FINAL REGISTRATION REPORT

Part B

Section 9

Ecotoxicology

Detailed summary of the risk assessment

Product code: DNT-162OD-R-CPd

Product name(s): EVRITELL 162 OD

Chemical active substance(s):

dicamba, 110 g/L
nicosulfuron, 40 g/L
thifensulfuron-methyl, 12 g/L

Central Zone

Zonal Rapporteur Member State: POLAND

CORE ASSESSMENT

(authorization)

Applicant: QEMETICA Agricultural Solutions Poland S.A. (formerly: CIECH Sarzyna S.A.).

Correction date: 08/2024

Correction on 10/2024

MS Finalisation date: 12/2024, 03/2025, 04/2025

Version history

When	What
January 2024	First submission to zRMS
August 2024	Applicant`s update
October 2024	Second Applicant's update
December 2024	Assessment by zRMS
March 2025	Final Registration Report
April 2025	zRMS updated Final Registration Report

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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	12	13	14	15	16	17	18	19	20	21
Use-No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	**Application**				**Application rate**			PHI (days)	Remarks: e.g. g safener/ synergist per ha	**Conclusion**						
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms	Non-target plants
Zonal uses (field or outdoor uses, certain types of protected crops)																				
1.	PL, HU, SK	Maize ZEAMX	F	Annual monocotyledonous weeds TTTMS; Annual dicotyledonous weeds TTTDS	Spraying, broadcast	P Post emergence of weeds; crop BBCH 12-16	a) 1 b) 1	n.a.	a) 1 L/ha b) 1 L/ha	a) as 1: 110 g as/ha as 2: 40 g as/ha as 3: 12 g as/ha b) as 1: 110 g as/ha as 2: 40 g as/ha as 3: 12 g as/ha	100 / 300	n.a.	Dose range 0.75-1.0 L/ha	**A**	**A**	**R**	**A**	**A**	**C**	**R**

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

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

Explanation for column 15 – 21 "Conclusion"

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

Remarks table:

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

9.1.1 Overall conclusions

zRMS comment: All comments and conclusions of the zRMS are presented in grey. Minor changes are introduced directly in the text and highlighted in grey. Not agreed or not relevant information is struck through and shaded for transparency.

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)

The risk for birds arising from acute and long-term exposure to EVRITELL 162 OD is acceptable. Moreover, the risk for birds due to uptake of contaminated drinking water and via secondary poisoning is also acceptable.
The TER values, calculated for recommended scenarios, exceed the trigger value of 10 for acute risk. However, first-tier TER value calculated for one scenario did not exceed the relevant trigger values of 5 for reproductive risk and acceptable risk to mammals was confirmed based on higher tier assessment. The risk to mammals is acceptable following use of EVRITELL 162 OD according to the proposed use pattern.

9.1.1.2 Effects on aquatic organisms (KCP 10.2)

Based on PEC/RAC calculations, no unacceptable risk is indicated for aquatic organisms considering all envisaged GAP uses for EVRITELL 162 OD, assuming that following risk mitigation measures are taken into account:

- a vegetative buffer strip of 10m to surface water bodies is required when conventional spraying techniques are applied.

9.1.1.3 Effects on bees (KCP 10.3.1)

The evaluation of the risk for bees was performed in accordance with the recommendations of the “Guidance Document on Terrestrial Ecotoxicology”, as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002).
EVRITELL 162 OD was tested in acute (oral and contact exposure) and chronic studies (oral exposure of adults and larvae). Data submitted with this application are listed in Appendix 1 and summarised in Appendix 2 of this part of Section.
The acute risk assessments for the active substance as well as for the formulated product EVRITELL 162 OD with Hazard Quotients well below the trigger for acceptability of effects indicate an acceptable risk for bees exposed in accordance with the intended uses in maize.
In addition, the chronic study for adult bees and chronic study for larvae were submitted according to EU Reg. 284/2009.

9.1.1.4 Effects on arthropods other than bees (KCP 10.3.2)

The evaluation of the risk for non-target arthropods was principally performed in accordance with the recommendations of the “Guidance Document on Terrestrial Ecotoxicology”, as provided by the Com-mission

Services (SANCO/10329/2002 rev.2 (final), October 17, 2002), and in consideration of the recommendations of the guidance document ESCORT 2.
The risk assessment was performed based on results obtained for EVRITELL 162 OD in extended laboratory studies on *A.rhopalosiphi*, *T.pyri, Ch.carnea* and *C.septempunctata* and age residue study on the most sensitive species with *Ch. carnea*.
The available data on aged residue study indicate that, any initial effects on non-target arthropods from the proposed uses of EVRITELL 162 OD will be short-lived and recovery/recolonisation will take place within an acceptable time frame, thus an acceptable in field risk can be concluded.

9.1.1.5 Effects on non-target soil meso- and macrofauna (KCP 10.4), Updated April 2025

9.1.1.6 The information regarding the analytical measurements of active substances during the soil studies for formulation of EVRITELL 162 OD (DNT-162OD-R-CPd) with earthworms and *Folsomia candida* and *Hypoapsis aculeifer* was provided by Applicant. Below is the Applicant's approach was provided:

Justification of active substances concentrations during studies on impact of DNT-162OD-R-CPd for soil organisms

The product DNT-162OD-R-CPd, contains 3 active substances: dicamba, nicosulfuron and thifensulfuron-methyl. Although concentration of the actives was not measured in the studies on earthworms, *Folsomia* and *Hypoaspis*, safe use of product DNT-162OD-R-CPd for this organisms can be ensured. Detailed justification in this regard is presented hereafter.

1. Nicosulfuron

It can be anticipated that concentration of nicosulfuron in tested soils was stable in the toxicity tests with DNT-162OD-R-CPd. The geometric mean soil DT_{90lab} value for nicosulfuron is equal to 78.3 d (EFSA Scientific Report (2007) 120, 1-91), which is greater than the longest exposure phase considered in the soil organism studies (56 days for the earthworms).

Consequently, the concentration of nicosulfuron does not need to be analysed in the soil organisms studies with product DNT-162OD-R-CPd. The current assessment is sufficient and safe use for earthworms and other soil macroorganism can be ensured in respect to nicosulfuron for the intended use of DNT-162OD-R-CPd.

2. Dicamba

Although geometric mean soil DT_{90lab} value for dicamba of 13.2 d (EFSA Journal 2011;9(1):1965) is lower than the exposure phases, which are considered in soil organisms studies, there are the other data which clearly proof that concentration of this molecule is stable in the standard tested soils, at least up to 28 days. According to *Folsomia* and *Hypoaspis* studies with a different product containing dicamba (Chwastox Nowy Trio 390 SL), concentration of dicamba was in tested soils stable. As presented in Table 1 and 2 it was within 80% of the nominal concentration, during whole study periods.

Table 1. Analitical measurements of dicamba in *Folsomia* study with product Chwastox Nowy Trio 190 SL (Holewik, 2019, study code: G/153/18, page 54)

Date of analysis	Nominal concentration of dicamba mg/kg	Concentration determined in particular replicates mg/kg			Average mg/kg	SD mg/kg	RSD %	Average %
		1	2	3				
14.05.2019 day 0	0.00 32.23	ND 31.92	ND 31.72	ND 30.95	ND 31.53	0.00 0.51	- 1.6	- 97.8
28.05.2019 day 14	0.00 32.23	ND 28.44	ND 28.58	ND 29.31	ND 28.78	0.00 0.47	- 1.6	- 89.3
11.06.2019 day 28	0.00 32.23	ND 26.15	ND 26.02	ND 26.11	ND 26.09	0.00 0.06	- 0.2	- 81.0

Table 2. Analitical measurements of dicamba in *Hypoaspis* study with product Chwastox Nowy Trio 190 SL (Holewik, 2019, study code: G/154/18, page 55)

Date of analysis	Nominal concentration of dicamba mg/kg	Concentration determined in particular replicates mg/kg			Average mg/kg	SD mg/kg	RSD %	Average %
		1	2	3				
10.07.2019 day 0	0.00 32.23	ND 31.07	ND 31.14	ND 31.85	ND 31.35	0.00 0.43	- 1.4	- 97.3
17.07.2019 day 7	0.00 32.23	ND 28.62	ND 29.39	ND 29.75	ND 29.25	0.00 0.58	- 2.0	- 90.8
24.07.2019 day 14	0.00 32.23	ND 30.28	ND 28.23	ND 29.89	ND 29.80	0.00 0.54	- 1.8	- 92.5

For further details on the studies on Chwastox Nowy Trio 390 SL, please refer to Appendix 1, where the whole study reports are enclosed.

Similar study with dicamba is not for *Eisenia* available. Nevertheless, safe use can be ensured for earthworm in respect to dicamba, even if degradation of this molecule would be considered in the study with DNT-162OD-R-CPd.

Taking into account:

- the lowest nominal endpoint for dicamba for earthworms from the studies on DNT-162OD-R-CPd (report presented in the current dRR with a study code: G-24-23),
- correction of nominal endpoints by the EU agreed DT_{50} soil endpoint for dicamba,
- and duration of the earthworm study,

the NOECs TWA for dicamba can be estimated by the following equation:

$$NOEC_{TWA} = NOEC_{nominal} * (DT_{50\ soil} / (Study\ duration * \ln(2)) * (1 - \exp(-Study\ duration * \ln(2) / DT_{50\ soil})$$

where:
$NOEC_{nominal\ for\ earthworm}$ = 62.53 mg dicamba/ kg dw soil
(based on nominal concentration from the study on DNT-162OD-R-CPd),

DT_{50} soil = 3.0 days
(half- life relevant for calculation of dicamba concentration in soil – please refer to section B8 for DNT-162OD-R-CPd),

Study duration – 56 days.

With this assumption, the following NOECs TWA would be then for dicamba achieved:

$NOEC_{TWA}$ for earthworms for dicamba= 4.83 mg/kg dws soil

Then, considering the worst-case PECsoil value for dicamba applied as DNT-162OD-R-CPd, in case of use in maize of 0.11 mg/kg dws soil (please refer to section B8 for DNT-162OD-R-CPd), a safe use for dicamba in respect to the earthworms can be demonstrated:

TER = 4.83/0.11 = 44 (TER > 5, safe use anticipated)

Consequently, the concentration of dicamba does not need to be analysed in the soil organisms studies with product DNT-162OD-R-CPd. The available data prove sufficiently, that concentration of dicamba will remain stable during whole studies on *Folsomia* and *Hyposaspis*. In case of the earthworms, even if degradation of dicamba, is considered during 56-day period, there will be no unacceptable risk to the earthworms. The TER for dicamba will be then much greater than 5.

Thifensulfuron-methyl

Thifensulfuron-methyl degradates in soil the most rapidly, when compared to dicamba and nicosulfuron. It`s geometric mean laboratory DT_{90} value is 4.4 days (EFSA Journal 2015;13(7):4201). Despite this, no measurement of thifensulfuron-methyl concentration in tested soils is also not an issue for DNT-162OD-R-CPd.

Earthworms

Starting from the longest study on earthworms, with 56 days of exposure, it can be clearly shown that nicosulfuron and dicamba are the toxicity drivers of product DNT-162OD-R-CPd to earthworms - NOT thifensulfuron-methyl. By comparison of toxicity units for all actives, as presented hereafter, it can be easily shown that nicosulfuron and dicamba are responsible for almost 100% (exactly 96%) effect, while thifensulfuron-methyl affects this toxicity in just 4%.

TU nicosulfuron = 40 g/L : 1000 mg/kg dws* = 0.04
TU dicamba = 110 g/L : 1000 mg/kg dws* = 0.11
TU thifensulfuron -methyl = 12 g/L : 2000 mg/kg dws = 0.006

% TUmix nicosulfuron = 0.04 : 0.156*100 % = 25%
% TU mix dicamba = 0.11 : 0.156* 100% = 71%
% TU mix thifensulfuron methyl = 0.006 : 0.156* 100% = 4%

* Endpoints (LC_{50} values for earthworms) taken from agreed LoEP for dicamba, nicosulfuron and thifensulfuron-methyl (EFSA Scientific Report (2007) 120, 1-91; EFSA Journal 2011;9(1):196; EFSA Journal 2015;13(7):4201)

Additionally, based on the lowest nominal endpoint for earthworms from the study on DNT-162OD-R-CPd, (report presented in the current dRR with a study code: G-24-23), corrected by EU approved DT_{50} soil value for thifensulfuron-methyl and the worst-case PECsoil value for this active presented in section B8 for DNT-1 DNT-162OD-R-CPd, it can be ensured that no risk is anticipated in relation to this active and earthworms. As presented below the expected TER for thifensulfuron-methyl would be far above the trigger value of 5.

$NOEC_{nominal}$ = 6.92 mg thifensulfuron-methyl/ kg dw soil
(based on nominal concentration from the study on DNT-162OD-R-CPd)

DT_{50} soil = 3.1 days (half- life relevant for concentration of thifensulfuron – methyl in soil – please refer to section B8 for DNT-162OD-R-CPd)

$NOEC_{TWA} = NOEC_{nominal} * (DT_{50\ soil} /(Study\ duration * ln(2)) * (1 - exp (-Study\ duration*ln(2)/ DT_{50\ soil})$

$NOEC_{TWA}$ for thifensulfuron-methyl= 0.55 mg/kg dws soil

PEC_{soil} = 0.0120 mg/kg dws soil (worst-case PEC_{soil} value for thifensulfuron-methyl, please refer to section B8 for DNT-162OD-R-CPd)

TER = 0.55/0.0120 = 46 (TER > 5, safe use anticipated)
Folsomia* and *Hypoaspis

There are no EU toxicity data of thifensulfuron-methyl for *Folsomia* or *Hypoaspis* and thus, approach with toxic units for DNT-162OD-R-CPd can not be for these species applied. However, as in the case of the earthworms, $NOEC/EC_{10}$ TWA can be calculated for thifensulfuron-methyl, in a manner presented above.

In case of *Folsomi*a the following can be for thifensulfuron - methyl considered:

$NOEC_{nominal}$ = 0.12 mg thifensulfuron-methyl/kg dw soil
(based on nominal concentration from the study on DNT-162OD-R-CPd for *Folsomia*)

DT_{50} soil = 3.1 days
(half- life relevant for TWA concentration of thifensulfuron – methyl in soil – please refer to section B8 for DNT-162OD-R-CPd)

$NOEC_{TWA} = NOEC_{nominal} * (DT_{50\ soil} /(Study\ duration * ln(2)) * (1 - exp (-Study\ duration*ln(2)/ DT_{50\ soil})$

$NOEC_{TWA}$ for thifensulfuron-methyl= 0.019 mg/kg dws soil

PECsoil = 0.0120 mg/kg dws soil (worst-case PECsoil value for thifensulfuron-methyl, please refer to section B8 for DNT-162OD-R-CPd)

TER = 0.019/0.0120 = 1.59

As presented above the provided approach is not sufficient to conclude on the safe risk for *Folsomia*. Nevertheless, as underlined already, content of thifensulfuron-methyl in product DNT-162OD-R-CPd is much lower than the other actives, and it is not expected to be the main driver of the product`s toxicity. As mentioned in paragraph on earthworms, dicamba and nicosulfuron are responsible for 96% toxicity of DNT-162OD-R-CPd, while thifensulfuron – methyl causes just 4% of the effect.
In addition, as thifensulfuron-methyl degradates very fast in soil, and as semi-static or flow-through testing is not possible in soil organisms studies, maintenance of its concentration as required in the guidelines might be not be an option. Degradation of a thifensulfuron-methyl during testing leads however to the formation degradation products (IN-L9223, IN-L9225 and IN-W8268, IN-A4098):

- which are stable in soil (their soil geometric mean DT_{90} values are, greater than the longest -56-d earthworm study; they are between 73.1 and 910 d; see EFSA Journal 2015;13(7):420),

- which toxicity to *Folsomia* is known (with NOEC between 0.045 and100 mg/kg dws, see EFSA Journal 2015;13(7):4201),
- and as presented in Section B9 of dRR for product DNT-162OD-R-CPd, no risk was for them indicated according to uses as intended. The achieved TER for all degradation products were higher than a trigger of 5. They were between 7 to even 50 000.

Consequently, a targeted risk assessment performed with the main emphasis on the degradation products of thifensulfuron-methyl is sufficient enough to concluded on the safe risk to *Folsomia*.

In case of *Hypoaspis* the following can be for thifensulfuron -methyl considered:

$NOEC_{nominal}$ = 12.36 mg thifensulfuron-methyl/ kg dw soil
(based on nominal concentration from the study on DNT-162OD-R-CPd for *Folsomia*)

DT_{50} soil = 3.1 days (half- life relevant for TWA concentration of thifensulfuron – methyl in soil – please refer to section B8 for DNT-162OD-R-CPd)

$NOEC_{TWA} = NOEC_{nominal} * (DT_{50\ soil} / (Study\ duration * \ln(2)) * (1 - \exp(-Study\ duration * \ln(2) / DT_{50\ soil})$

$NOEC_{TWA}$ for thifensulfuron-methyl= 3.78 mg/kg dws soil

PECsoil = 0.0120 mg/kg dws soil (worst-case PECsoil value for thifensulfuron-methyl, please refer to section B8 for DNT-162OD-R-CPd)

TER = 3.78/0.0120 = 315 (TER >5, safe use anticipated)

Therefore, as presented above, it can be ensured that there are no issues with risk related to thifensulfuron-methyl and predatory mites. Even, when rapid degradation of this molecule in the soil is considered, the expected TER for thifensulfuron-methyl is much higher than the trigger of 5.

Based on above it can be concluded that there is no need to monitor thifensulfuron-methyl concentration during the studies on Eisenia, *Folsomia* or *Hypoapsis* with product DNT-162OD-R-CPd. Even if degradation of thifensulfuron-methyl is considered during studies, there will be no unacceptable risk to the earthworms or the other soil macroorganisms. The TER values will be then much greater than 5.

Conclusions for DNT-162OD-R-CPd:

In case of all actives, present in product DNT-162OD-R-CPd, safe use can be demonstrated to earthworms, *Folsomia* and *Hypoaspis*. The presented data allow to conclude sufficiently on the acceptable risk for DNT-162OD-R-CPd.

zRMS comment: The information regarding the analytical measurements of active substances during the soil studies for formulation of EVRITELL 162 OD (DNT-162OD-R-CPd) with earthworms and *Folsomia candida* and *Hypoapsis aculeifer* was accepted by zRMS. No additional risk assessment for earthworms and other soil macroorganism is required.

It should be considered at MSs level.

9.1.1.7 Effects on soil microbial activity (KCP 10.5)

The risk from exposure to dicamba, nicosulfuron and thifensulfuron-methyl and relevant soil degradation products applied as EVRITELL 162 OD for all intended uses is indicated to be acceptable for the soil meso- and macrofauna.
Risk assessments conducted with relevant PEC_{soil} for active substances dicamba, nicosulfuron, thifensulfuron-methyl and its relevant metabolites and in EVRITELL 162 OD indicate a low risk to soil microorganisms when applied to the proposed use rates.

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

For the proposed use of EVRITELL 162 OD, and depending on the approach considered, a safe risk for non-target plants is indicated when:

Deterministic approach: either 1 m buffer strip with 90% drift reduction or a 5 m buffer strip with 50% drift reduction, or 10 m buffer strip with no drift reduction is applied as risk mitigation measure.

Probabilistic approach: either 1 m buffer strip with 50% drift reduction or a 5 m buffer strip with no drift reduction is applied as risk mitigation measure.

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

Additional tests on other non-target species are not required.

9.1.2 Grouping of intended uses for risk assessment

No risk envelope approach (according to SANCO/11244/2011) is applied.

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 EVRITELL 162 OD is indicated in the table.

