 7, 14 and 21 days after 50% emergence in controls for emergence, mortality and visual phytotoxicity. Biomass and height were assessed at test termination
Replication:	Five pots per treatment, 4 seeds per pot
Duration:	21 days after 50% emergence in the controls
Environmental conditions	
Test temperature:	Mean: 20.9 °C (Min: 14.9 °C, Max: 28.9 °C)
Humidity:	Mean: 71.9 % (Min: 44.8 %, Max: 88.7 %)
Soil pH:	7.4
Lighting:	Ambient lighting was supplemented by sodium vapour lamps giving at least a 16 hour day. The mean ambient light intensity for the study period was 17.3 kilo lux (Kl), and the maximum intensity was 56.9 Kl

Study Design and Methods

Experimental dates: 29 June 2012 to 21 August 2012

Planted seeds of three monocot species (*Avena sativa*, *Lolium perenne* and *Oryza sativa*) and seven dicot species (*Beta vulgaris*, *Brassica napus*, *Cucumis sativus*, *Daucus carota*, *Lactuca sativa*, *Lycopersicon esculentum* and *Raphanus sativus*) were sprayed with a series of at least six test concentrations of A18032E together with the adjuvant A12127R, with a fixed mixture ratio of 1:2.5 for the two respective components. Nominal test concentrations used in the definitive test ranged from 0.914 to 6000 g A18032E/ha. The number of emerged seedlings, number of surviving seedlings, seedling height and weight were determined at test termination.

Seeds were sown directly into plastic pots (8 cm diameter and 8 cm deep), 1 - 2 cm deep, four seedlings per pot.

Observations were made 6 or 7, 14 and 21 days after 50% emergence in controls to document seedling emergence, mortality and visual phytotoxicity. Plant height was recorded at the final assessment. Plant condition was described by noting the presence or absence of possible signs of phytotoxicity such as chlorosis, leaf distortion and stunting. Each plant was then assigned a numerical score that described the plant condition. This was a scale from 0 to 100% - a subjective or qualitative assessment that determines whether damage is absent (0%), slight (1 – 39%), moderate (40 – 69%), severe (70 – 99%) or all plants dead (100%). A score of 10 does not mean that 10% of the plant is showing the effect (e.g. chlorosis), merely that the severity of the effect (e.g. chlorosis) is very slight.

The growth of emerged seedlings was evaluated at the end of the test by assessing the height and biomass of seedlings. Plant biomass was estimated by measuring the total dry weight of the shoots within each replicate. Seedling height was measured from the surface of the soil to the top of the tallest leaf. Dead or non-emerged seedlings were assigned a height of 0 cm. Seedlings were then clipped at soil level, the shoots of all living seedlings within a replicate were placed in a labelled paper bag, dried in an oven, and weighed as a group. Mean seedling height and replicate biomass were determined for each treatment group containing living seedlings at test termination.

Results and Discussion
Statistical analyses were used to evaluate effects of test substance application on plant emergence, height and biomass. Least Significant Difference (LSD) was used to establish the NOER by determining which treatment groups differed significantly ($p \leq 0.05$) from the control group. The ER_{50} and corresponding confidence limits were determined using simple probit - maximum likelihood estimation method. Statistical analysis was not conducted for plant condition and visual phytotoxicity because those data are qualitative.

The NOER and ER_{50} for biomass, height and emergence for each of the ten test species are presented in table below:

Table A 55: Effect Rates of A18032E + A12127R on 21-Day Biomass, Height and Emergence

Species	ER_{50} (g A18032E/ha)			NOER (g A18032E/ha)		
	Dry weight	Height	Emergence	Dry weight	Height	Emergence
Monocots						
Avena sativa **(oat)**	913.22	1031.79	>6000	222	74.1	6000
Lolium perenne **(ryegrass)**	79.51	143.10	>6000	24.7	24.7	6000
Oryza sativa **(rice)**	28.62	74.17	>667	24.7	24.7	667
Dicots						
Beta vulgaris **(sugar beet)**	7.72	8.31	>667	2.74	2.74	667
Brassica napus **(oilseed rape)**	53.85	144.59	>667	8.23	24.7	667
Cucumis sativus **(cucumber)**	60.33	227.13	>667	24.7	74.1	667
Daucus carota **(carrot)**	13.22	42.06	>667	8.23	8.23	667
Lactuca sativa **(lettuce)**	6.97	23.76	>667	2.74	24.7	667
Lycopersicon esculentum **(tomato)**	43.91	81.24	>667	24.7	24.7	667
Raphanus sativus **(radish)**	33.61	50.18	>667	8.23	8.23	667

Validity criteria
The validity criteria for the test were met:
- There was at least 70% emergence in the controls
- The control seedlings did not exhibit any phytotoxic effects
- The mean survival of the emerged control seedlings was at least 90%
- The environmental conditions were identical for all the tested species

Conclusions
A pre-emergent application of A18032E plus A12127R, at rates up to 6000 g A18032E/ha resulted in ER_{50} values ranging from 6.97 to > 6000 g A18032E/ha.
Lactuca sativa (lettuce) was the most sensitive species, with ER_{50} values based on dry weight and final height of 6.97 and 23.76 g A18032E/ha, respectively, and a NOER based on dry weight and final height of 2.74 and 24.7 g A18032E/ha, respectively. For all species, ER_{50} values based on emergence were higher than the highest concentrations tested, i.e. 667 or 6000 g A18032E/ha.

A 2.6.3 KCP 10.6.3 Extended laboratory studies on non-target plants

A 2.6.4 KCP 10.6.4 Semi-field and field tests 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