Table 9.1-2-1 Metabolites of Dicamba

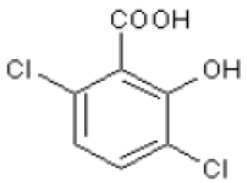
Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?
DCSA (3,60dichloro-2-hydroxybenzoic acid)		207	Soil: 58.8 % Water/sediment: 31.4 %	Yes, aquatic and soil organisms

Table 9.1-3-2 Metabolites of nicosulfuron

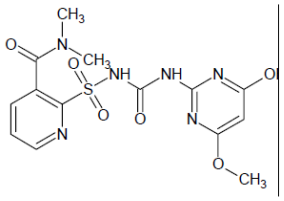
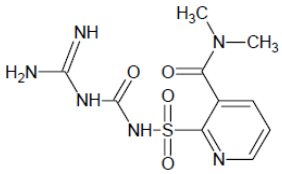
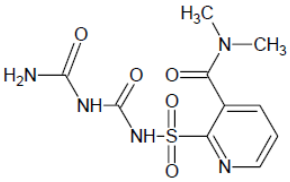
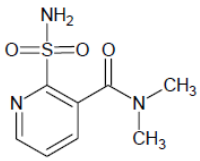
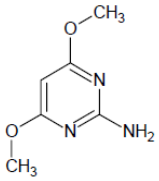
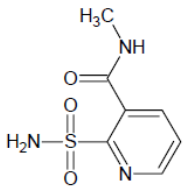
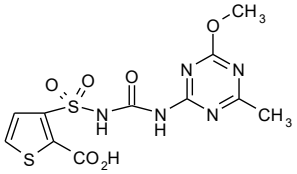
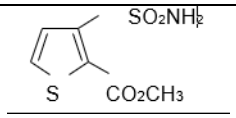
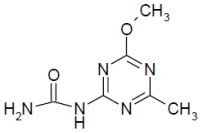
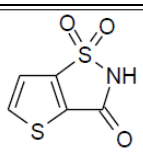
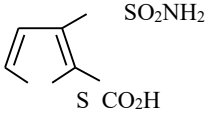
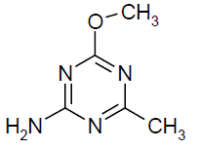
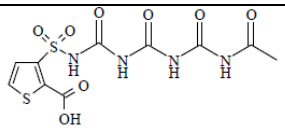
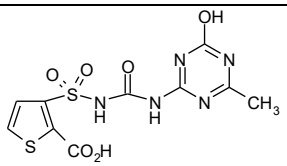
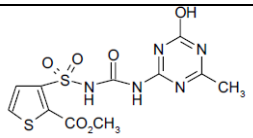
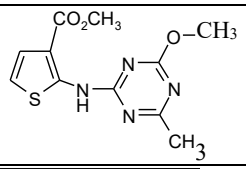
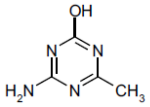
Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?
HMUD (2-[(4,6-dimethoxypyrimidin-2-ylcarbamoyl)sulfamoyl]-N,N-dimethylnicotinamide)		396.4	Soil: aerob; max 14.4 % on day 28 Water: max. 14.1 % on day 62 Sediment: max. 5.7 % on day 30	Yes, for aquatic
AUSN 2-[(carbamimidoylcarbamoyl)sulfamoyl]-N,N-dimethylpyridine-3-carboxamide		314.3	Soil: aerob; max 19.5 % on day 112 Water: max. 9.1 % on day 177	Yes, for aquatic and soil organisms
UCSN 2-[(carbamoylcarbamoyl)sulfamoyl]-N,N-dimethylpyridine-3-carboxamide		315.3	Soil: aerob; max 11 % on day 238 Water: max. 5.4 % on day 177	Yes, for aquatic and soil organisms
ASDM N,N-dimethyl-2-sulfamoylpyridine-3-carboxamide		229.2	Soil: aerob; max 21.50 % on day 189 Water: max. 6.9 % on day 177	Yes, for aquatic and soil organisms
ADMP 4,6-dimethoxypyrimidin-2-amine		155.2	Soil: aerob; max 7.2 % on day 31 Water/Sediment study: not investigated	No
MU-466 N-methyl-2-sulfamoylpyridine-3-carboxamide		215.1	Soil: aerob; < 5% Water/Sediment study: not investigated	No

Table 9.1-3-3 Metabolites of thifensulfuron-methyl

Metabolite	Molar weight (g/mol)	Chemical structure	Maximum observed occurrence in compartments (%)	Risk assessment required due to
IN-L9225	373.4		Soil: 94% aerobic Total Water/sediment system: 55% (water); 7.0% (Sediment)	PECsoil, PECsw/sed
IN-A5546	221.2		Soil: 10.5% aerobic, 27.7% photolysis Hydrolysis: 64.2% (pH 4), 7.6% (pH 7)	PECsoil, PECsw/sed
IN-V7160	183.2		Soil photolysis: 9.6% Total Water/sediment system: 25% (water); 6% (sediment)	PECsoil, PECsw/sed
IN-W8268	189.2		Soil: 29.6%	PECsoil, PECsw/sed
IN-L9223	207.2		Soil: 19% aerobic Total Water/sediment system: 39% (water); 8% (sediment)	PECsoil, PECsw/sed
IN-A4098	140.1		Soil: 18% aerobic, 32.3% photolysis Total Water/sediment system: 20.0% (water); 7.0% (sediment)	PECsoil, PECsw/sed
IN-U5F72 (2-acid-3triuret)	378.3		Soil: 17% aerobic	PECsoil, PECsw/sed
IN-JZ789	359.3		Soil: 10% aerobic Total Water/sediment system: 21% (water) ; 4% (sediment)	PECsw/sed, PECsoil
IN-L9226	373.4		Soil: 18.5% aerobic Total Water/sediment system: 7.8% (water); 7.2% (sediment)	PECsoil, PECsw/sed

Metabolite	Molar weight (g/mol)	Chemical structure	Maximum observed occurrence in compartments (%)	Risk assessment required due to
IN-D8858	280.3		Aqueous photolysis: 15.3%	PECsw/sed
IN-B5528	126.1		Hydrolysis: 25.3% (pH 4), not formed at pH 7	PECsw/sed

9.2 Effects on birds (KCP 10.1.1)

9.2.1 Toxicity data

Avian toxicity studies have been carried out with dicamba, nicosulfuron and thifensulfuron-methyl its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.
Effects on birds of EVRITELL 162 OD were not evaluated as part of the EU assessment of dicamba, nicosulfuron and thifensulfuron-methyl. No new studies for the formulation are required.

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

Species	Substance	Exposure System	Results	Reference
Colinus virginianus	Dicamba	Acute	**LD_{50} = 216 mg a.s./kg bw**	EFSA Scientific Report 2011;9(1):1965
Anas platyrhynchos	Dicamba	Acute	LD_{50} = 1373 mg a.s./kg bw	EFSA Scientific Report 2011;9(1):1965
Anas platyrhynchos	Dicamba	Short-term	LD_{50} > 1567 mg a.s./kg bw	EFSA Scientific Report 2011;9(1):1965
Colinus virginianus	Dicamba	Short-term	LD_{50} > 995 mg a.s./kg bw	EFSA Scientific Report 2011;9(1):1965
Anas platyrhynchos	Dicamba	Reproductive toxicity	**NOEL = 89 mg a.s./kg bw/day**	EFSA Scientific Report 2011;9(1):1965
Colinus virginianus	Dicamba	Reproductive toxicity	NOEL = 170 mg a.s./kg bw/day	EFSA Scientific Report 2011;9(1):1965
Bobwhite quail, Mallard duck	nicosulfuron	Acute	**LD_{50} > 2000 mg/kg b.w./day**	EFSA Scientific Report (2007) 120, 1-91

Species	Substance	Exposure System	Results	Reference
Bobwhite quail	nicosulfuron	Short-term (5 days)	LD_{50} > 1603 mg/kg b.w./day NOEL = 1603 mg/kg b.w./day	EFSA Scientific Report (2007) 120, 1-91
Mallard duck	nicosulfuron	Short-term (5 days)	LD_{50} > 911 mg/kg b.w./day NOEL = 911 mg/kg b.w./day	EFSA Scientific Report (2007) 120, 1-91
Japanese quail	nicosulfuron	Reproductive toxicity	**NOEC = 171 mg/kg b.w./day**	EFSA Scientific Report (2007) 120, 1-91
Mallard duck (*Anas platyrhynchos*)	thifensulfuron-methyl	Acute	LD_{50} > 2510 (4739)* mg/kg bw/day	EFSA Journal 2015;13(7):420
Bobwhite quail (*Colinus virginianus*)	thifensulfuron-methyl	Short-term	LDD_{50} > 1524 mg/kg bw/day	EFSA Journal 2015;13(7):420
Mallard duck (*Anas platyrhynchos*)	thifensulfuron-methyl	Short-term	**LDD_{50} > 1306 mg/kg bw/day**	EFSA Journal 2015;13(7):420
Bobwhite quail (*Colinus virginianus*)	thifensulfuron-methyl	Long-term	**NOAEL = 23 mg/kg bw/day**	EFSA Journal 2015;13(7):420
Mallard duck (*Anas platyrhynchos*)	thifensulfuron-methyl	Long-term	NOAEL = 172 mg/kg bw/day	EFSA Journal 2015;13(7):420

*extrapolated endpoint based on no mortality in the acute bird study in accordance with EFSA (2009)

9.2.1.1 Justification for new endpoints

No deviation from EU agreed endpoints.

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).

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.

Table 9.2-2: Screening assessment of the acute and long-term/reproductive risk for birds due to the use of EVRITELL 162 OD in maize

Intended use	**Maize**
Active substance/product	dicamba
Application rate (g/ha)	1 x 110

Acute toxicity (mg/kg bw)	216				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Maize	Small omnivorous bird	158.8	1.0	17.47	12.37
Reprod. toxicity (mg/kg bw/d)	89				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Maize	Small omnivorous bird	64.8	1.0 × 0.53	3.78	23.56

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.

Table 9.2-3: Screening assessment of the acute and long-term/reproductive risk for birds due to the use of EVRITELL 162 OD in maize

Intended use	**Maize**				
Active substance/product	nicosulfuron				
Application rate (g/ha)	1 x 40				
Acute toxicity (mg/kg bw)	2000				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Maize	Small omnivorous bird	158.8	1.0	6.35	314.86
Reprod. toxicity (mg/kg bw/d)	171				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Maize	Small omnivorous bird	64.8	1.0 × 0.53	1.37	124.48

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.

Table 9.2-4: Screening assessment of the acute and long-term/reproductive risk for birds due to the use of EVRITELL 162 OD in maize

Intended use	**Maize**				
Active substance/product	Thifensulfuron-methyl				
Application rate (g/ha)	1 x 12				
Acute toxicity (mg/kg bw)	1306				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Maize	Small omnivorous bird	158.8	1.0	1.91	685.35

Reprod. toxicity (mg/kg bw/d)	23				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Maize	Small omnivorous bird	64.8	1.0 × 0.53	0.41	55.81

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.

Mixture toxicity for birds - acute

Toxicity studies for birds with formulated products are typically not available. For the assessment of acute effects, a surrogate LD_{50} was calculated. A model often used to estimate the toxicity of mixtures is the assumption of dose/concentration additivity of toxicity (Loewe and Muischnek, 1926).
The following formula was used to derive a surrogate LD_{50} for the mixture of active substances with known toxicity assuming dose additivity:

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

With:

$X_{(a.s._i)}$ – fraction of active substance [i] in the mixture,

$LD_{50(a.s._i)}$ – acute toxicity value for active substance [i].

Active substance	$LD_{50(a.s._i)}$ **(mg a.s./kg b.w./day)**	**Content in the product [g/l]**	$X_{(a.s._i)}$	$\frac{X_{(a.s._i)}}{LD_{50(a.s._i)}}$	$LD_{50}(mix)$ **(mg a.s./kg b.w./day)**
Dicamba	216	110	0.6790	0.00314	301.2
Nicosulfuron	2000	40	0.2469	0.000123	
Thifenslfuron-methyl	1306	12	0.0741	0.0000567	

According to the EFSA/2009/1438 an endpoint for a mixture of active substances calculated assuming dose additivity should be conceived as an endpoint of a single virtual compound. Therefore, the exposure calculation for the risk assessment is based as well on this assumption. Content in the formulation and application rate per hectare should thus be expressed in terms of that virtual compound. Therefore, the overall application rate for active substances combined of 0.162 kg virtual compound/ha is considered the acute risk assessment.

Table 9.2-5: Screening assessment of the acute risk for birds due to the use of EVRITELL 162 OD in maize

Intended use	**maize**				
Active substance/product	Dicamba + nicosulfuron + thifensulfuron-methyl / EVRITELL 162 OD				
Application rate (g/ha)	1 × 162				
Acute toxicity (mg/kg bw)	301.2				
TER criterion	10				
Crop scenario	**Indicator focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Maize	Small omnivorous bird	158.8	1	25.73	11.71

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor

TER_A values are above the trigger value of 10, indicating low acute risk to birds following application of EVRITELL 162 OD. Therefore, no additional risk assessment is necessary for virtual compound toxicity.

Mixture toxicity for birds – long-term
A combined risk assessment was performed using the following equation:

TER_{LT}, combi = trigger / ((trigger/lowest TERsubst 1) + (trigger/lowest TERsubst 2)) + (trigger/lowest TERsubst 3))

The combined risk assessment was conducted based on screening assessment for dicamba nicosulfuron and thifensulfuron-methyl:
TER_{LT}, combi = 5 / ((5/23.56) + (5/124.48) +(5/55.81)) = 14.62

TER_{LT} values are above the trigger value of 5, indicating that EVRITELL 162 OD poses low long-term risk to birds according to proposed use.

zRMS comment: Agreed. The risk assessment at screening and Tier 1 is considered acceptable. 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). Safe use of active substance for birds such as dicamba, nicosulfuron and thifensulfuron-methyl were confirmed based on TER_A and TER_{LT} above the trigger values of 10 and 5, respectively, indicating the acute and long-term risk is acceptable. Combined acute and log-term risk assessment for birds was accepted by RMS.

9.2.2.2 Higher-tier risk assessment

No higher-tier assessment required.

zRMS comment: Agreed.

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 EVRITELL 162 OD 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.

zRMS comment: Agreed.

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).

With a geomean K(f)oc of 9.8, dicamba belongs to the group of less sorptive substances.

Effective application rate (g/ha)	=	110		
Acute toxicity (mg/kg bw)	=	216	quotient =	0.51
Reprod. toxicity (mg/kg bw/d)	=	89	quotient =	1.24

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

Effective application rate (g/ha)	=	40		
Acute toxicity (mg/kg bw)	=	2000	quotient =	0.02
Reprod. toxicity (mg/kg bw/d)	=	171	quotient =	0.23

With a K(f)oc of 9.0 L/kg thifensulfuron - methyl belongs to the group of less sorptive substances.

Effective application rate (g/ha)	=	12		
Acute toxicity (mg/kg bw)	=	1306	quotient =	0.009
Reprod. toxicity (mg/kg bw/d)	=	23	quotient =	0.52

zRMS comment: Agreed.

9.2.2.4 Effects of secondary poisoning

The log Pow of dicamba amounts to 0.55 – 1.9 at pH 5.0 – 8.9 and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

The log Pow of nicosulfuron amounts to 0.61 and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

The log Pow of thifensulfuron - methyl amounts to 0.0253 at pH 5, -1.65 at pH 7 and -2.10 at pH 9 and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

zRMS comment: Agreed.

Risk assessment for earthworm-eating birds via secondary poisoning

Not required

zRMS comment: Agreed.

Risk assessment for fish-eating birds via secondary poisoning

Not required

zRMS comment: Agreed.

9.2.2.5 Biomagnification in terrestrial food chains

Not relevant.

zRMS comment: Agreed.

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

Not relevant.

zRMS comment: Agreed.

9.2.4 Overall conclusions

The risk for birds arising from acute and long-term exposure to EVRITELL 162 OD is acceptable. Moreover, the risk for birds due to uptake of contaminated drinking water and via secondary poisoning is also acceptable.

zRMS comment: Agreed.

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, nicosulfuron and thifensulfuron-methyl and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents as well as in Section 6 (Mammalian Toxicology) of this report (new studies).

Effects on mammals of EVRITELL 162 OD were not evaluated as part of the EU assessment of dicamba, nicosulfuron and thifensulfuron-methyl.

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

Species	Substance	Exposure System	Results	Reference
Rat	dicamba	Acute	**LD_{50} = 1581 mg/kg bw**	EFSA Scientific Report (2007) 122, 1-84
Rat	dicamba	Long-term (multigeneration)	**NOAEL = 150 mg/kg bw**	EFSA Scientific Report (2007) 122, 1-84
Rat/ mouse	nicosulfuron	Acute toxicity	**LD_{50} > 5000 mg/kg bw/day**	EFSA Scientific Report (2007) 120, 1-91
Rat	nicosulfuron	Reproductive toxicity	**NOAEL = 3861 mg/kg b.w./day (male)**	EFSA Scientific Report (2007) 120, 1-91
Rat	ASDM (nicosulfuron metabolite)	Acute toxicity	LD_{50} > 5000 mg/kg b.w./day	EFSA Scientific Report (2007) 120, 1-91
Rat	AUSN (nicosulfuron metabolite)	Acute toxicity	LD_{50} > 2000 mg/kg b.w./day	EFSA Scientific Report (2007) 120, 1-91
Rat	thifensulfuron-methyl	Acute	LD_{50} > 5000 mg/kg b.w./day	EFSA Journal 2015;13(7):420
Rat	IN-L9225	Acute	LD_{50} > 2000 mg/kg b.w./day	EFSA Journal 2015;13(7):420
Rat	IN-A4098	Acute	LD_{50} > 2000 mg/kg b.w./day (males) LD_{50} = 1000 mg/kg b.w./day (females)	EFSA Journal 2015;13(7):420
Rat	IN-W8268	Acute	LD_{50} > 2000 mg/kg b.w./day	EFSA Journal 2015;13(7):420
Rat	thifensulfuron-methyl	Long-term	**NOAEL = 1.3 mg/kg bw day***	EFSA Journal 2015;13(7):420
Rat	thifensulfuron-methyl	Long-term	NOAEL = 43 mg/kg bw day*	EFSA Journal 2015;13(7):420

*The NOAEL of 1.3 mg/kg b.w per day was used in the screening step assessment as it was used in the human risk assessment to set the ADI. This NOAEL was further refined at first tier to 43 mg/kg b.w per day. Should a higher tier assessment be required in the future then the ecological relevance of this NOAEL should be considered further.

zRMS comment: zRMS confirms that the reported toxicity data in table 9.3-1 are in accordance with the EU agreed end-points and will be used for risk assessment.

9.3.1.1 Justification for new endpoints

No deviation from EU agreed endpoints.

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).

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.

Table 9.3-2: Screening assessment of the acute and long-term/reproductive risk for mammals due to the use of EVRITELL 162 OD in maize

Intended use	Maize				
Active substance/product	dicamba				
Application rate (g/ha)	1 × 110				
Acute toxicity (mg/kg bw)	1581				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Maize	Small herbivorous mammal	136.4	1.0	15.0	105.40
Reprod. toxicity (mg/kg bw/d)	150				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Maize	Small herbivorous mammal	72.3	1.0 × 0.53	4.22	35.59

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.

Table 9.3-3: Screening assessment of the acute and long-term/reproductive risk for mammals due to the use of EVRITELL 162 OD in maize

Intended use	Maize				
Active substance/product	nicosulfuron				
Application rate (g/ha)	1 × 40				
Acute toxicity (mg/kg bw)	5000				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Maize	Small herbivorous mammal	136.4	1.0	5.46	915.75
Reprod. toxicity (mg/kg bw/d)	3861				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Maize	Small herbivorous mammal	72.3	1.0 × 0.53	1.53	2518.59

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.

Table 9.3-4: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of EVRITELL 162 OD in maize

Intended use **Active substance/product** **Application rate (g/ha)**	Maize Thifensulfuron-methyl 1 × 12				
Acute toxicity (mg/kg bw) **TER criterion**	1000 (to cover metabolite IN-A4098) 10				
Crop scenario **Growth stage**	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Maize	Small herbivorous mammal	136.4	1.0	1.64	609.76
Reprod. toxicity (mg/kg bw/d) **TER criterion**	1.3 5				
Crop scenario **Growth stage**	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Maize BBCH 10-19	Small insectivorous mammal "shrew"	4.2	1.0 × 0.53	0.03	48.15
Maize BBCH 10-29	Small herbivorous mammal "vole"	72.3	1.0 × 0.53	0.46	**2.83**
Maize BBCH 10-29	Small herbivorous mammal "mouse"	7.8	1.0 × 0.53	0.05	26.0

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.

Mixture toxicity for mammals - acute

For the assessment of acute effects, a surrogate LD_{50} was calculated. A model often used to estimate the toxicity of mixtures is the assumption of dose/concentration additivity of toxicity (Loewe and Muischnek, 1926).

The following formula was used to derive a surrogate LD_{50} for the mixture of active substances with known toxicity assuming dose additivity:

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

With:

$X_{(a.s._i)}$ – fraction of active substance [i] in the mixture,

$LD_{50\,(a.s._i)}$ – acute toxicity value for active substance [i].

Active substance	$\boldsymbol{LD_{50\,(a.s._i)}}$ **(mg a.s./kg b.w./day)**	**Content in the product [g/l]**	$\boldsymbol{X_{(a.s._i)}}$	$\frac{\boldsymbol{X_{(a.s._i)}}}{\boldsymbol{LD_{50\,(a.s._i)}}}$	$\boldsymbol{LD_{50}(mix)}$ **(mg a.s./kg b.w./day)**
Dicamba	1581	110	0.6790	0.000429	1809.95
nicosulfuron	5000	40	0.2469	0.0000494	

Thifensulfuron-methyl	1000	12	0.0741	0.0000741	

According to the EFSA/2009/1438 an endpoint for a mixture of active substances calculated assuming dose additivity should be conceived as an endpoint of a single virtual compound. Therefore the exposure calculation for the risk assessment is based as well on this assumption. Content in the formulation and application rate per hectare should thus be expressed in terms of that virtual compound. Therefore, the overall application rate for active substances combined of 0.162 kg virtual compound/ha is considered for the acute risk assessment.

Table 9.3-5: Screening assessment of the acute and long-term/reproductive risk for mammals due to the use of EVRITELL 162 OD in maize

Intended use	maize				
Active substance/product	dicamba + nicosulfuron + thifensulfuron-methyl / EVRITELL 162 OD				
Application rate (g/ha)	1 × 162				
Acute toxicity (mg/kg bw)	1809.95				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Maize	Small herbivorous mammal	136.4	1.0	22.10	81.90

TER_A values are above the trigger value of 10, indicating low acute risk to mammals following application of EVRITELL 162 OD. Therefore, no additional risk assessment is necessary for virtual compound toxicity.

Mixture toxicity for mammals – long-term

A combined risk assessment was performed using the following equation:

TER_{LT}, combi = trigger / ((trigger/lowest TERsubst 1) + (trigger/lowest TERsubst 2) + (trigger/lowest TERsubst 3))

The combined risk assessment was conducted based on the screening and Tier 1 assessment (lowest acceptable value) for dicamba, nicosulfuron and thifensulfuron-methyl

TER_{LT}, combi = 5 / ((5/35.59) + (5/2518.59) + (5/**2.83**)) = **2.62**

The combined long-term risk assessment is not acceptable and needs to be refined.

zRMS comment: Agreed.

9.3.2.2 Higher-tier risk assessment

The risk assessment conducted at Tier 1 for Thifensulfuron-methyl indicates an unacceptable chronic risk to small herbivorous mammal “vole” exposed to EVRITELL 162 OD.

Therefore, additional risk assessment is required.

The refined risk assessment is based on toxicological endpoint of NOAEL = 43 mg/kg bw/d (for details see

EFSA Journal 2015;13(7):420).

Table 9.3-6: Higher-tier assessment of the long-term/reproductive risk for mammals due to the use of EVRITELL 162 OD in maize – refined parameters (*) are further described and justified in the text

Intended use	Maize				
Active substance/product	Thifensulfuron-methyl				
Application rate (g/ha)	1 × 12				
Acute toxicity (mg/kg bw)	43				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Maize BBCH 10-29	Small herbivorous mammal "vole"	72.3	1.0 × 0.53	0.46	93.48

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; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Additional evaluation of TERmix has to be performed, as refined TER_{lt} value for thifensulfuron-methyl is lower than lowest acceptable Tier 1 value used in combined risk assessment for EVRITELL 162 OD mixture.

The refined combined risk assessment is based on the higher tier assessment for thifensulfuron-methyl and screening assessment for dicamba and for nicosulfuron for the indicator species with the lowest TER_{lt} (Small herbivorous mammal).

TER_{LT}, combi = 5 / ((5/35.59) + (5/2518.59) + (5/93.48)) = 25.52

The combined long-term risk assessment is acceptable.

zRMS comment: Agreed. The risk assessment at screening and Tier 1 is considered acceptable. 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). Safe use of active substance for mammals such as dicamba, nicosulfuron were confirmed based on TER_A and TER_{LT} above the trigger values of 10 and 5, respectively, indicating the acute and long-term risk is acceptable. The risk assessment conducted at Tier 1 for Thifensulfuron-methyl indicates an unacceptable chronic risk to small herbivorous mammal "vole" exposed to EVRITELL 162 OD. The refined risk assessment is based on toxicological endpoint of NOAEL = 43 mg/kg bw/d. The refined combined risk assessment is based on the higher tier assessment for thifensulfuron-methyl and screening assessment for dicamba and for nicosulfuron for the indicator species with the lowest TER_{LT} (Small herbivorous mammal). The refinement risk assessment for mammals should be confiremed by MSs level.

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).

With a geomean K(f)oc of 9.8, dicamba belongs to the group of less sorptive substances.

Effective application rate (g/ha)	=	110		
Acute toxicity (mg/kg bw)	=	1581	quotient =	0.07
Reprod. toxicity (mg/kg bw/d)	=	150	quotient =	0.73

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

Effective application rate (g/ha)	=	40		
Acute toxicity (mg/kg bw)	=	5000	quotient =	0.008
Reprod. toxicity (mg/kg bw/d)	=	3861	quotient =	0.01

With a K(f)oc of 9.0 L/kg thifensulfuron - methyl belongs to the group of less sorptive substances.

Effective application rate (g/ha)	=	12		
Acute toxicity (mg/kg bw)	=	1000	quotient =	0.012
Reprod. toxicity (mg/kg bw/d)	=	1.3	quotient =	9.23

zRMS comment: Agreed.

9.3.2.4 Effects of secondary poisoning

The log Pow of dicamba amounts to 0.55 – 1.9 at pH 5.0 – 8.9 and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

The log Pow of nicosulfuron amounts to 0.61 and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

The log Pow of thifensulfuron - methyl amounts to 0.0253 at pH 5, -1.65 at pH 7 and -2.10 at pH 9 and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

zRMS comment: Agreed.

Risk assessment for earthworm-eating mammals via secondary poisoning

Not required.

zRMS comment: Agreed.

Risk assessment for fish-eating mammals via secondary poisoning

Not required.

zRMS comment: Agreed.

9.3.2.5 Biomagnification in terrestrial food chains

Not relevant.

zRMS comment: Agreed.

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

Not relevant.

zRMS comment: Agreed.

9.3.4 Overall conclusions

The TER values, calculated for recommended scenarios, exceed the trigger value of 10 for acute risk. However, first-tier TER value calculated for one scenario did not exceed the relevant trigger values of 5 for reproductive risk and acceptable risk to mammals was confirmed based on higher tier assessment. The risk to mammals is acceptable following use of EVRITELL 162 OD according to the proposed use pattern.

zRMS comment: Agree with the presented risk assessment.

The refined risk assessment for mammals was accepted by zRMS.
Safe use of active substance for mammals such as dicamba, nicosulfuron and thifensulfuron-methyl were confirmed based on TER_A and TER_{LT} above the trigger values of 10 and 5, respectively, indicating the acute and long-term risk is acceptable.
Combined acute and log-term risk assessment for mammals was accepted by RMS.
The refinement risk assessment for mammals should be confiremed by MSs level.

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

No available data considered as relevant.

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, nicosulfuron and thifensulfuron methyl and their relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on aquatic organisms of EVRITELL 162 OD were not evaluated as part of the EU assessments of dicamba, nicosulfuron and thifensulfuron methyl. 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 9.5-1: Endpoints and effect values relevant for the risk assessment for aquatic organisms –dicamba, nicosulfuron and thifensulfuron methyl and its metabolite

Species	Substance	Exposure System	Results	Reference
Dicamba				
Cyprinus carpio	dicamba	96 h static	EC_{50} > 100 mg a.s./L	EFSA Journal 2011; 9(1):1965
Oncorhynchus mykiss	dicamba	96 h	**LC_{50} > 41 mg a.s./L**	EFSA Journal 2011; 9(1):1965
Oncorhynchus mykiss	dicamba	28 d	**NOEC = 180 mg a.s./L**	EFSA Journal 2011; 9(1):1965
Daphnia magna	dicamba	48 h	**EC_{50} > 41 mg a.s./L**	EFSA Journal 2011; 9(1):1965
Daphnia magna	dicamba	21 d	**NOEC = 97 mg a.s./L**	EFSA Journal 2011; 9(1):1965
Skeletonema costatum	dicamba	72 h	E_rC_{50} > 4.1 mg a.s./L E_bC_{50} = 1.8 mg a.s./L*	EFSA Journal 2011; 9(1):1965
Navicula pelliculosa	dicamba	96 h static	**E_rC_{50}/E_bC_{50} > 3.8 mg a.s./L**	EFSA Journal 2011; 9(1):1965
Anabaena flos-aquae	dicamba	96 h static	E_rC_{50}/E_bC_{50} > 32 mg a.s./L	EFSA Journal 2011; 9(1):1965
Lemna gibba	dicamba	7 d static	E_rC_{50} n.a. E_bC_{50} > 3.25 mg a.s/L	EFSA Journal 2011; 9(1):1965
Myriophyllum spicatum	dicamba	26 d	**IC_{50} > 0.45 mg a.s./L**	EFSA Journal 2011; 9(1):1965
Oncorhynchus mykiss	DCSA	96 h.	**LC_{50} >100mg a.s./L**	EFSA Journal 2011; 9(1):1965
Daphnia magna	DCSA	48 h.	**EC_{50} > 89 mg a.s./L**	EFSA Journal 2011; 9(1):1965
Selenastrum capricornutum	DCSA	72 h	**E_rC_{50} > 138 mg a.s./L** E_bC_{50} = 118 mg a.s./L	EFSA Journal 2011; 9(1):1965
Lemna gibba	DCSA	7 days	**E_rC_{50} > 73 mg a.s./L** E_bC_{50} = 11.9 mg a.s/L	EFSA Journal 2011; 9(1):1965

Nicosulfuron				
Species	**Substance**	**Exposure System**	**Results**	**Reference**
Fish				
Oncorhynchus mykiss	nicosulfuron	96 h, s	**LC_{50} = 65.7 mg a.s./L** mm	EFSA Scientific Report (2007) 120, 1-91
Oncorhynchus mykiss	nicosulfuron	28 d, f	**NOEC = 10 mg a.s./L** mm	EFSA Scientific Report (2007) 120, 1-91
Lepomis macrochirus	ASDM	96 h, s	LC_{50} >100 mg ASDM/L	EFSA Scientific Report (2007) 120, 1-91
Brachydanio rerio (zebra fish)	AUSN	96 h, s	LC_{50} >100 mg AUSN/L	EFSA Scientific Report (2007) 120, 1-91
Oncorhyncus mykiss	MU-466	96 h, s	LC_{50} >100 mg MU-466/L	EFSA Scientific Report (2007) 120, 1-91
Oncorhyncus mykiss	HMUD	96 h, s	LC_{50} >100 mg HMUD/L	EFSA Scientific Report (2007) 120, 1-91
Oncorhyncus mykiss	ADMP	96 h, s	LC_{50} >100 mg ADMP/L	EFSA Scientific Report (2007) 120, 1-91
Aquatic invertebrates				
Daphnia magna	nicosulfuron	48 h, s	**EC_{50} = 90 mg a.s./L** mm	EFSA Scientific Report (2007) 120, 1-91
Daphnia magna	nicosulfuron	21d, ss	**NOEC = 5.2 mg a.s./L** mm	EFSA Scientific Report (2007) 120, 1-91
Daphnia magna	ASDM	48 h, s	EC_{50} > 954 mg metabolite/L mm	EFSA Scientific Report (2007) 120, 1-91
Daphnia magna	AUSN	48 h, s	EC_{50} >100 mg metabolite./L mm	EFSA Scientific Report (2007) 120, 1-91
Daphnia magna	MU-466	48 h, s	EC_{50} >100 mg metabolite./L mm	EFSA Scientific Report (2007) 120, 1-91
Daphnia magna	HMUD	48 h, s	EC_{50} >100 mg metabolite./L mm	EFSA Scientific Report (2007) 120, 1-91
Daphnia magna	UCSN	48 h, s	EC_{50} >100 mg metabolite./L mm	EFSA Scientific Report (2007) 120, 1-91
Daphnia magna	ADMP	48 h, s	EC_{50} >100 mg metabolite./L mm	EFSA Scientific Report (2007) 120, 1-91

Algae				
Anabaena flos-*aquae*	nicosulfuron	72 h, s	**E_bC_{50} = 7.8 mg a.s./L**	EFSA Scientific Report (2007) 120, 1-91
Pseudokirchneriella subcapitata	ASDM	72 h, s	E_rC_{50}>336 mg metabolite./L	EFSA Scientific Report (2007) 120, 1-91
Scenedesmus subspicatus	AUSN	72 h, s	E_rC_{50}>100 mg metabolite./L	EFSA Scientific Report (2007) 120, 1-91
Scenedesmus subspicatus	MU-466	72 h, s	E_rC_{50}>100 mg metabolite./L	EFSA Scientific Report (2007) 120, 1-91
Scenedesmus subspicatus	HMUD	72 h, s	E_rC_{50}>100 mg metabolite./L	EFSA Scientific Report (2007) 120, 1-91
Scenedesmus subspicatus	UCSN	72 h, s	E_rC_{50}>100 mg metabolite./L	EFSA Scientific Report (2007) 120, 1-91
Scenedesmus subspicatus	ADMP	72 h, s	E_rC_{50}>100 mg metabolite./L	EFSA Scientific Report (2007) 120, 1-91
Aquatic plants				
Lemna gibba	nicosulfuron	7 d, ss	E_rC_{50} = 0.0027 mg a.s./L	EFSA Scientific Report (2007) 120, 1-91
Lemna gibba	nicosulfuron	7 d, ss	**E_rC_{50} = 0.00139 mg a.s./L**	Dengler D. S08-00936 2008
Lemna gibba	ASDM	7 d, ss	EC_{50} >100 mg metabolite/L	EFSA Scientific Report (2007) 120, 1-91
Lemna gibba	AUSN	7 d, ss	EC_{50} >100 mg metabolite/L	EFSA Scientific Report (2007) 120, 1-91
Lemna gibba	HMUD	7 d, ss	EC_{50} >1.0 mg metabolite/L	EFSA Scientific Report (2007) 120, 1-91
Lemna gibba	UCSN	7 d, ss	EC_{50} >100 mg metabolite/L	EFSA Scientific Report (2007) 120, 1-91

Thifensulfuron methyl				
Species	**Substance**	**Exposure System**	**Results**	**Reference**
Acute toxicity to fish				
Oncorhynchus mykiss	Thifensulfuron methyl 50SG	96 h, s	LC_{50} >120 mg product/L $_{nom}$ **LC_{50} >56.4 mg a.s./L $_{mm}$**	EFSA 2015 Not available, 2001 (DuPont-11440)
Oncorhynchus mykiss	IN-L9225	96 h, s	**LC_{50} >120 mg met./L $_{mm}$**	EFSA 2015 Not available, 2001 (DuPont-5622)
Oncorhynchus mykiss	IN-JZ789	96 h, s	**LC_{50} >0.94 mg met./L $_{mm}$**	EFSA 2015 Not available, 1991 (DuPont-1655)
Oncorhynchus mykiss	IN-V7160	96 h, s	**LC_{50} >1.0 mg met./L $_{mm}$**	EFSA 2015 Not available, 1999 (DuPont-3561)
Oncorhynchus mykiss	IN-A4098	96 h, s	LC_{50} >200 mg met./L $_{nom}$	EFSA 2015 Not available, 1984 (Ciba 87 26)
Oncorhynchus mykiss	IN-A4098	96 h, s	**LC_{50} >0.93 mg met./L $_{mm}$**	EFSA 2015 Not available, 1999 (DuPont-3559)
Oncorhynchus mykiss	IN-W8268	96 h, s	**LC_{50} >115 mg met./L $_{mm}$**	EFSA 2015 Not available, 2000 (DuPont-4683)
Acute toxicity to invertebrates				
Chironomus riparius	Thifensulfuron methyl	48 h, s	**EC_{50} >100 mg a.s./L $_{nom}$**	EFSA 2015 Juckeland, D, 2012 (11 10 48 045 W)
Daphnia magna	IN-L9225	48 h, s	**EC_{50} >130 mg met./L $_{mm}$**	EFSA 2015 Samel, A., 2001 (DuPont-5621)
Daphnia magna	IN-L9223	48 h, s	**EC_{50} >1.2 mg met./L $_{mm}$**	EFSA 2015 Samel, A., 1999 (DuPont-3216)
Daphnia magna	IN-JZ789	48 h, s	**EC_{50} >1.1 mg met./L $_{mm}$**	EFSA 2015 Hoke, R., 1999 (DuPont-1654)
Daphnia magna	IN-V7160	48 h, s	**EC_{50} >1.3 mg met./L $_{mm}$**	EFSA 2015 Hoke, R.A., 2001 (DuPont-4507)
Daphnia magna	IN-A4098	48 h, s	**EC_{50} >99 mg met./L $_{mm}$**	EFSA 2015 Samel, A., 1999 (DuPont-3247)
Daphnia magna	IN-W8268	48 h, s	**EC_{50} >125 mg met./L $_{mm}$**	EFSA 2015 Samel, A., 2000 (DuPont-4682)
Daphnia magna	Thifensulfuron methyl 50 SG	48 h, s	EC_{50} >120 mg product/L $_{nom}$ **EC_{50} >60.7 mg a.s./L $_{nom}$**	EFSA 2015 Hoke, R.A., 2003 (DuPont-11439)
Chronic toxicity to invertebrates				
Daphnia magna	Thifensulfuron methyl	21 d, ss	**NOEC = 99 mg/L**	Confirmatory data submitted by FMC Hutton, D.G., 1989 (HLR 70-89)

				EFSA Supporting publication 2020:EN-1627
Daphnia magna	IN-L9223	21 d, ss	**NOEC(reproduction) = 13 mg met./L mm**	EFSA 2015 Samel, A., 2000 (DuPont-4487)
Daphnia magna	IN-V7160	21 d, ss	**NOEC(adult body length) = 11 mg met./L mm**	EFSA 2015 Hoke, R., 2001 (DuPont-4507)
Daphnia magna	IN-A4098	21 d, ss	**NOEC(reproduction) = 32 mg met./L nom**	EFSA 2015 Grade, R., Wydra, V., Moll, M., 2006 (168 MEM)
Toxicity to algae				
Pseudokirchneriella subcapitata	IN-L9225	72 h, s	**E_rC_{50} = 36.5 mg met./L nom** E_bC_{50} (cell density) = 33.4 mg met./L nom	EFSA 2015 Sloman, T., 2001 (DuPont-5620)
Pseudokirchneriella subcapitata	IN-L9223	72 h, s	**EC_{50} >1.3 mg met./L mm**	EFSA 2015 Sloman, T., 1999 (DuPont-3012)
Pseudokirchneriella subcapitata	IN-JZ789	72 h, s	**EC_{50} >1.28 mg met./L mm**	EFSA 2015 Sloman, T., 1999 (DuPont-2850)
Pseudokirchneriella subcapitata	IN-V7160	72 h, s	**EC_{50} >11 mg met./L mm**	EFSA 2015 Sloman, T., 1999 (DuPont-3190)
Pseudokirchneriella subcapitata	IN-A4098	72 h, s	E_bC_{50} >10 mg met./L nom **E_rC_{50} >10 mg met./L nom**	EFSA 2015
Pseudokirchneriella subcapitata	IN-W8268	72 h, s	E_bC_{50} (Cell density) = 29.9 mg met./L nom **E_rC_{50} = 31.6 mg met./L nom**	EFSA 2015 Sloman, T., 2000 (DuPont-4680)
Pseudokirchneriella subcapitata	IN-L9226	72 h, s	EC_{50} (yield, biomass and growth rate) **>89 mg met./L nom**	EFSA 2015 Vinken & Wydra, 2007 (51 TIM)
Pseudokirchneriella subcapitata	IN-A5546	72 h, s	E_bC_{50} = 48 mg met./L mm **E_rC_{50} >110 mg met./L mm**	EFSA 2015 Hoberg, J.R., 2007 (DuPont-21528)
Pseudokirchneriella subcapitata	2-acid-3-triuret	72 h, s	E_yC_{50} >100 mg met./L nom **E_rC_{50} >100 mg met./L nom**	EFSA 2015 Falk, S., 2012 (S12-01019)
Pseudokirchneriella subcapitata	Thifensulfuron methyl 50 SG (DuPont)	72 h, s	E_bC_{50} 0.30325 mg a.s./L nom **E_rC_{50} = 0.8 mg a.s./L nom**	EFSA 2015
Toxicity to macrophytes				
Ceratophyllum demersum	Thifensulfuron methyl	14 d, s	E_rC_{50} = 32.15 mg a.s./L mm	EFSA 2015** Hoberg, J., 2011
Elodea canadensis	Thifensulfuron methyl	14 d, s	E_rC_{50} = 0.0217 mg a.s./L mm	EFSA 2015** Hoberg, J., 2011
Myriophyllum aquaticum	Thifensulfuron methyl	14 d, s	E_rC_{50} = 0.1871 mg a.s./L $_{mm}$	EFSA 2015** Hoberg, J., 2011
Vallisneria americana	Thifensulfuron methyl	14 d, s	**E_rC_{50} = 0.00023 mg a.s./L $_{mm}$** NOEC = 0.00011 mg a.s./L $_{mm}$	EFSA 2015** Hoberg, J., 2011

Lemna gibba	IN-L9225	14 d, s	EC_{50} = 36.76 mg met./L $_{mm}$ **E_rC_{50} (frond count) = 82.2 mg met./L $_{mm}$**	EFSA 2015 Boeri, R., 2001 (DuPont-4654)
Lemna gibba	IN-L9223	14 d, s	EC_{50} (frond count and biomass) >172.1 mg met./L $_{nom}$ **E_rC_{50} (frond count) >172.1 mg met./L $_{nom}$**	EFSA 2015 Sloman, T., 2001 (DuPont-5618)
Lemna gibba	IN-JZ789	14 d, s	EC_{50} (frond count and biomass) >100 mg met./L $_{nom}$ **E_rC_{50} (frond count) >100 mg met./L $_{nom}$**	EFSA 2015 Sloman, T., 2001 (DuPont-5617)
Lemna gibba	IN-V7160	14 d, s	EC_{50} (frond count and biomass) >100 mg met./L $_{nom}$ **E_rC_{50} (frond count) >100 mg met./L $_{nom}$**	EFSA 2015 Sloman, T., 2001 (DuPont-5619)
Lemna gibba	IN-A4098	7 d, s	**E_rC_{50} >100 mg met./L $_{nom}$** E_bC_{50} >100 mg met./L $_{nom}$	EFSA 2015 Sowing, P., 2002 (CE01/072)
Lemna gibba	IN-W8268	14 d, s	E_bC_{50} >100 mg met./L $_{nom}$ EC_{50} = 39.5 mg met./L $_{nom}$ **E_rC_{50} >100 mg met./L $_{nom}$**	EFSA 2015 Sloman, T., 2000 (DuPont-4681)
Lemna gibba	IN-L9226	7 d, s	E_yC_{50} = 0.17 mg met./L $_{mm}$ **E_rC_{50} = 0.31 mg met./L $_{mm}$**	EFSA 2015 Vinken & Wydra 2007 (54 TIM)
Lemna gibba	IN-A5546	7 d, s	**EC_{50} (yield, biomass, and growth rate) >40.4 mg met./L $_{mm}$**	EFSA 2015 Sloman, T., 2006 (DuPont-19856)
Lemna gibba	2-acid-3-triuret	7 d, s	E_yC_{50} >100 mg met./L $_{nom}$ **E_rC_{50} >100 mg met./L $_{nom}$**	EFSA 2015 Weber, 2012 (S1201020)
Lemna gibba	IN-B5528	7 d, s	E_yC_{50} >119.52 mg met./L $_{nom}$ **E_rC_{50} >119.52 mg met./L $_{nom}$** E_bC_{50} >119.52 mg met./L $_{nom}$	EFSA 2015 Chandrasehar, 2010 (DuPont-29481)
Lemna gibba	Thifensulfuron methyl 50 SG	7 d, s	E_yC_{50} = 0.0014 mg product/L $_{nom}$ E_yC_{50} = 0.00071 mg a.s./L $_{nom}$ E_rC_{50} = 0.0026 mg product/L $_{nom}$ E_rC_{50} = 0.0013 mg a.s./L $_{nom}$	EFSA 2015
Higher-tier studies (micro- or mesocosm studies)				
No data (not required)				
Lemna gibba	Thifensulfuron methyl	7 d, variable exposure duration	E_yC_{50} (12 hr exposure) = 0.149 mg a.s./L $_{nom}$ E_rC_{50} (12 hr exposure) = 0.632 mg a.s./L $_{nom}$ E_yC_{50} (24 hr exposure) = 0.0149 mg a.s./L $_{nom}$ E_rC_{50} (24 hr exposure) >0.198 mg a.s./L $_{nom}$ E_yC_{50} (48 hr exposure) = 0.0035 mg a.s./L $_{nom}$ E_rC_{50} (48 hr exposure) >0.0593 mg a.s./L $_{nom}$ E_yC_{50} (96 hr exposure) = 0.00045 mg a.s./L $_{nom}$ E_rC_{50} (96 hr exposure) = 0.0032 mg a.s./L $_{nom}$	EFSA 2015***

Lemna gibba	Thifensulfuron methyl	16 d (9 d period at lower temperature)	E_yC_{50} = 0.068 mg a.s./L $_{mm}$ E_rC_{50} >0.447 mg a.s./L $_{mm}$	EFSA 2015***

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
* The accurate value; worst case-cover other algae species
** Endpoints from these studies were not deemed to be appropriate for use in a higher tier risk assessment but were used in a qualitative way together with the ErC50 proposed by the RMS for *Vallisneria americana.*
*** These studies were considered to be valid however they were not considered to be suitable for use in a refined risk assessment, please refer to the RAR for further information

Table 9.5-2: Endpoints and effect values relevant for the risk assessment for aquatic organisms – EVRITELL 162 OD

Species	Substance	Exposure System	Results	Reference
Navicula pelliculosa	EVRITELL 162 OD	72 h	**E_rC_{50} = 42.80 mg formulation/L** E_yC_{50} = 28.09 mg formulation/L	Kacperek-Karetta Z. 2023, W-11-23
Daphnia magna	EVRITELL 162 OD	48 h, s	**EC_{50} = 292.631 mg/L $_{nom}$**	Szlauer S. 2022, EMI/4/70/2022
Pseudokirchneriella subcapitata	EVRITELL 162 OD	72 h	**E_rC_{50} = 7.836 mg formulation/L** E_yC_{50} = 2.390 mg formulation/L	Szlauer S. 2023, EMI/4/71/2022
Lemna gibba	EVRITELL 162 OD	7d, ss	Frond number **E_rC_{50} = 0.155 mg formulation/L** E_yC_{50} = 0.037 mg ormulation/L Dry weight E_rC_{50} = 1.663 mg formulation/L E_bC_{50} = 0.083 mg formulation/L	Kacperek-Karetta Z. 2023, W-12-23
Myriophyllum spicatum	EVRITELL 162 OD	14 d,s	E_rC_{50} = 0.106 mg/L $_{mm}$ (average spcific growth rate for shoot lenght) NOEC = 0.016 mg/L	Brzozowska-Wojoczek K. 2024, ETOX-2024-39
Higher-tier studies (micro- or mesocosm studies)				

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

9.5.1.1 Justification for new endpoints

No new data for active substances is presented with this application.

9.5.2 Risk assessment

The evaluation of the risk for aquatic and sediment-dwelling organisms was performed in accordance with the recommendations of the "Guidance document on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters in the context of Regulation (EC) No 1107/2009", as provided by the Commission Services (SANTE-2015-00080, 15 January 2015).
The relevant global maximum FOCUS Step 1, 2 and 3 PEC_{SW} for risk assessments covering the proposed use pattern and the resulting PEC/RAC ratios are presented in the table below.

In the following table, 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 and each organism group.

Table 9.5-3: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for EVRITELL for each organism group for the use of EVRITELL 162 OD in maize

Group			**Inverteb. acute**	**Algae**	**Algae**	**Aquatic plants**	**Aquatic plants**
Test species			***Daphnia magna***	***Navicula pelliculosa***	***Pseudokirchneriella subcapitata***	***Lemna gibba***	**Myriophyllum spicatum**
Endpoint (µg/L)			EC_{50} 292631	E_rC_{50} 42800	E_rC_{50} 7836	E_rC_{50} 155	E_rC_{50} 106
AF			100	10	10	10	10
RAC (µg/L)			2926.31	4280	783.6	15.5	10.6
		PEC $_{gl-max}$ (µg/L)					
No buffer	Pond	0.2156	<0.001	<0.001	<0.001	0.014	0.020
	Ditch	5.3969	0.002	0.001	0.007	0.348	0.509
	stream	4.2037	0.001	0.001	0.005	0.271	0.397
10 m buffer	Pond	0.1385	<0.001	<0.001	<0.001	0.009	0.013
	Ditch	0.9384	<0.001	<0.001	0.001	0.061	0.089
	stream	0.9384	<0.001	<0.001	0.001	0.061	0.089
20 m buffer	Pond	0.0925	<0.001	<0.001	<0.001	0.006	0.009
	Ditch	0.4876	<0.001	<0.001	0.001	0.031	0.046
	stream	0.4876	<0.001	<0.001	0.001	0.031	0.046

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

Table 9.5-4: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for dicamba for each organism group based on FOCUS Steps 1 and 2 calculations for the use of EVRITELL 162 OD in maize

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Aquatic plants**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Navicula pelliculosa***	***Myriophyllum spicatum***
Endpoint (µg/L)		LC_{50} >41000	NOEC 180000	EC_{50} >41000	NOEC 97000	E_rC_{50} 3800	IC_{50} >450
AF		100	10	100	10	10	10
RAC (µg/L)		>410	18000	> 410	9700	380	>45
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**						
Step 1							
	37.2054	0.091	0.002	0.091	0.004	0.098	0.827
Step 2							
N-Europe March-May	3.6522	0.009	<0.001	0.009	<0.001	0.010	0.081
N-Europe Jun-Sept	3.6522	0.009	<0.001	0.009	<0.001	0.010	0.081

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

Metabolites of dicamba

Table 9.5-5: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for DCSA for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of EVRITELL 162 OD in maize

Group		**Fish acute**	**Inverteb. acute**	**Algae**	**Aquatic plants**
Test species		***Oncorhynchus mykiss***	***Daphnia magna***	***Selenastrum capricornutum***	***Lemna gbba***
Endpoint (µg/L)		LC_{50} >100000	EC_{50} >89000	E_rC_{50} >138000	E_rC_{50} 73000
AF		100	100	10	10
RAC (µg/L)		>1000	> 890	>13800	7300
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**				
Step 1					
	14.5776	0.015	0.016	0.001	0.002
Step 2					
N-Europe March-May	1.5794	0.002	0.002	<0.001	<0.001
N-Europe Jun-Sept	1.5794	0.002	0.002	<0.001	<0.001

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

Table 9.5-6: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for nicosulfuron for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of EVRITELL 162 OD in maize

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Aquatic plants**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Anabaena flos-aquae***	***Lemna gibba***
Endpoint (µg/L)		LC_{50} 65700	NOEC 10000	EC_{50} 90000	NOEC 5200	E_bC_{50} 7800	E_rC_{50} 1.39
AF		100	10	100	10	10	10
RAC (µg/L)		657	1000	900	520	780	0.139
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**						
Step 1							
	13.3431	0.020	0.013	0.015	0.026	0.017	**95.994**
Step 2							
N-Europe March-May	1.9890	0.003	0.002	0.002	0.004	0.003	**14.309**
Step 3							
D3/ditch	0.2193	<0.001	<0.001	<0.001	<0.001	<0.001	**1.578**
D4/pond	0.03311	<0.001	<0.001	<0.001	<0.001	<0.001	0.238
D4/stream	0.1864	<0.001	<0.001	<0.001	<0.001	<0.001	**1.341**
D5/pond	0.01823	<0.001	<0.001	<0.001	<0.001	<0.001	0.131
D5/stream	0.1909	<0.001	<0.001	<0.001	<0.001	<0.001	**1.373**

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Aquatic plants
D6/ditch	0.2107	<0.001	<0.001	<0.001	<0.001	<0.001	**1.516**
R1/pond	0.01621	<0.001	<0.001	<0.001	<0.001	<0.001	0.117
R1/ stream	0.4547	0.001	<0.001	0.001	0.001	0.001	**3.271**
R2/ stream	1.273	0.002	0.001	0.001	0.002	0.002	**9.158**
R3/ stream	1.640	0.002	0.002	0.002	0.003	0.002	**11.799**
R4/ stream	1.708	0.003	0.002	0.002	0.003	0.002	**12.288**

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

For the intended uses, calculated PEC/RAC ratios did not indicate an acceptable risk for the most sensitive group of aquatic organisms for nicosulfuron (risk for *Lemna gibba* as characterised by an EC_{50} for species of 1.39 µg/L in connection with an assessment factor of 10) in several FOCUS Steps 1-3 scenarios. Therefore, further PEC/RAC ratios were calculated based on FOCUS Step 4 PEC_{SW} considering reduced exposure of surface water bodies.

Table 9.5-7: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for nicosulfuron based on FOCUS Step 4 calculations and toxicity data for most sensitive species *Lemna Gibba* with mitigation of spray drift and run-off for the use of EVRITELL 162 OD in maize (buffer zone of 5 m)

Intended use		maize
Active substance		nicosulfuron
Application rate (g/ha)		1 × 1 L/ha
Nozzle reduction	**No-spray buffer (m)**	5m
	Vegetated filter strip (m)	None
None	D3 ditch	0.07816
None	D4 pond	0.03305
None	D4 stream	0.08231
None	D5 pond	0.01733
None	D5 stream	0.08237
None	D6 ditch	0.06986
None	R1 pond	0.01543
50 %		0.01217
75 %		0.01054
90 %		0.009566
None	R1 stream	0.4547
50 %		0.4547
75 %		0.4547
90 %		0.4547
None	R2 stream	1.273
50 %		1.273
75 %		1.273
90 %		1.273
None	R3 stream	1.640
50 %		1.640
75 %		1.640
90 %		1.640
None	R4 stream	1.708
50 %		1.708
75 %		1.708

90 %		1.708
RAC (µg/L) 0.139		**PEC/RAC ratio**
None	D3 ditch	0.562
None	D4 pond	0.238
None	D4 stream	0.592
None	D5 pond	0.125
None	D5 stream	0.593
None	D6 ditch	0.503
None	R1 pond	0.111
50 %		0.088
75 %		0.076
90 %		0.069
None	R1 stream	**3.271**
50 %		**3.271**
75 %		**3.271**
90 %		**3.271**
None	R2 stream	**9.158**
50 %		**9.158**
75 %		**9.158**
90 %		**9.158**
None	R3 stream	**11.799**
50 %		**11.799**
75 %		**11.799**
90 %		**11.799**
None	R4 stream	**12.288**
50 %		**12.288**
75 %		**12.288**
90 %		**12.288**

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

Table 9.5-8: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for nicosulfuron based on FOCUS Step 4 calculations and toxicity data for most sensitive species *Lemna Gibba* with mitigation of spray drift and run-off for the use of EVRITELL 162 OD in maize

Intended use **Active substance** **Application rate (g/ha)**		maize nicosulfuron 1 × 1 L/ha	
Nozzle reduc-tion	**Width of planted buffer strip (m)**	10-12 m	18-20 m
None	R1 pond	0.008286	0.004939
None	R1 stream	0.1867	0.09413
None	R2 stream	**0.5620**	**0.2910**
None	R3 stream	**0.7414**	**0.3880**
None	R4 stream	**0.7766**	**0.4070**
RAC (µg/L) 0.139		**PEC/RAC ratio**	
None	R1 pond	0.060	0.036
None	R1 stream	**1.343**	0.677
None	R2 stream	**4.043**	**2.094**
None	R3 stream	**5.334**	**2.791**
None	R4 stream	**5.587**	**2.928**

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

Table 9.5-9: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for nicosulfuron based on FOCUS Step 4 calculations and toxicity data for most sensitive species *Lemna Gibba* with mitigation of spray drift and run-off for the use of EVRITELL 162 OD in maize (VFSmod model)

Intended use **Active substance** **Application rate (g/ha)**		maize nicosulfuron 1 × 1 L/ha
Nozzle reduc-tion	**Vegetative strip**	**VFSmod = 5 m**
None	R1 pond	0.007567
None	R1 stream	0.06008
None	R2 stream	0.08191
None	R3 stream	0.08596
None	R4 stream	0.06105
RAC (µg/L) 0.139		**PEC/RAC ratio**

None	R1 pond	0.054
None	R1 stream	0.432
None	R2 stream	0.589
None	R3 stream	0.618
None	R4 stream	0.439

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

Metabolites of nicosulfuron

Table 9.5-10: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for ASDM for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of EVRITELL 162 OD in maize

Group		**Fish acute**	**Inverteb. acute**	**Algae**	**Aquatic plants**
Test species		***Lepomis macrochirus***	***Daphnia magna***	***Pseudokirchneriella subcapitata***	***Lemna gibba***
Endpoint (µg/L)		LC_{50} >100000	EC_{50} >954000	E_rC_{50} >336000	EC_{50} >100000
AF		100	100	10	10
RAC (µg/L)		>1000	>9540	>33600	>10000
FOCUS Scenario	**PEC gl-max (µg/L)**				
Step 1					
	5.4237	0.005	0.001	<0.001	0.001
Step 2					
N-Europe March-May	0.8054	0.001	0.000	0.000	0.000

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

Table 9.5-11: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AUSN for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of EVRITELL 162 OD in maize

Group		**Fish acute**	**Inverteb. acute**	**Algae**	**Aquatic plants**
Test species		***Brachydanio rerio***	***Daphnia magna***	***Scenedesmus subspicatus***	***Lemna gibba***
Endpoint (µg/L)		LC_{50} >100000	EC_{50} >100000	E_rC_{50} >100000	EC_{50} >100000
AF		100	100	10	10
RAC (µg/L)		>1000	>1000	>10000	>10000
FOCUS Scenario	**PEC gl-max (µg/L)**				
Step 1					
	3.83554	0.004	0.004	<0.001	<0.001
Step 2					

Group		Fish acute	Inverteb. acute	Algae	Aquatic plants
N-Europe March-May	0.5697	0.001	0.001	<0.001	<0.001

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

Table 9.5-12: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for HMUD for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of EVRITELL 162 OD in maize

Group		Fish acute	Inverteb. acute	Algae	Aquatic plants
Test species		***Oncorhynchus mykiss***	***Daphnia magna***	***Scenedesmus subspicatus***	***Lemna gibba***
Endpoint (µg/L)		LC_{50} >100000	EC_{50} >100000	E_rC_{50} >100000	EC_{50} >1000
AF		100	100	10	10
RAC (µg/L)		>1000	>1000	>10000	>100
FOCUS Scenario	**PEC gl-max (µg/L)**				
Step 1					
	4.4035	0.004	0.004	<0.001	0.044
Step 2					
N-Europe March-May	0.6317	0.001	0.001	<0.001	0.006

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

Table 9.5-13: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for UCSN for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of EVRITELL 162 OD in maize

Group		Inverteb. acute	Algae	Aquatic plants
Test species		***Daphnia magna***	***Scenedesmus subspicatus***	***Lemna gibba***
Endpoint (µg/L)		EC_{50} >100000	E_rC_{50} >100000	EC_{50} >100000
AF		100	10	10
RAC (µg/L)		>1000	>10000	>10000

Group		Inverteb. acute	Algae	Aquatic plants
FOCUS Scenario	**PEC gl-max (µg/L)**			
Step 1				
	1.8084	0.002	<0.001	<0.001
Step 2				
N-Europe March-May	0.2696	<0.001	<0.001	<0.001

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

Table 9.5-14: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for thifensulfuron-methyl for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of EVRITELL 162 OD in maize

Group		Fish acute	Inverteb. acute	Inverteb. prolonged	Algae	Aquatic plants
Test species		***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchneriella subcapitata***	***Vallisneria americana***
End-point (µg/L)		LC_{50} >56400	EC_{50} >60700	NOEC 99000	E_bC_{50} 800	E_rC_{50} 0.23
AF		100	100	10	10	10
RAC (µg/L)		>564	>607	9900	80	0.023
FOCUS Scenario	**PEC gl-max (µg/L)**					
Step 1						
	4.0629	0.007	0.007	<0.001	0.051	**176.648**
Step 2						
N-Europe March-May	0.1777	<0.001	<0.001	<0.001	0.002	**7.726**
N-Europe Jun-Sept	0.1777	<0.001	<0.001	<0.001	0.002	**7.726**
Step 3						
D3/ditch	0.06298	<0.001	<0.001	<0.001	0.001	**2.738**

Group		Fish acute	Inverteb. acute	Inverteb. prolonged	Algae	Aquatic plants
D4 /pond	0.002543	<0.001	<0.001	<0.001	<0.001	0.111
D4 /stream	0.05392	<0.001	<0.001	<0.001	0.001	**2.344**
D5 / pond	0.002543	<0.001	<0.001	<0.001	<0.001	0.111
D5 / stream	0.05627	<0.001	<0.001	<0.001	0.001	**2.447**
D6 / ditch	0.06285	<0.001	<0.001	<0.001	0.001	**2.733**
R1 / pond	0.002895	<0.001	<0.001	<0.001	<0.001	0.126
R1 / stream	0.09669	<0.001	<0.001	<0.001	0.001	**4.204**
R2 / stream	0.1559	<0.001	<0.001	<0.001	0.002	**6.778**
R3 / stream	0.1797	<0.001	<0.001	<0.001	0.002	**7.813**
R4 / stream	0.1667	<0.001	<0.001	<0.001	0.002	**7.248**

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

For the intended uses, calculated PEC/RAC ratios did not indicate an acceptable risk for the most sensitive group of aquatic organisms for thifensulfuron-methyl (risk for *Vallisneria americana* as characterised by an EC_{50} for species of 0.23 µg/L in connection with an assessment factor of 10) in several FOCUS Steps 1-3 scenarios. Therefore, further PEC/RAC ratios were calculated based on FOCUS Step 4 PEC_{SW} considering reduced exposure of surface water bodies.

Table 9.5-15: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for thifensulfuron-methyl based on FOCUS Step 4 calculations and toxicity data for most sensitive species *Vallisneria americana* with mitigation of spray drift and run-off for the use of EVRITELL 162 OD in maize (buffer zone of 5 m)

Intended use		maize
Active substance		thifensulfuron-methyl
Application rate (g/ha)		1 × 1 L/ha
Nozzle reduction	**No-spray buffer (m)**	5m
	Vegetated filter strip (m)	None
None	D3 ditch	0.02064
50 %		0.01032
75 %		0.005159
90 %		0.002064

None	D4 pond	0.002272
50 %		0.001135
75 %		0.000568
90 %		0.000227
None	D4 stream	0.02270
50 %		0.01135
75 %		0.005676
90 %		0.002270
None	D5 pond	0.002272
50 %		0.001135
75 %		0.000568
90 %		0.000227
None	D5 stream	0.02369
50 %		0.01185
75 %		0.005923
90 %		0.002369
None	D6 ditch	0.02060
50 %		0.01030
75 %		0.005150
90 %		0.002061
None	R1 pond	0.002681
50 %		0.001780
75 %		0.001330
90 %		0.001060
None	R1 stream	0.09669
50 %		0.09669
75 %		0.09669
90 %		0.09669
None	R2 stream	0.1559
50 %		0.1559
75 %		0.1559
90 %		0.1559
None	R3 stream	0.1797
50 %		0.1797
75 %		0.1797
90 %		0.1797
None	R4 stream	0.1667
50 %		0.1667
75 %		0.1667
90 %		0.1667

RAC (µg/L) 0.023		**PEC/RAC ratio**
None	D3 ditch	0.897
None		0.449
None		0.224
None		0.090
None	D4 pond	0.099
50 %		0.049
75 %		0.025
90 %		0.010
None	D4 stream	0.987
50 %		0.493
75 %		0.247
90 %		0.099
None	D5 pond	0.099
50 %		0.049
75 %		0.025
90 %		0.010
None	D5 stream	**1.030**
50 %		0.515
75 %		0.258
90 %		0.103
None	D6 ditch	0.896
50 %		0.448
75 %		0.224
90 %		0.090
None	R1 pond	0.117
50 %		0.077
75 %		0.058
90 %		0.046
None	R1 stream	**4.204**
50 %		**4.204**
75 %		**4.204**
90 %		**4.204**
None	R2 stream	**6.778**
50 %		**6.778**
75 %		**6.778**

90 %		**6.778**
None	R3 stream	**7.813**
50 %		**7.813**
75 %		**7.813**
90 %		**7.813**
None	R4 stream	**7.248**
50 %		**7.248**
75 %		**7.248**
90 %		**7.248**

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

Table 9.5-16: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for for thifensulfuron-methyl based on FOCUS Step 4 calculations and toxicity data for most sensitive species *Vallisneria americana* with mitigation of spray drift and run-off for the use of EVRITELL 162 OD in maize

Intended use **Active substance** **Application rate (g/ha)**		maize thifensulfuron-methyl 1 × 1 L/ha	
Nozzle reduction	**Width of planted buffer strip (m)**	10-12 m	18-20 m
None	D3 ditch	0.01095	0.005689
None	D4 pond	0.001633	0.001091
None	D4 stream	0.01204	0.006256
None	D5 pond	0.001633	0.001090
None	D5 stream	0.01256	0.006528
None	D6 ditch	0.01093	0.005679
None	R1 pond	0.001653	0.001090
None	**R1 stream**	0.03968	0.02002
None	**R2 stream**	0.06883	0.03565
None	**R3 stream**	0.08117	0.04248
None	**R4 stream**	0.07577	0.03971
RAC (µg/L) 0.023		**PEC/RAC ratio**	
None	D3 ditch	0.476	0.247
None	D4 pond	0.071	0.047
None	D4 stream	0.523	0.272
None	D5 pond	0.071	0.047
None	D5 stream	0.546	0.284

None	D6 ditch	0.475	0.247
None	R1 pond	0.072	0.047
None	**R1 stream**	**1.725**	0.87
None	**R2 stream**	**2.993**	**1.55**
None	**R3 stream**	**3.529**	**1.847**
None	**R4 stream**	**3.294**	**1.727**

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

Table 9.5-17: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for thifensulfuron-methyl based on FOCUS Step 4 calculations and toxicity data for most sensitive species *Vallisneria americana* with mitigation of spray drift and run-off for the use of EVRITELL 162 OD in maize (VFSmod model)

Intended use		maize	
Active substance		thifensulfuron-methyl	
Application rate (g/ha)		1 × 1 L/ha	
Nozzle reduc-tion	**Vegetative strip**	**VFSmod = 5 m**	**VFSmod = 10 m**
None	D3 ditch	0.02064	0.01095
None	D4 pond	0.002272	0.001633
None	D4 stream	0.02270	0.01204
None	D5 pond	0.002272	0.001633
None	D5 stream	0.02369	0.01256
None	D6 ditch	0.02060	0.01093
None	R1 pond	0.002270	0.001632
None	R1 stream	0.01802	0.009558
None	R2 stream	0.02457	0.01303
None	R3 stream	0.02579	0.01368
None	R4 stream	0.01831	0.009713
RAC (µg/L)			
0.023		**PEC/RAC ratio**	
None	D3 ditch	0.897	0.476
None	D4 pond	0.099	0.071
None	D4 stream	0.987	0.523
None	D5 pond	0.099	0.071
None	D5 stream	1.030	0.546
None	D6 ditch	0.896	0.475
None	R1 pond	0.099	0.071
None	R1 stream	0.783	0.416
None	R2 stream	**1.068**	0.567

None	R3 stream	**1.121**	0.595
None	R4 stream	0.796	0.422

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

Metabolites of thifensulfuron-methyl

Table 9.5-18: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for IN-L9225 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of EVRITELL 162 OD in maize

Group		**Fish acute**	**Inverteb. acute**	**Algae**	**Aquatic plants**
Test species		***Oncorhynchus mykiss***	***Daphnia magna***	***Pseudokirchneriella subcapitata***	***Lemna gibba***
Endpoint (µg/L)		LC_{50} >120000	EC_{50} >130000	E_rC_{50} 36500	E_rC_{50} 82200
AF		100	100	10	10
RAC (µg/L)		>1200	>13000	3650	8220
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**				
Step 1					
	8.1104	0.007	0.006	0.002	0.001
Step 2					
N-Europe Mar-May	1.3067	0.001	0.001	<0.001	<0.001
N-Europe Jun-Sep	1.3067	0.001	0.001	<0.001	<0.001

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

Table 9.5-19: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for IN-JZ789 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of EVRITELL 162 OD in maize

Group		**Fish acute**	**Inverteb. acute**	**Algae**	**Aquatic plants**
Test species		***Oncorhynchus mykiss***	***Daphnia magna***	***Pseudokirchneriella subcapitata***	***Lemna gibba***
Endpoint (µg/L)		LC_{50} >940	EC_{50} >1100	EC_{50} >1280	E_rC_{50} >100000
AF		100	100	10	10
RAC (µg/L)		>9.4	>11	>128	>10000

Group		Fish acute	Inverteb. acute	Algae	Aquatic plants
FOCUS Scenario	**PEC gl-max (µg/L)**				
Step 1					
	8.1104	0.863	0.737	0.063	0.001
Step 2					
N-Europe Mar-May	1.3067	0.139	0.119	0.010	<0.001
N-Europe Jun-Sep	1.3067	0.139	0.119	0.010	<0.001

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

Table 9.5-20: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for IN-V7160 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of EVRITELL 162 OD in maize

Group		Fish acute	Inverteb. acute	Inverteb. chronic	Algae	Aquatic plants
Test species		***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchneriella subcapitata***	***Lemna gibba***
Endpoint (µg/L)		LC_{50} >1000	EC_{50} >1300	NOEC 11000	E_bC_{50} >11000	E_rC_{50} >100000
AF		100	100	10	10	10
RAC (µg/L)		>10	>13	1100	>1100	>10000
FOCUS Scenario	**PEC gl-max (µg/L)**					
Step 1						
	8.1104	0.811	0.624	0.007	0.007	0.001
Step 2						
N-Europe Mar-May	1.3067	0.131	0.101	0.001	0.001	<0.001
N-Europe Jun-Sep	1.3067	0.131	0.101	0.001	0.001	<0.001

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

Table 9.5-21: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for IN-L9223 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of EVRITELL 162 OD in maize

Group		**Inverteb. acute**	**Inverteb. chronic**	**Algae**	**Aquatic plants**
Test species		***Daphnia magna***	***Daphnia magna***	***Pseudokirchneriella subcapitata***	***Lemna gibba***
Endpoint (µg/L)		EC_{50} >1200	NOEC 13000	EC_{50} >1300	E_rC_{50} >172100
AF		100	10	10	10
RAC (µg/L)		>12	1300	>130	>17210
FOCUS Scenario	**PEC gl-max (µg/L)**				
Step 1					
	8.1104	0.676	0.006	0.062	<0.001
Step 2					
N-Europe Mar-May	1.3067	0.109	0.001	0.010	<0.001
N-Europe Jun-Sep	1.3067	0.109	0.001	0.010	<0.001

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

Table 9.5-22: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for IN-A4098 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of EVRITELL 162 OD in maize

Group		**Fish acute**	**Inverteb. acute**	**Inverteb. chronic**	**Algae**	**Aquatic plants**
Test species		***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchneriella subcapitata***	***Lemna gibba***
Endpoint (µg/L)		LC_{50} >930	EC_{50} >99000	NOEC 32000	E_rC_{50} >10000	E_rC_{50} >100000
AF		100	100	10	10	10
RAC (µg/L)		>9.3	>990	3200	>1000	>10000
FOCUS Scenario	**PEC gl-max (µg/L)**					
Step 1						
	8.1104	0.872	0.008	0.003	0.008	0.001
Step 2						

Group		Fish acute	Inverteb. acute	Inverteb. chronic	Algae	Aquatic plants
N-Europe Mar-May	1.3067	0.141	0.001	<0.001	0.001	<0.001
N-Europe Jun-Sep	1.3067	0.141	0.001	<0.001	0.001	<0.001

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

Table 9.5-23: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for IN-W8268 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of EVRITELL 162 OD in maize

Group		Fish acute	Inverteb. acute	Algae	Aquatic plants
Test species		***Oncorhynchus mykiss***	***Daphnia magna***	***Pseudokirchneriella subcapitata***	***Lemna gibba***
Endpoint (µg/L)		LC_{50} >115000	EC_{50} >125000	E_rC_{50} >31600	E_rC_{50} >100000
AF		100	100	10	10
RAC (µg/L)		>1150	>1250	>3160	>10000
FOCUS Scenario	**PEC gl-max (µg/L)**				
Step 1					
	8.1104	0.007	0.006	0.003	0.001
Step 2					
N-Europe Mar-May	1.3067	0.001	0.001	<0.001	<0.001
N-Europe Jun-Sep	1.3067	0.001	0.001	<0.001	<0.001

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

Table 9.5-24: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for IN-L9226 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of EVRITELL 162 OD in maize

Group		Algae	Aquatic plants
Test species		***Pseudokirchneriella subcapitata***	***Lemna gibba***
Endpoint (µg/L)		EC_{50} >89000	E_rC_{50} 310
AF		10	10
RAC (µg/L)		>8900	31

Group		**Algae**	**Aquatic plants**
FOCUS Scenario	**PEC gl-max (µg/L)**		
Step 1			
	8.1104	0.001	0.262
Step 2			
N-Europe Mar-May	1.3067	<0.001	0.042
N-Europe Jun-Sep	1.3067	<0.001	0.042

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

Table 9.5-24: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for IN-A5546 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of EVRITELL 162 OD in maize

Group		**Algae**	**Aquatic plants**
Test species		***Pseudokirchneriella subcapitata***	***Lemna gibba***
Endpoint (µg/L)		E_rC_{50} >110000	EC_{50} >40400
AF		10	10
RAC (µg/L)		>11000	>4040
FOCUS Scenario	**PEC gl-max (µg/L)**		
Step 1			
	8.1104	0,001	0,002
Step 2			
N-Europe Mar-May	1.3067	<0.001	<0.001
N-Europe Jun-Sep	1.3067	<0.001	<0.001

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

Table 9.5-26: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for 2-acid-3-triuret for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of EVRITELL 162 OD in maize

Group		**Algae**	**Aquatic plants**
Test species		***Pseudokirchneriella subcapitata***	***Lemna gibba***
Endpoint (µg/L)		E_rC_{50} >100000	E_rC_{50} >100000
AF		10	10
RAC (µg/L)		>10000	>10000
FOCUS Scenario	**PEC gl-max (µg/L)**		
Step 1			
	8.1104	0.001	0.001
Step 2			
N-Europe Mar-May	1.3067	<0.001	<0.001
N-Europe Jun-Sep	1.3067	<0.001	<0.001

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

Table 9.5-27: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for IN-B5528 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of EVRITELL 162 OD in maize

Group		**Aquatic plants**
Test species		***Lemna gibba***
Endpoint (µg/L)		E_rC_{50} >119520
AF		10
RAC (µg/L)		>11952
FOCUS Scenario	**PEC gl-max (µg/L)**	
Step 1		
	8.1104	0.001
Step 2		
N-Europe Mar-May	1.3067	<0.001

Group		Aquatic plants
N-Europe Jun-Sep	1.3067	<0.001

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

Risk assessment for the combinations of a.s. in the formulation

Following the dilution and spraying of the formulated product, much of the formulation constituents are likely to be lost by volatilisation. Therefore, shortly after application of a formulated product, aquatic organisms are mainly exposed to the active substance present in the formulation. In addition, as demonstrated in the short-term studies here above there are no indications for interactions of the active substances (no synergisms or additional toxicity occurs due to the co-formulants) given that the formulation does not cause an (unexpected) increased toxicity compared to the active substances. An evaluation of the risk posed by the intact formulation is therefore relevant only for the acute/short-term assessment. The long-term risk was assessed considering data for the active substances in the formulation and no chronic combined risk assessment has been performed.

According to the new EFSA Scientific Opinion (EFSA, 2013) measured and calculated mixture toxicity should be compared to determine synergistic, additive or antagonistic effects of the formulation. In the following the concentration addition (CA) model is used as proposed by EFSA.

To determine the respective formulation effect, EFSA proposed to calculate the model deviation ratio (MDR), which divides the calculated mixture toxicity ($LC_{50}/EC_{50\ mix\text{-}CA}$) by the measured mixture toxicity ($LC_{50}/EC_{50\ EVRITELL}$). Ecotoxicity studies are biological test systems which underlie a certain natural biological variability when repeating a study. Hence, a threshold has to be defined when an increased/decreased mixture toxicity effect cannot be seen as only additive any longer. EFSA proposes a factor of 5, *i.e.* if the MDR is between 0.2 and 5 the observed and calculated mixture toxicities are considered in agreement.

Active susbtance / species	Test system	Endpoint (mg a.s./L)
dicamba		
Oncorhynchus mykiss	LC_{50} 96h	> 41
Daphnia magna	EC_{50} 48h	> 41
Navicula pelliculosa	EC_{50} 96h	> 3.8
M. spicatum	IC_{50}	> 0.45
nicosulfuron		
Oncorhynchus mykiss	LC_{50} 96h	65.7
Daphnia magna	EC_{50} 48h	90
Anabaena flos-aquae	E_rC_{50} 72h	7.8
Lemna gibba	E_rC_{50}	0.00139
Thifensulfuron-methyl		
Oncorhynchus mykiss	LC_{50} 96h	>56.4
Daphnia magna	EC_{50} 48h	> 60.7
P. subcapitata	E_bC_{50} 72h	0.8
V. americana	E_rC_{50}	0.00023

The calculated MDR values are between 0.2 and 5 for each organism except makrophytes (see Table 9.5-28), indicating that the formulation does not cause an (unexpected) increased toxicity compared to the active substances for these organisms. No synergisms or additional toxicity occurs due to the co-formulants. The apparent antagonism for makrophytes (toxicity of the formulation lower than expected) can be explained

by the fact that endpoints for individual active substances are "higher than" values.

Table 9.5-28: Summary of results obtained in the studies with the formulated product EVRITELL 162 OD and comparison of calculated and measured mixture toxicity

Test species	Endpoint & Test system	LC_{50} / EC_{50} [mg/L]			
		Measured toxicity of EVRITELL (LC_{50} EVRITELL or EC_{50} EVRITELL) (mg/L)	Measured toxicity of EVRITELL (converted to be a.i. based) (LC_{50} EVRITELL or EC_{50} EVRITELL) (mg a.s./L)	Calculated mixture toxicity[a] LC_{50} mix-CA or EC_{50} mix-CA	Model deviation ratio (MDR = EC_{50} mix-CA / EC_{50} EVRITELL)
O. mykiss	LC_{50}, acute, 96 h	-	-	46.226	-
D. magna	EC_{50}, acute, 48 h	292.631	46.89	48.721	1.039
P. subcapitata	E_rC_{50}, 72 h	7.836	1.256	3.301	2.629
Lemna gibba	E_rC_{50}	0.155	0.025	0.002	0.080

[a] The mixture toxicity of the formulation was re-calculated based on the nominal contents of dicamba (110 g/L*)* nicosulfuron (40 g/L) and thifensulfuron-methyl (12 g/L) within the formulation and based on a density of formulation of 1.011 g/mL.

The calculated factors fall outside 0.8-1.2 for fish (see Table 9.5-29), indicating that the mixture composition in the formulation study giving the measured mixture toxicity is not similar to the mixture composition at the PECmix.

Table 9.5-29: Comparison of mixture composition in the formulation study (giving the measured mixture toxicity) and mixture composition at the PEC_{mix}

Test species	Endpoint & Test system	LC_{50} / EC_{50} [mg/L]		
		Calculated mixture toxicity (a.s. in EVRITELL) LC_{50} mix-CA or EC_{50} mix-CA	Calculated mixture toxicity (a.s. in PEC_{mix})[b] LC_{50} mix-CA or EC_{50} mix-CA at lower exposure tier	Factors (EC_{50} mix-CA (a.s. in EVRITELL)/EC_{50} mix-CA (a.s. in PEC_{mix})) at lower exposure tier
O. mykiss	LC_{50}, acute, 96 h	46.226	47.50	0.973
D. magna	EC_{50}, acute, 48 h	48.721	50.996	0.955
P. subcapitata	E_rC_{50}, 72 h	3.301	4.046	0.816
Lemna gibba	E_rC_{50}	0.002	0.003	0.758

[a] The mixture toxicity of the formulation was re-calculated based on the nominal contents of dicamba (110 g/L*)* nicosulfuron (40 g/L) and thifensulfuron-methyl (12 g/L) within the formulation and based on a density of formulation of 1.011 g/mL.

[b] The mixture toxicity of the formulation was re-calculated based on the mixture composition at the PEC_{mix} for dicamba (0.003652 mg/L at Step 2), nicosulfuron (0.001989 mg/L at Step 2) and thifensulfuron-methyl (0.000178 mg/L at Step 2).

Table 9.5-50: Comparison of calculated mixture toxicity and toxicity per fraction of a single a.s.

Test species	Endpoint & Test system	LC_{50} / EC_{50} [mg/L]		
		Calculated mixture toxicity (a.s. in EVRITELL) $LC_{50\ mix\text{-}CA}$ or $EC_{50\ mix\text{-}CA}$	Calculated toxicity per fraction of EVRITELL (based on each a.s.) $(1/TU_i)$[a]	Deviation from mixture toxicity (1-$EC_{x\ mix\text{-}CA}$ x $(1/EC_{x\ mix\text{-}CA} - TU_i)$) [%]
O. mykiss	LC_{50}, acute, 96 h	46.226	dicamba: 60.382 nicosulfuron: 266.085 thifensulfuron-methyl: 761.4	dicamba: 76.6% nicosulfuron: 17.37% thifensulfuron-methyl: 6.1%
D. magna	EC_{50}, acute, 48 h	48.721	dicamba: 60.382 nicosulfuron: 364.50 thifensulfuron-methyl: 819.45	dicamba: 80.7% nicosulfuron: 13.37% thifensulfuron-methyl: 5.9%
P. subcapitata	E_rC_{50}, 72 h	3.301	dicamba: 5.596 nicosulfuron: 31.59 thifensulfuron-methyl: 10.8	dicamba: 59.0% nicosulfuron: 10.4% thifensulfuron-methyl: 30.6%
Lemna gibba	E_rC_{50}	0.002	dicamba: 0.663 nicosulfuron: 0.0056 thifensulfuron-methyl: 0.0031	dicamba: 0.3% nicosulfuron: 35.4% thifensulfuron-methyl: 64.3%

[a] TUi is defined as the concentration of the i^{th} a.s. at the $EC_{50\ EVRITELL}$ (re-caculated to the sum of a.s.) divided by the respective single-substance toxicity ($EC_{50\ a.s.}$). ~~This is calculated based on the nominal contents of Boscalid (233 g/L) and Difenoconazole (66 g/L) within the formulation and based on a density of formulation of 1.111 g/mL~~ This is calculated based on the nominal contents of dicamba (110 g/L) nicosulfuron (40 g/L) and thifensulfuron-methyl (12 g/L) within the formulation and based on a density of formulation of 1.011 g/mL

Regarding EVRITELL 162 OD, no active substance is clearly driving the acute risk for fish, aquatic invertebrates, algae and makrophytes. The studies performed with the formulated product EVRITELL 162 OD do not reflect the toxicity of one particular active substance, as the formulation toxicity – endpoint recalculated to each active substance concentrations – does not come for 90 % (of more) from the toxicity per fraction of a single a.s. (TUi) (see Table 9.5-30).

Table 9.5-31: Conduct a mixture RA based on calculated mixture toxicity according to 10.3.8 from EFSA AGD in maize for fish

Exposure	Higher exposure tier		
	dicamba	**nicosulfuron**	**Thifensulfuron-methyl**
Exposure tier (FOCUS step)	Step 2	Step4 (R1 pond)	Step4 (R3 stream)
PECsw [mg a.s./L]	0.003652	0.000007567	0.00001368
Relative proportions of the individual mixture components in the environment (pi PEC)	0.994	0.002	0.004
Total exposure concentration of the mixture (a.s. based) (PECmix) [mg/L]	0.003673		
Calculated mixture toxicity (a.s. in PECmix) (ECx mix-CA = ∑ (pi PEC/ECx i)) [mg a.s./L]	41.074		
ETRmix = PECmix/ECx PPP	0.001		
Trigger	0.01		

Table 9.5-32: Conduct a mixture RA based on calculated mixture toxicity according to 10.3.8 from EFSA AGD in maize for invertebrates

Exposure	Higher exposure tier		
	dicamba	**nicosulfuron**	**Thifensulfuron-methyl**
Exposure tier (FOCUS step)	Step 2	Step4 (R1 pond)	Step4 (R3 stream)
PECsw [mg a.s./L]	0.003652	0.000007567	0.00001368
Relative proportions of the individual mixture components in the environment (pi PEC)	0.994	0.002	0.004
Total exposure concentration of the mixture (a.s. based) (PECmix) [mg/L]	0.003673		
Calculated mixture toxicity (a.s. in PECmix) (ECx mix-CA = ∑ (pi PEC/ECx i)) [mg a.s./L]	41.096		
ETRmix = PECmix/ECx PPP	0.0001		
Trigger	0.01		

Table 9.5-33: Conduct a mixture RA based on calculated mixture toxicity according to 10.3.8 from EFSA AGD in maize for alga

Exposure	Higher exposure tier		
	dicamba	**nicosulfuron**	**Thifensulfuron-methyl**
Exposure tier (FOCUS step)	Step 2	Step4 (R1 pond)	Step4 (R3 stream)
PECsw [mg a.s./L]	0.003652	0.000007567	0.00001368
Relative proportions of the individual mixture components in the environment (pi PEC)	0.994	0.002	0.004
Total exposure concentration of the mixture (a.s. based) (PECmix) [mg/L]	0.003673		
Calculated mixture toxicity (a.s. in PECmix) (ECx mix-CA = ∑ (pi PEC/ECx i)) [mg a.s./L]	3.752		
ETRmix = PECmix/ECx PPP	0.00098		
Trigger	0.1		

Table 9.5-34: Conduct a mixture RA based on calculated mixture toxicity according to 10.3.8 from EFSA AGD in maize for lemna

Exposure	Higher exposure tier		
	dicamba	**nicosulfuron**	**Thifensulfuron-methyl**
Exposure tier (FOCUS step)	Step 2	Step4 (R1 pond)	Step4 (R3 stream)
PECsw [mg a.s./L]	0.003652	0.000007567	0.00001368
Relative proportions of the individual mixture components in the environment (pi PEC)	0.994	0.002	0.004
Total exposure concentration of the mixture (a.s. based) (PECmix) [mg/L]	0.003673		
Calculated mixture toxicity (a.s. in PECmix) (ECx mix-CA = ∑ (pi PEC/ECx i)) [mg a.s./L]	0.05		
ETRmix = PECmix/ECx PPP	0.073		
Trigger	0.1		

FINAL CONCLUSION

Steps	**Conclusion on the steps**			
	Fish	**Invetebrates**	**Algae**	**Macrophytes**
Step 1: data available?	Endpoints only available for the a.s., go to 7.	Endpoints available for a.s. and the ppp, go to 2.	Endpoints available for a.s. and the ppp, go to 2.	Endpoints available for a.s. and the ppp, go to 2.
Step 2: apparent synergism or antagonism?		The MDR is between 0.2-5. No antagonism or synergism is indicated. Thus, the "concentration addition" concept holds, go to 3.	The MDR is between 0.2-5. No antagonism or synergism is indicated. Thus, the "concentration addition" concept holds, go to 3.	The MDR is <0.2. Thus, antagonism is indicated, go to 9.
Step 3: mixture similar or not?	Mixture similar every scenario. All scenarios can be assessed via product test, go to 4.	Mixture similar every scenario. All scenarios can be assessed via product test, go to 4.	Mixture similar every scenario. All scenarios can be assessed via product test, go to 4.	
Step 4: ETRmix assessment (ECxPPP)	**Acceptable risk have been found in all scenarios in FOCUS step 1-3.**	**Acceptable risk have been found in all scenarios in FOCUS step 1-3.**	**Acceptable risk have been found in all scenarios in FOCUS step 1-3.**	**Acceptable risk have been found in all scenarios if risk mitigation is applied (FOCUS Step 4)**
Step 5: driver available?				
Step 6: driver assessment			Risk acceptable for all scenarios, if risk mitigation is applied (FOCUS Step 4).	Risk acceptable for all scenarios, if risk mitigation is applied FOCUS Step 4)
Step 7: synergism assessment (few data)	Mixture toxicity calculation feasible: Go to 8			
Step 8a: ETRmix assessment	Risk acceptable for all scenarios in FOCUS step 1-3.			Risk acceptable for all scenarios, if risk mitigation is applied FOCUS Step 4)
Step 8b: RQmix assessment				
Step 9: anatagonism assessment				Measured mixture toxicity not plausible: Go to 8
Step 10: synergism assessment				

9.5.3 Overall conclusions

Based on PEC/RAC calculations, no unacceptable risk is indicated for aquatic organisms considering all envisaged GAP uses for EVRITELL 162 OD, assuming that following risk mitigation measures are taken into account:

- a vegetative buffer strip of 10m to surface water bodies is required when conventional spraying techniques are applied.

zRMS comment:

The evaluation of the risk for aquatic 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" (EFSA Journal 2013;11(7):3290). Based on PEC/RAC calculations, no unacceptable risk is indicated for aquatic organisms considering all envisaged GAP uses for **EVRITELL 162 OD**, assuming that following risk mitigation measures are taken into account: However, as aquatic plants are the most sensitive group of aquatic organisms, further studies should be provided at Member State level. The study with *Myriophyllum* and product **EVRITELL 162 OD** should be conducted in accordance with OECD 239. A final conclusion on the risk to the aquatic environment from the formulation **EVRITELL 162 OD** can only be drawn after the studies with the formulation and aquatic plants are made available. This should be addressed during product authorisation at Member State level. **Justification**: Evritell 162 OD is a herbicide containing 3 active substances, where the data on the toxicity of the active substance dicamba on aquatic plants show that *Myriophyllum spicatum* is the most sensitive species.

DATA GAP: In case formulation *Myriophyllum*: 1. Risk assessment for aquatic plants (*M. spicatum*) has been not performed (insufficient data set - data gap). 2. The new study the product **EVRITELL 162 OD** and *M.spicatum* should be performed.

Updated November 2024

To address the current data gap for *Myriophyllum spicatum* conducted by Applicant according to the OECD Guidelines. The new study for *Myriophyllum spicatum* with formulated product **EVRITELL 162 OD** has been accepted by zRMS. Toxicity data and risk assessment for *Myriophyllum spicatum* was available for the PPP **EVRITELL 162 OD** and a low risk was demonstrated for this species. The use **EVRITELL 162 OD** according to the label will not pose risk to aquatic organisms (ratio PEC/RAC is below 1) with apply 10 meters vegetative buffer zone.

The use EVRITELL 162 OD according to the label will not pose risk to aquatic organisms (ratio PEC/RAC is below 1) with apply 10 meters vegetative buffer zone. The risk assessment for aquatic organisms should be considered by MSs level.

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, nicosulfuron and thifensulfuron-methyl. 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 bees of EVRITELL 162 OD were not evaluated as part of the EU assessment of dicamba, nicosulfuron and thifensulfuron-methyl. New data submitted with this application are listed in table 9.6-1 and summarised in Appendix 2.

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

Species	Substance	Exposure System	Results	Reference
Apis mellifera	Dicamba	Oral	LD_{50} > 100 µg a.s./bee	EFSA Journal 2011;9(1):1965
		Contact	LD_{50} > 100 µg/bee	
Apis mellifera	Banvel 480 SL	Oral	LD_{50} > 100 µg a.s./bee	EFSA Journal 2011;9(1):1965
		Contact	LD_{50} > 100 µg/bee	
Apis mellifera	Technical nicosulfuron	Oral	Study details did not allow calculation of oral LD_{50} in terms of µg a.s./bee [LC_{50} > 1000 mg a.s. /litre in diet]	EFSA Scientific Report (2007) 120, 1-91, Conclusion on the peer review of nicosulfuron
		Contact	LD_{50} = 76 µg a.s./bee	
Apis mellifera	Formulation: 'SL-950 4% SC'	Oral	>131 µg product/bee – equivalent to 5.24 µg a.s./bee	EFSA Scientific Report (2007) 120, 1-91, Conclusion on the peer review of nicosulfuron
		Contact	-	
Apis mellifera	Thifensulfuron - methyl	Oral	LD_{50} > 7.1 µg as/bee	Thifensulfuron-methyl SANCO/7577/VI/97-final 12 December 2001
		Contact	LD_{50} > 100 µg as/bee	
Apis mellifera	Thifensulfuron methyl 50 SG	Oral	LD_{50} >181.92 (>90.96 µg a.s.)	EFSA Journal 2015;13(7):4201
		Contact	LD_{50} >200 (>100 µg a.s.)	

Species	Substance	Exposure System	Results	Reference
Apis mellifera	DNT-162OD-R-CPd (formulation EVRITELL 162 OD)	Oral	LD_{50} >200 µg t.i/bee (> 22.7[a] + 7.8[b] + 2.4[c] µg a.s./bee)	Parma P., 2022, EMI/4/64/2022
Apis mellifera	DNT-162OD-R-CPd (formulation EVRITELL 162 OD)	Contact	LD_{50} >200 µg t.i/bee (> 22.7[a] + 7.8[b] + 2.4[c] µg a.s./bee)	Parma P., 2022, EMI/4/65/2022
Apis mellifera	DNT-162OD-R-CPd (formulation EVRITELL 162 OD)	10 days Chronic	LDD_{50} > 29.83 µg t.i./bee/day (> 3.39[a] + 1.16[b] + 0.35[c] µg/bee/day) NOEDD = 1.86 µg t.i./bee/day (0.21[a] + 0.07[b] + 0.02[c] µg/ bee/day)	Parma P., 2023, EMI/4/62/2022
Apis mellifera	DNT-162OD-R-CPd (formulation EVRITELL 162 OD)	Larval (repeated exposure)	ED_{50} > 100.0 µg t.i/larva (>11.37[a] + 3.9[b] + 1.19[c] µg a.s/larva) NOED > 100.0 µg t.i/larva (>11.37[a] + 3.9[b] + 1.19[c] µg a.s/larva)	Parma P., 2023, EMI/4/63/2022
Higher-tier studies (tunnel test, field studies)				
Not relevant				

a: dicamba ; b: nicosulfuron ; c: thifensulfuron-methyl; t.i.: test item

9.6.1.1 Justification for new endpoints

To assess potential effects of EVRITELL 162 OD on bees, the studies were performed for this formulation and the results can be used for the risk assessment.
A chronic 10 day laboratory feeding study for adult honey bees and larval toxicity study exposed to DNT-162OD-R-CPd (EVRITELL 162 OD) are submitted to fulfil the requirements. These studies are required according to Regulation (EC) No. 284/2013

9.6.2 Risk assessment

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

9.6.2.1 Hazard quotients for bees

Table 9.6-2: First-tier assessment of the risk for bees due to the use of EVRITELL 162 OD in maize

Intended use **Active substance** **Application rate (g a.s/ha)**	maize dicamba 1 x 110		
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	110	< 1.1
Contact toxicity	>100		< 1.1
Intended use **Active substance** **Application rate (g a.s/ha)**	Maize Nicosulfuron 1 x 40		
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	Study details did not allow calculation of oral LD50 in terms of µg a.s./bee [LC50 > 1000 mg a.s. /litre in diet]	40	n.a
Contact toxicity	76		0.52
Intended use **Active substance** **Application rate (g a.s/ha)**	maize thifensulfuron 1 x 12		
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	> 7.1	12	< 1.7
Contact toxicity	> 100		< 0.12
Product **Application rate (g/ha)**	EVRITELL 162 OD 1 x 1016 *		
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	>200	1016	< 5.08
Contact toxicity	>200		< 5.08

* Application rate of = 1.0 L product ha-1 x 1.016 (relative density) = 1.016 kg product/ha
Q_{HO}, Q_{HC}: Hazard quotients for oral and contact exposure. Q_H values shown in bold breach the relevant trigger.

Applicant update – August 2024

Since toxicity endpoints derived from chronic bee and bee larvae studies were available, a risk assessment

for chronic toxicity to bees was done following the EFSA bee GD (2013), even although the bee GD is currently not in force. Therefore, the risk assessment performed has to be considered only for demonstration.

Table 9.6-3 Screening assessment of the acute and chronic risk for bees due to the use of EVRITELL 162 OD in maize (EFSA Journal 2013; 11(7): 3295)

Intended use	**maize**			
Active substance	EVRITELL 162 OD			
Application rate (g/ha)	1 x 1016 *			
Contact route of exposure				
	"calculation factor" (linked with dust)	**HQ**	**Trigger**	**Risk indicator**
Honey bee	1	5.1	42	OK
Oral route of exposure (pollen and nectar)				
	"calculation factor" (Ef x SV)	**ETR**	**Trigger**	**Risk indicator**
Honey bee acute	7.6	0.04	0.2	OK
Honey bee chronic	7.6	0259	0.03	!
Honey bee larvae	4.4	0.04	0.2	OK

Accumulative effects			
Has the substance a potential for accumulative toxicity (see section 8.1.1.3 and pertinent part of Appendix O in the GD)?	*No*		

Exposure to contaminated water				
Guttation				
According to the planned implementation, this assessment is not to be used for applications submitted after 31st January 2018. Also, for application submitted after that date it was noted that "the assessment scheme as such is considered to be acceptable but more information is needed on which crops and under what circumstances guttation droplets are produced and to what extent guttation droplets are used as a water source. It should also be clarified if it is useful to provide water to honeybees as a possible risk mitigation measures".				
Surface water				
	water consumption (µL)	**ETR**	**Trigger**	**Risk indicator**
acute	11.4	0.00	0.2	OK
chronic	11.4	0.000	0.03	OK
larvae	111	0.00	0.2	OK
Puddle water				
	water consumption (µL)	**ETR**	**Trigger**	**Risk indicator**
acute	11.4	0.00	0.2	OK
chronic	11.4	0.000	0.03	OK
larvae	111	0.00	0.2	OK

Results of the screening step indicated that further risk assessment at tier 1 is required for chronic risk to adult bees via oral (pollen and nectar) exposure.

Table 9.6-4 First-tier assessment of the chronic risk for bees due to the use of EVRITELL 162 OD in maize

Intended use	**maize**				
Active substance	EVRITELL 162 OD				
Application rate (g/ha)	1 x 1016 *				
Category	**Endpoint**	**BBCH**	**Scenario**	**ETR**	**trigger**
Chronic	LDD_{50} > 29.83 µg a.s./bee	10 - 29	treated crop	0.023	0.03
			weeds	**0.071**	
			field margin	0.001	
			adjacent crop	0.000	
			next crop	0.013	

The chronic risk assessment has been conducted for demonstration. The ETR values for chronic bees is above the trigger for weeds scenario. However, maize are not a melliferous plant, so it is not attractive to the bees and other pollinators. Also EVRITELL 162 OD is used at early stage (BBCH 12-16), when the treated plant are small and do not have flowers. It should also be taken into account that LDD_{50} are expressed as greater than 29.83 µg t.i./bee/day (the highest tested dose). Although, the risk assessment was presented only for demonstration, the EFSA bee GD (2013) is still not implemented and currently is undergoing a revision. Therefore, no refinements are included.

zRMS comment: The HQ values are lower than the trigger of 50, indicating low risk to bees from following application of **EVRITELL 162 OD**. In addition, the chronic studies for bees were submitted by the applicant. The chronic study for adult bees and a study effects on honey bee development and other honey bee life stages were accepted by zRMS. The risk assessment based on these studies should be considered when GD for Bees, 2013 is implemented at EU level. Final decision should be taken into account at MSs level.

Updated November 2024

The risk assessment for bees according EFSA 2013 guidance was performed by Applicant. The risk assessment was accepted by zRMS. First tier chronic evaluation of the risk to adult bees exposed to **EVRITELL 162 OD** resulted with ETR value above the trigger in weeds scenario indicating potentially unacceptable risk. No data enabling refinement of the risk was available. Nevertheless, since the EFSA Bee Guidance Document is yet to be implemented (2013), this result should be treated as indication of area that should be covered in the future, once the guidance document is officially noted and accepted. Further assessments from chronic exposure could be required at national level. It also should be noted that product **EVRITELL 162 OD** is an herbicide that is mostly applied **at early stage (BBCH 12-16), when the treated plant are small and do not have flowers**. Flowering weeds in fields treated with EVRITELL 162 OD might be attractive to bees. However, in the case of weeds present, application of **EVRITELL 162 OD** leads to a significant reduction/unattractiveness of the ground cover in the treated fields within a very few days, so flowering, if any, will take place only a very short time period. Thus, the likelihood of bees found in treated fields can be considered as low and exposure to bees, if any, is restricted to a very short time period and a limited number of individuals. On the other hand, maize are not a melliferous plant, so it is not attractive to the bees and other pollinators**.** In conclusion, no adverse effects on populations and

communities need to be expected in consideration of the intended GAP uses of **EVRITELL 162 OD.**

The chronic risk assessment for bees should be considered by MSs level.

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

Not relevant.

9.6.3 Effects on bumble bees

Studies on the toxicity to bumble bees have been carried out with dicamba, nicosulfuron and thifensulfuron-methyl. Full details of these studies are provided in Appendix 2 of this document (new studies).

Table 9.6-3.1: Endpoints and effect values relevant for the risk assessment for bumble bees

Species	Substance	Time scale/type of endpoint	Toxicity	Reference
Bombus spp.	EVRITELL 162 OD (DNT-162OD-R-CPd)	Acute oral	LD_{50} > 400 µg test item/bumble bee (>44.67[a] + >15.55[b] + > 4.95[c] µg/ bumble bee)	Wojciech A., 2023, B-56-23
Bombus spp.	EVRITELL 162 OD (DNT-162OD-R-CPd)	Acute contact	LD_{50} > 400 µg test item/bumble bee (>44.67[a] + >15.55[b] + > 4.95[c] µg/ bumble bee)	Wojciech A., 2023, B-57-23

a: dicamba; b: nicosulfuron; c: thifensulfuron-methyl

The risk assessment for bumble bees is performed in accordance with the recommendations of the "Guidance on the risk assessment of plant protection products on bees (*Apis mellifera, Bombus* spp. and solitary bees)", as provided by the European Food Safety Authority (EFSA Journal 2013;11(7):3295 doi: 10.2903/j.efsa.2013.3295, July 04, 2014). All calculations are based on the EFSA Screening Step and 1st Tier calculator (BeeTool v3).

The Screening Step was conducted considering all recommended application rates according to the proposed use pattern (downwards spray).

Screening assessment of the risk of dicamba, nicosulfuron and thifensiulfuron-methyl for bumble-bees due to the use of EVRITELL 162 OD

Intended use	Maize				
Application method	downward spraying				
Active substance	< EVRITELL 162 OD >				
Use pattern	1 x 162 g a.s./ha,				
Type design	**LD_{50} (µg a.s./bee)**	**Max. single application rate (g a.s./ha)**		**$HQ_{contact}$ criterion**	**Trigger**
Adult acute contact toxicity	65.17	162		<2.5	≤7
Type design	**LD_{50} (µg a.s./bee)**	**Max. single application rate (kg a.s./ha)**	**E_f × SV**	**ETR**	**Trigger**
Adult acute oral toxicity	65.17	162	11.2	<0.03	≤ 0.036

Ef: exposure factor; SV: shortcut value; $HQ_{contact}$: Hazard quotient for contact exposure; ETR: Exposure toxicity ratio; ETR values shown in **bold** breach the relevant trigger.
a.s. =active substance

The exposure toxicity ratio (ETR) for oral exposure of bumble bees to EVRITELL 162 OD is below the trigger value of 0.036. Therefore, Tier 1 assessment is not required.
The risk for bumble bees is acceptable.

zRMS comment: Accepted. The exposure toxicity ratio (ETR) for oral exposure of bumble bees to **EVRITELL 162 OD** is below the trigger value of 0.036. Therefore, Tier 1 assessment is not required. The risk for bumble bees is acceptable. The risk assessment for bumble bees should be considered by MSs level.

9.6.4 Effects on solitary bees

No data/information available.

9.6.5 Overall conclusions

Both the oral and contact hazard quotients are less than 50 and thus no unacceptable risk to bees is expected from the proposed use of EVRITELL 162 OD when used as recommended.

zRMS comment: Accepted.

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 dicamba, nicosulfuron and thifensulfuron-methyl. 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 nontarget arthropods of EVRITELL 162 OD were not evaluated as part of the EU assessment of dicamba, nicosulfuron and thifensulfuron-methyl. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

The risk assessment was performed based on results obtained for EVRITELL 162 OD in extended laboratory studies on *A.rhopalosiphi*, *T.pyri, Ch.carnea* and *C.septempunctata* and age residue studies on ~~the~~ two ~~-~~most sensitive species with *Ch. carnea* and *C.septempunctata* .

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

Species	Substance	Exposure System	Results	Reference
Typhlodromus pyri (protonymphs)	DNT-162OD-R-CPd (formulation EVRITELL 162 OD)	Extended laboratory test bean leaves (2D)	LR_{50} = 1403.1 mL of the test item /ha (Dicamba: 161.4 g a.s./ha Nicosulfuron 55.3 g a.s./ha Thifensulfuron-methyl 16.9 g a.s./ha ER_{50} >1000.0 mL of the test item /ha (Dicamba > 115.0 g a.s./ha Nicosulfuron > 39.4 g a.s./ha Thifensulfuron-methyl > 12.0 g a.s./ha)	Kręglewska M., 2022, 0016/0175/E
Aphidius rhopalosiphi (adults)	DNT-162OD-R-CPd (formulation EVRITELL 162 OD)	Extended laboratory Test barley seedlings (3D)	LR_{50} / ER_{50} > 2000 mL of the test item /ha (Dicamba > 230.0 g a.s./ha Nicosulfuron > 78.8 g a.s./ha Thifensulfuron-methyl > 24.1 g a.s./ha)	Kręglewska M., 2022, 0016/0178/E
Chrysoperla carnea	DNT-162OD-R-CPd	Extended laboratory	LR_{50} = 205.006 mL of	Kubisiak K., 2023,

Species	Substance	Exposure System	Results	Reference
(larvae)	(formulation EVRITELL 162 OD)	test bean leaves (2D)	the test item /ha (Dicamba: 23.576 g Nicosulfuron: 8.077 g Thifensulfuron - methyl: 2.466 g/ha)	0016/0176/E
Coccinella septempunctata L. (larvae)	DNT-162OD-R-CPd (formulation EVRITELL 162 OD)	Extended laboratory test bean leaves (2D)	LR_{50} = 283.137 mL of the test item /ha (Dicamba: 32.561g/ha Nicosulfuron: 11.156 g/ha Thifensulfuron-methyl: 3.406 g/ha)	Domagała J., 2023, 0016/0177/E
Chrysoperla carnea	DNT-162OD-R-CPd (formulation EVRITELL 162 OD)	Aged-residue test French bean plants (3D)	Mortality at 1.0 L/ha: 65% at 0 DAT 37.5 % at 14 DAT 12.5 % at 28 DAT	Domagała J., 2023, 0016/0224/E
Coccinella septempunctata L.	DNT-162OD-R-CPd (formulation EVRITELL 162 OD)	Aged-residue test French bean plants, leaves (3D)	Mortality at 1.0 L/ha: 87.5% at 0 DAT 50.0 % at 14 DAT 35.0 % at 28 DAT	Domagała J., 2024, 0016/0244/E
Field or semi-field tests				
Not relevant				

9.7.1.1 Justification for new endpoints

Endpoints are provided for the actual formulated product EVRITELL 162 OD for the standard test species *Aphidius rhopalosiphi*, *Typhlodromus pyri*, *Chrysoperla carnea* and *Coccinella septempunctata*. Therefore, risk assessments are based on these data.

9.7.2 Risk assessment

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

9.7.2.1 Risk assessment for in-field exposure

Table 9.7-2: First- and higher-tier assessment of the in-field risk for non-target arthropods due to the use of EVRITELL 162 OD in maize

Intended use	Maize (field crop scenario)		
Active substance/product	EVRITELL 162 OD		
Application rate (L product/ha)	1 ×1		
MAF	1		
Test species Tier II (Extended lab testing)	**LR_{50} (lab.) (L product/ha)**	**$PER_{in\text{-}field}$ (L product/ha)**	**HQin-field criterion: HQ ≤ 1**
Aphidius rhopalosiphi	>2	1	< 0.5
Typhlodromus pyri	1.4031		0.71
Chrysoperla carnea	0.205		**4.87**
Coccinella septempunctata	0.283		**3.53**

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient; DALT: Days after last treatment.
Criteria values shown in **bold** breach the relevant trigger.

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

Based on results obtained for EVRITELL 162 OD in extended laboratory studies the corresponding "in-field" hazard quotients are above the trigger value of 1 for *Chrysoperla carnea* and *Coccinella septempunctata* indicating an unacceptable "in-field" risk to non-target arthropods, following application of EVRITELL 162 OD according to the proposed GAP.
In connection with that and to complete risk assessment for non-target arthropods Applicant decided to conduct additional study. Additional studies will include aged residue studies on ~~the~~ two most sensitive species i.e. *Chrysoperla carnea,* and *Coccinella septempunctata* for plant protection product EVRITELL 162 OD.

zRMS comment: The field risk for non-target arthropod species *Coccinella septempunctata* (TER = 3.53) is not clarified. The field risk assessment fails for the two indicator species *Coccinella septempunctata* and *Chrysoperla carnea*. Only the risk for the most sensitive species *Chrysoperla carnea* is clarified. However, clarification of the risk for *Coccinella septempunctata* is also required. Therefore the refinement risk for *Coccinella septempunctata* is also required.

Updated November 2024

The study for *Coccinella septempunctata* and **EVRITELL 162 OD** conducted by Applicant. The new study for *Coccinella septempunctata* with formulated product **EVRITELL 162 OD** has been accepted by zRMS.

9.7.2.2 Risk assessment for off-field exposure

The risk envelope approach is not applied here, since application rate and the highest drift rate is the same for all of the intended uses (respectively 1.0 L product/ha and 2.77%).

The worst PERoff field value is equal 2.77 g formulation/ha for intended use.

Table 9.7-3: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of EVRITELL 162 OD in maize.

Intended use	Maize (field crop scenario)
Active substance/product	EVRITELL 162 OD
Application rate (L product/ha)	1 × 1
MAF	1
Vdf	10 [a)] 5[b)]

Test species Tier II (Extended lab testing)	**L/ER$_{50}$ (lab.) (L product/ha)**	**Drift percentile [%]**	**Drift rate**	**PER$_{off\text{-}field}$ (L product/ha)**	**CF**	**HQoff-field criterion: HQ ≤ 1**
Aphidius rhopalosiphi	>2	2.77	0.0277	0.00277 0.00554	5	< 0.0069 0.01385
Typhlodromus pyri	1.4031					0.0099 0.0197
Chrysoperla carnea	0.205					0.068 0.135
Coccinella septempunctata	0.283					0.049 0.098

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

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

a) In accordance with Working document on Risk Assessment of Plant Protection Products in the Central Zone (Version 2.0, August 2023) .

b) CZSC meeting 2024

Based on results obtained for EVRITELL 162 OD in extended laboratory studies on *A. rhopalosiphi, T. pyri,* and *Ch. carnea* and *C. septempunctata* the corresponding "off-field" hazard quotients are below the trigger value of 1 indicating an acceptable "off-field" risk to non-target arthropods, following application of EVRITELL 162 OD according to the proposed GAP.

zRMS comment: The off-field risk of **EVRITELL 162 OD** to non-target arthropods was assessed from Hazard Quotients (HQ) between toxicity endpoints estimated from the formulated product **EVRITELL 162 OD** as well as off-field predicted environmental rate. No risk was determined off-field after application of **EVRITELL 162 OD** at maximum rate of 1 L/ha. No additional action required. Risk is acceptable.

9.7.2.3 Additional higher-tier risk assessment

To complete "in-field" risk assessment for non-target arthropods additional higher tier study on *Ch. carnea* was performed. The effects of both fresh and aged foliar residues of EVRITELL 162 OD on the green lacewing *Chrysoperla carnea* were evaluated under extended laboratory test conditions. When applied at a rate equivalent to 1.0 L product/ha, fresh (0-day-old) foliar residues of EVRITELL 162 OD proved harmful to the mites, but both 14-day-old and 28-day-old residues resulted in no unacceptable effects on either the survival or the subsequent reproductive capacity of the green lacewing.

In terms of the in-field risk, acceptable effects were noted after 14 and 28 days at 1.0 L product/ha. As the estimated in-field exposure level is 1.0 L product/ha for the use maize, in-field recovery is expected within this time scale (14 - 28 days).

Risk mitigation measures
Not relevant.

Moreover to complete the "in-field" risk assessment the Applicant perform additional age residue study on *Coccinella septempunctata* with the product EVRITELL 162 OD.
The impact of the freshly-dried and field-aged residues of the test item on the *Coccinella septempunctata* were evaluated under extended laboratory conditions on following bioassays: 0 DAT, 14 DAT and 28 DAT. The test item achieved <50% mortality and <50% reduction in reproductive performance (relative to the control) in two subsequent bioassays at 14 DAT and 28 DAT for rate 1000 mL of the test item/200 L of water/ha thus in-field recovery is expected within this time scale (14 - 28 days).

Risk mitigation measures:
Not relevant.

zRMS comment: The field risk for non-target arthropod species *Coccinella septempunctata* (TER = 3.53) is not clarified. The field risk assessment fails for the two indicator species *Coccinella septempunctata* and *Chrysoperla carnea*. Only the risk for the most sensitive species *Chrysoperla carnea* is clarified. However, clarification of the risk for *Coccinella septempunctata* is also required. After re-examining the documentation and your arguments, I maintain my request to clarify the risk for the second species Coccinella septempunctata. Justification: 1. Escort 2 recommends that the most sensitive species should be indicated in laboratory tests and then more detailed tests should be performed on the most sensitive species. However, ESCORT 3 clearly indicates that tests should not be limited to the most sensitive species, but should include other important non-target organisms in order to ensure a comprehensive assessment. ESCORT 3 emphasizes a more comprehensive assessment, covering many species, realistic exposure scenarios and a broader view of ecological aspects. This approach ensures a thorough risk assessment and protection of the entire ecosystem. *Chrysoperla carnea* has been identified as the most sensitive species taking into account the mortality parameter and in this case conducting tests - aged residue study - can provide key information on the persistence and impact of pesticide residues on this species. However, this does not necessarily provide adequate protection for *Coccinella septempunctata*. Although ESCORT 2 suggests using the most sensitive species, in my opinion the risk assessment should not overlook other species that have shown unacceptable risk in the initial assessments. Given that the risk quotient for *Coccinella septempunctata* is also above the threshold, it is prudent to conduct an aged residue study for both species to obtain a more comprehensive risk profile. Differences in morphology, ecological roles, physiological responses and behavioural patterns between *Chrysoperla carnea* and *Coccinella septempunctata* significantly influence their sensitivity to pesticides. Conducting an aged residue study on both species will allow for a more comprehensive risk assessment, providing a thorough assessment of the ecological impact on a range of beneficial arthropods. 2. Additionally, in the extended laboratory study, the emergence rate for Coccinella septempunctata is reduced by approximately 16.2% at the highest dose tested compared to the control. However, the hatch rate for *Chrysoperla carnea* is reduced by about 9.5% and we do not observe a clear dose-response pattern here regarding the reproduction parameter. In the extended laboratory study, the average number of eggs that one female is able to lay per day and that are capable of hatching for *Coccinella*

septempunctata is reduced by about 40% for the highest tested dose compared to the control and a clear dose-response pattern is visible. Considering the reproduction parameter, it is *Coccinella septempunctata* and not *Chrysoperla carnea* that is more sensitive. Therefore the refinement risk for *Coccinella septempunctata* is also required.

Updated November 2024
The additional age residue for *Coccinella septempunctata* and **EVRITELL 162 OD** conducted by Applicant. The new study for *Coccinella septempunctata* with formulated product **EVRITELL 162 OD** has been accepted by zRMS.
Moreover to complete the "in-field" risk assessment the Applicant perform additional age residue study on Coccinella septempunctata with the product EVRITELL 162 OD. The impact of the freshly-dried and field-aged residues of the test item on the *Coccinella sep-tempunctata* were evaluated under extended laboratory conditions on following bioassays: 0 DAT, 14 DAT and 28 DAT. The test item achieved <50% mortality and <50% reduction in reproductive performance (relative to the control) in two subsequent bioassays at 14 DAT and 28 DAT for rate 1000 mL of the test item/200 L of water/ha thus in-field recovery is expected within this time scale (14 - 28 days). The risk assessment was accepted by RMS. Further refinement is not needed.

9.7.2.4 Risk mitigation measures

No risk mitigation needed.

9.7.3 Overall conclusions

Based on results obtained for EVRITELL 162 OD in extended laboratory studies on *A. rhopalosiphi* , *T. pyri*, the corresponding "in-field" hazard quotients are below the trigger value of indicating an acceptable "in-field" risk to non-target arthropods, following application of EVRITELL 162 OD according to the proposed GAP.
However, for *Ch. carnea* and *C. septempunctata* the in-field hazard quotient are above the trigger value of 1 indicating an unacceptable "in-field" risk. In connection with that and to complete risk assessment for non-target arthropods Applicant decided to conduct and aged residue study on the most sensitive species *Ch. carnea* for plant product protection EVRITELL 162 OD.

The available data on aged residue study on *Ch.carnea* indicate that, any initial effects on non-target arthropods from the proposed uses of EVRITELL 162 OD will be short-lived and recovery/recolonisation will take place within an acceptable time frame, thus an acceptable risk can be concluded.

Based on results obtained for EVRITELL 162 OD in extended laboratory studies on *A. rhopalosiphi, T. pyri*, *Ch. carnea* and *C. septempunctata* the corresponding "off-field" hazard quotients are be-low the trigger value of 1 indicating an acceptable "off-field" risk to non-target arthropods, following application of EVRITELL 162 OD according to the proposed GAP.

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, nicosulfuron, thifensulfuron-methyl and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on earthworms and other non-target soil organisms (meso- and macrofauna) of EVRITELL 162 OD were not evaluated as part of the EU assessment of dicamba, nicosulfuron and thifensulfuron-methyl. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

Species	Substance	Exposure System	Results	Reference
Nicosulfuron				
Eisenia fetida	AUSN	Chronic, 8 weeks (reproductive toxicity study)	**NOEC 0.100 mg** AUSN /kg d.w. soil (highest test dose)	EFSA Scientific Report (2007) 120, 1-91
Eisenia fetida	UCSN	Chronic, 8 weeks (reproductive toxicity study)	**NOEC 0.050 mg** UCSN /kg d.w. soil (highest test dose)	EFSA Scientific Report (2007) 120, 1-91
Eisenia fetida	ASDM	Chronic, 8 weeks (reproductive toxicity study)	**NOEC 0.350 mg** ASDM /kg d.w. soil (highest test dose)	EFSA Scientific Report (2007) 120, 1-91
Folsomia candida, (Collembola)	AUSN	Chronic, 28 days (reproductive toxicity study)	**NOEC 0.100 mg** AUSN /kg d.w. soil (highest test dose)	EFSA Scientific Report (2007) 120, 1-91
Folsomia candida, (Collembola)	UCSN	Chronic, 28 days (reproductive toxicity study)	**NOEC 0.050 mg** UCSN /kg d.w. soil (highest test dose)	EFSA Scientific Report (2007) 120, 1-91
Folsomia candida, (Collembola)	ASDM	Chronic, 28 days (reproductive toxicity study)	**NOEC 0.350 mg** ASDM /kg d.w. soil (highest test dose)	EFSA Scientific Report (2007) 120, 1-91
Thifensulfuron-methyl				
Eisenia fetida	IN-A4098	Chronic 56 days	**NOEC = 0.2 mg/kg dw**	EFSA Journal 2015;13(7):4201
Eisenia fetida	IN-A4098	Chronic 56 days	NOEC = 0.202 mg/kg dw	EFSA Journal 2015;13(7):4201
Eisenia fetida	IN-A4098	Chronic 56 days	NOEC = 8.0 mg/kg dw	EFSA Journal 2015;13(7):4201
Eisenia fetida	IN-L9223	Chronic 56 days	**NOEC = 10.0 mg/kg dw**	EFSA Journal 2015;13(7):4201
Eisenia fetida	IN-L9225	Chronic 56 days	**NOEC = 0.4 mg/kg dw**	EFSA Journal 2015;13(7):4201

Species	Substance	Exposure System	Results	Reference
Nicosulfuron				
Eisenia fetida	IN-L9225	Chronic 56 days	NOEC = 8.0 mg/kg dw	EFSA Journal 2015;13(7):4201
Eisenia fetida	IN-W8268	Chronic 56 days	NOEC = 80.0 mg/kg dw	EFSA Journal 2015;13(7):4201
Eisenia fetida	IN-W8268	Chronic 56 days	**NOEC = 8.0 mg/kg dw**	EFSA Journal 2015;13(7):4201
Collembola (*F.candida*)	IN-A4098	Chronic 28 days	**NOEC = 0.045 mg/kg dw** NOEC = 31.7 mg/kg dw	EFSA Journal 2015;13(7):4201
Predatory mite (*H.aculeifer*)	IN-A4098	Chronic 14 days	**NOEC = 100.0 mg/kg dw**	EFSA Journal 2015;13(7):4201
Collembola (*F.candida*)	IN-L9223	Chronic 28 days	**NOEC = 100.0 mg/kg dw**	EFSA Journal 2015;13(7):4201
Collembola (*F.candida*)	IN-L9225	Chronic 28 days	**NOEC = 10.0 mg/kg dw** NOEC = 100.0 mg/kg dw	EFSA Journal 2015;13(7):4201
Predatory mite (*H.aculeifer*)	IN-L9225	Chronic 14 days	**NOEC = 100.0 mg/kg dw**	EFSA Journal 2015;13(7):4201
Collembola (*F.candida*)	IN-W8268	Chronic 14 days	**NOEC = 100.0 mg/kg dw**	EFSA Journal 2015;13(7):4201
Predatory mite (*H.aculeifer*)	IN-W8268	Chronic 14 days	**NOEC = 50.0 mg/kg dw**	EFSA Journal 2015;13(7):4201
Eisenia fetida	EVRITELL 162 OD	Chronic, 56 days	**NOEC = 560 mg/kg dw** EC_{10} = 586.8 mg/kg dw	P. Pieczka, 2023, G-24-23
Folsomia candida	EVRITELL 162 OD	Chronic, 28 days	**NOEC = 10.0 mg/kg dw** EC_{10} = 12.96 mg/kg dw	P. Pieczka, 2023, G-31-23
Hypoaspis aculeifer	EVRITELL 162 OD	Chronic, 14 days	**NOEC ≥ 1000.0 mg/kg dw** EC_{10} > 1000.0 mg/kg dw	P. Pieczka, 2023, G-32-23
Field studies				
Litter bag test				

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

9.8.1.1 Justification for new endpoints

No deviation from EU agreed endpoints.

9.8.2 Risk assessment

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

9.8.2.1 First-tier risk assessment

The relevant PEC_{soil} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.2, Table 8.7-3. According to the assessment of environmental-fate data, multi-annual accumulation in soil is considered for dicamba, nicosulfuron and thifensulfuron-methyl.

Table 9.8-2: First-tier assessment of the acute and chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of EVRITELL 162 OD in maize

Intended use	maize		
Chronic effects on earthworms			
Product/active substance	**NOEC (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
AUSN	0.100	0.0119*	8.40
UCSN	0.050	0.0061*	8.20
ASDM	0.350	0.0233*	15.02
IN-A4098	0.2	0.0063*	31.75
IN-L9223	10.0	0.0020*	5000
IN-L9225	0.4	0.0026*	153.85
IN-W8268	8.0	0.0017*	4705.88
EVRITELL 162 OD	560	1.02	549.02
Chronic effects on other soil macro- and mesofauna			
Product/active substance	**NOEC (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
AUSN (*F. candida*)	0.100	0.0119*	8.40
UCSN (*F. candida*)	0.050	0.0061*	8.20
ASDM (*F. candida*)	0.350	0.0233*	15.02
IN-A4098 (*F. candida*)	0.045	0.0063*	7.14
IN-A4098 (*H. aculeifer*)	100	0.0063*	15873
IN-L9223 (*F. candida*)	100	0.0020*	50000
IN-L9225 (*F. candida*)	10	0.0026*	3846.2
IN-L9225 (*H. aculeifer*)	100	0.0026*	38462

IN-W8268 (*F. candida*)	100	0.0017*	58824
IN-W8268 (*H. aculeifer*)	50	0.0017*	29422
EVRITELL 162 OD (*F. candida*)	10	1.02	9.80
EVRITELL 162 OD (*H. aculeifer*)	1000	1.02	980.39

TER values shown in bold fall below the relevant trigger.
* $PEC_{accumulation}$

9.8.2.2 Higher-tier risk assessment

Not relevant.

9.8.3 Overall conclusions

The risk from exposure to dicamba, nicosulfuron and thifensulfuron-methyl and relevant soil degradation products applied as EVRITELL 162 OD for all intended uses is indicated to be acceptable for the soil meso- and macrofauna.

zRMS comment: Agreed. The risk assessment for earthworms and other soil macroorganism for a.s. (dicamba and nicosulfuron and thifensulfuron-methyl) and their metabolites as well as, the product **EVRITELL 162 OD** was accepted by zRMS only provisionally. Risk assessments for **EVRITELL 162 OD** with the proposed use pattern was carried out according to the guidance for risk assessment for terrestrial ecotoxicology "Guidance Document on Terrestrial Ecotoxicology", (SANCO/10329/2002 rev.2 final, 2002). However, risk assessments are accepted provisionally. The studies for formulation of EVRITELL 162 OD for earthworms and *Folsomia candida* and *Hypoapsis aculeifer* with risk assessment was accepted by zRMS only provisionally. The toxicity endpoints were based on nominal concentration. At the end on the studies concentration of substances active were not reported. The analytical measurements should be performed and reported at least at the start, middle, and end of the study. The intermediate measurements should be to capture the degradation of the substance (i.e., designed substance property dependent). The TWA or geometric mean measured concentration should be calculated over the duration of the test and used if the concentration falls under 80% of nominal. Please complete the information regarding the analytical measurements of active substances during the study.

It should be considered at MSs level.

Updated April 2025

The information regarding the analytical measurements of active substances during the soil studies for formulation of EVRITELL 162 OD (DNT-162OD-R-CPd) with earthworms and *Folsomia candida* and *Hypoapsis aculeifer* was provided by Applicant. Below is the Applicant's approach was provided:

Justification of active substances concentrations during studies on impact of DNT-162OD-R-CPd for soil organisms

The product DNT-162OD-R-CPd, contains 3 active substances: dicamba, nicosulfuron and thifensulfuron-methyl. Although concentration of the actives was not measured

in the studies on earthworms, *Folsomia* and *Hypoaspis*, safe use of product DNT-162OD-R-CPd for this organisms can be ensured. Detailed justification in this regard is presented hereafter.

3. Nicosulfuron

It can be anticipated that concentration of nicosulfuron in tested soils was stable in the toxicity tests with DNT-162OD-R-CPd. The geometric mean soil DT_{90lab} value for nicosulfuron is equal to 78.3 d (EFSA Scientific Report (2007) 120, 1-91), which is greater than the longest exposure phase considered in the soil organism studies (56 days for the earthworms).

Consequently, the concentration of nicosulfuron does not need to be analysed in the soil organisms studies with product DNT-162OD-R-CPd. The current assessment is sufficient and safe use for earthworms and other soil macroorganism can be ensured in respect to nicosulfuron for the intended use of DNT-162OD-R-CPd.

4. Dicamba

Although geometric mean soil DT_{90lab} value for dicamba of 13.2 d (EFSA Journal 2011;9(1):1965) is lower than the exposure phases, which are considered in soil organisms studies, there are the other data which clearly proof that concentration of this molecule is stable in the standard tested soils, at least up to 28 days. According to *Folsomia* and *Hypoaspis* studies with a different product containing dicamba (Chwastox Nowy Trio 390 SL), concentration of dicamba was in tested soils stable. As presented in Table 1 and 2 it was within 80% of the nominal concentration, during whole study periods.

Table 1. Analitical measurements of dicamba in *Folsomia* study with product Chwastox Nowy Trio 190 SL (Holewik, 2019, study code: G/153/18, page 54)

Date of analysis	Nominal concentration of dicamba mg/kg	Concentration determined in particular replicates mg/kg			Average mg/kg	SD mg/kg	RSD %	Average %
		1	*2*	*3*				
14.05.2019 day 0	0.00 32.23	ND 31.92	ND 31.72	ND 30.95	ND 31.53	0.00 0.51	- 1.6	- 97.8
28.05.2019 day 14	0.00 32.23	ND 28.44	ND 28.58	ND 29.31	ND 28.78	0.00 0.47	- 1.6	- 89.3
11.06.2019 day 28	0.00 32.23	ND 26.15	ND 26.02	ND 26.11	ND 26.09	0.00 0.06	- 0.2	- 81.0

Table 2. Analitical measurements of dicamba in *Hypoaspis* study with product Chwastox Nowy Trio 190 SL (Holewik, 2019, study code: G/154/18, page 55)

Date of analysis	Nominal concentration of dicamba mg/kg	Concentration determined in particular replicates mg/kg			Average mg/kg	SD mg/kg	RSD %	Average %
		1	2	3				
10.07.2019 day 0	0.00 32.23	ND 31.07	ND 31.14	ND 31.85	ND 31.35	0.00 0.43	- 1.4	- 97.3
17.07.2019 day 7	0.00 32.23	ND 28.62	ND 29.39	ND 29.75	ND 29.25	0.00 0.58	- 2.0	- 90.8
24.07.2019 day 14	0.00 32.23	ND 30.28	ND 28.23	ND 29.89	ND 29.80	0.00 0.54	- 1.8	- 92.5

For further details on the studies on Chwastox Nowy Trio 390 SL, please refer to Appendix 1, where the whole study reports are enclosed.

Similar study with dicamba is not for *Eisenia* available. Nevertheless, safe use can be ensured for earth-worm in respect to dicamba, even if degradation of this molecule would be considered in the study with DNT-162OD-R-CPd.

Taking into account:

- the lowest nominal endpoint for dicamba for earthworms from the studies on DNT-162OD-R-CPd (report presented in the current dRR with a study code: G-24-23),
- correction of nominal endpoints by the EU agreed DT_{50} soil endpoint for dicamba,
- and duration of the earthworm study,

the NOECs TWA for dicamba can be estimated by the following equation:

$NOEC_{TWA} = NOEC_{nominal} * (DT_{50\ soil} / (\text{Study duration} * \ln(2)) * (1 - \exp(-\text{Study duration} * \ln(2) / DT_{50\ soil})$

where:
$NOEC_{nominal\ for\ earthworm}$ = 62.53 mg dicamba/ kg dw soil
(based on nominal concentration from the study on DNT-162OD-R-CPd),

DT_{50} soil = 3.0 days
(half- life relevant for calculation of dicamba concentration in soil – please refer to section B8 for DNT-162OD-R-CPd),

Study duration – 56 days.

With this assumption, the following NOECs TWA would be then for dicamba achieved:

$NOEC_{TWA}$ for earthworms for dicamba= 4.83 mg/kg dws soil

Then, considering the worst-case PECsoil value for dicamba applied as DNT-162OD-R-CPd, in case of use in maize of 0.11 mg/kg dws soil (please refer to section B8 for DNT-162OD-R-CPd), a safe use for dicamba in respect to the earthworms can be demonstrated:

TER = 4.83/0.11 = 44 (TER > 5, safe use anticipated)

Consequently, the concentration of dicamba does not need to be analysed in the soil organisms studies with product DNT-162OD-R-CPd. The available data prove sufficiently, that concentration of dicamba will remain stable during whole studies

on *Folsomia* and *Hyposaspis*. In case of the earthworms, even if degradation of dicamba, is considered during 56-day period, there will be no unacceptable risk to the earthworms. The TER for dicamba will be then much greater than 5.

Thifensulfuron-methyl

Thifensulfuron-methyl degradates in soil the most rapidly, when compared to dicamba and nicosulfuron. It`s geometric mean laboratory DT_{90} value is 4.4 days (EFSA Journal 2015;13(7):4201). Despite this, no measurement of thifensulfuron-methyl concentration in tested soils is also not an issue for DNT-162OD-R-CPd.

Earthworms

Starting from the longest study on earthworms, with 56 days of exposure, it can be clearly shown that nicosulfuron and dicamba are the toxicity drivers of product DNT-162OD-R-CPd to earthworms - NOT thifensulfuron-methyl. By comparison of toxicity units for all actives, as presented hereafter, it can be easily shown that nicosulfuron and dicamba are responsible for almost 100% (exactly 96%) effect, while thifensulfuron-methyl affects this toxicity in just 4%.

TU nicosulfuron = 40 g/L : 1000 mg/kg dws* = 0.04
TU dicamba = 110 g/L : 1000 mg/kg dws* = 0.11
TU thifensulfuron -methyl = 12 g/L : 2000 mg/kg dws = 0.006

% TUmix nicosulfuron = 0.04 : 0.156*100 % = 25%
% TU mix dicamba = 0.11 : 0.156* 100% = 71%
% TU mix thifensulfuron methyl = 0.006 : 0.156* 100% = 4%

* Endpoints (LC_{50} values for earthworms) taken from agreed LoEP for dicamba, nicosulfuron and thifensulfuron-methyl (EFSA Scientific Report (2007) 120, 1-91; EFSA Journal 2011;9(1):196; EFSA Journal 2015;13(7):4201)

Additionally, based on the lowest nominal endpoint for earthworms from the study on DNT-162OD-R-CPd, (report presented in the current dRR with a study code: G-24-23), corrected by EU approved DT_{50} soil value for thifensulfuron-methyl and the worst-case PECsoil value for this active presented in section B8 for DNT-1 DNT-162OD-R-CPd, it can be ensured that no risk is anticipated in relation to this active and earthworms. As presented below the expected TER for thifensulfuron-methyl would be far above the trigger value of 5.

$NOEC_{nominal}$ = 6.92 mg thifensulfuron-methyl/ kg dw soil
(based on nominal concentration from the study on DNT-162OD-R-CPd)

DT_{50} soil = 3.1 days (half- life relevant for concentration of thifensulfuron – methyl in soil – please refer to section B8 for DNT-162OD-R-CPd)

$NOEC_{TWA} = NOEC_{nominal} * (DT_{50\ soil} / (Study\ duration * \ln(2)) * (1 - \exp(-Study\ duration * \ln(2) / DT_{50\ soil})$

$NOEC_{TWA}$ for thifensulfuron-methyl= 0.55 mg/kg dws soil

PEC_{soil} = 0.0120 mg/kg dws soil (worst-case PEC_{soil} value for thifensulfuron-methyl, please refer to section B8 for DNT-162OD-R-CPd)

TER = 0.55/0.0120 = 46 (TER > 5, safe use anticipated)
Folsomia* and *Hypoaspis

There are no EU toxicity data of thifensulfuron-methyl for *Folsomia* or *Hypoaspis* and thus, approach with toxic units for DNT-162OD-R-CPd can not be for these species applied. However, as in the case of the earthworms, NOEC/EC_{10} TWA can be calculated for thifensulfuron-methyl, in a manner presented above.

In case of *Folsomi*a the following can be for thifensulfuron - methyl considered:

$NOEC_{nominal}$ = 0.12 mg thifensulfuron-methyl/kg dw soil
(based on nominal concentration from the study on DNT-162OD-R-CPd for *Folsomia*)

DT_{50} soil = 3.1 days
(half- life relevant for TWA concentration of thifensulfuron – methyl in soil – please refer to section B8 for DNT-162OD-R-CPd)

$NOEC_{TWA} = NOEC_{nominal} * (DT_{50\ soil} /(\text{Study duration} * \ln(2)) * (1 - \exp(-\text{Study duration}*\ln(2)/ DT_{50\ soil})$

$NOEC_{TWA}$ for thifensulfuron-methyl= 0.019 mg/kg dws soil

PECsoil = 0.0120 mg/kg dws soil (worst-case PECsoil value for thifensulfuron-methyl, please refer to section B8 for DNT-162OD-R-CPd)

TER = 0.019/0.0120 = 1.59

As presented above the provided approach is not sufficient to conclude on the safe risk for *Folsomia*. Nevertheless, as underlined already, content of thifensulfuron-methyl in product DNT-162OD-R-CPd is much lower than the other actives, and it is not expected to be the main driver of the product`s toxicity. As mentioned in paragraph on earthworms, dicamba and nicosulfuron are responsible for 96% toxicity of DNT-162OD-R-CPd, while thifensulfuron – methyl causes just 4% of the effect.
In addition, as thifensulfuron-methyl degradates very fast in soil, and as semi-static or flow-through testing is not possible in soil organisms studies, maintenance of its concentration as required in the guidelines might be not be an option. Degradation of a thifensulfuron-methyl during testing leads however to the formation degradation products (IN-L9223, IN-L9225 and IN-W8268, IN-A4098):

- which are stable in soil (their soil geometric mean DT_{90} values are, greater than the longest -56-d earthworm study; they are between 73.1 and 910 d; see EFSA Journal 2015;13(7):420),
- which toxicity to *Folsomia* is known (with NOEC between 0.045 and100 mg/kg dws, see EFSA Journal 2015;13(7):4201),
- and as presented in Section B9 of dRR for product DNT-162OD-R-CPd, no risk was for them indicated according to uses as intended. The achieved TER for all degradation products were higher than a trigger of 5. They were between 7 to even 50 000.

Consequently, a targeted risk assessment performed with the main emphasis on the degradation products of thifensulfuron-methyl is sufficient enough to concluded on the safe risk to *Folsomia*.

In case of *Hypoaspis* the following can be for thifensulfuron -methyl considered:

$NOEC_{nominal}$ = 12.36 mg thifensulfuron-methyl/ kg dw soil
(based on nominal concentration from the study on DNT-162OD-R-CPd for *Folsomia*)

DT_{50} soil = 3.1 days (half- life relevant for TWA concentration of thifensulfuron – methyl in soil – please refer to section B8 for DNT-162OD-R-CPd)

$NOEC_{TWA} = NOEC_{nominal} * (DT_{50\ soil} / (Study\ duration * \ln(2)) * (1 - \exp(-Study\ duration * \ln(2) / DT_{50\ soil})$

$NOEC_{TWA}$ for thifensulfuron-methyl= 3.78 mg/kg dws soil

PECsoil = 0.0120 mg/kg dws soil (worst-case PECsoil value for thifensulfuron-methyl, please refer to section B8 for DNT-162OD-R-CPd)

TER = 3.78/0.0120 = 315 (TER >5, safe use anticipated)

Therefore, as presented above, it can be ensured that there are no issues with risk related to thifensulfuron-methyl and predatory mites. Even, when rapid degradation of this molecule in the soil is considered, the expected TER for thifensulfuron-methyl is much higher than the trigger of 5.

Based on above it can be concluded that there is no need to monitor thifensulfuron-methyl concentration during the studies on Eisenia, *Folsomia* or *Hypoapsis* with product DNT-162OD-R-CPd. Even if degradation of thifensulfuron-methyl is considered during studies, there will be no unacceptable risk to the earthworms or the other soil macroorganisms. The TER values will be then much greater than 5.

Conclusions for DNT-162OD-R-CPd:

In case of all actives, present in product DNT-162OD-R-CPd, safe use can be demonstrated to earthworms, *Folsomia* and *Hypoaspis*. The presented data allow to conclude sufficiently on the acceptable risk for DNT-162OD-R-CPd.

zRMS comment: The information regarding the analytical measurements of active substances during the soil studies for formulation of EVRITELL 162 OD (DNT-162OD-R-CPd) with earthworms and *Folsomia candida* and *Hypoapsis aculeifer* was accepted by zRMS. No additional risk assessment for earthworms and other soil macroorganism is required.

It should be considered at MSs level.

9.9 Effects on soil microbial activity (KCP 10.5)

9.9.1 Toxicity data

Studies on effects soil microorganisms have been carried out with dicamba, nicosulfuron, thifensulfuron-methyl and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on soil microorganisms of EVRITELL 162 OD were not evaluated as part of the EU assessment of dicamba, nicosulfuron, thifensulfuron- methyl. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2. Only endpoints on N-mineralisation are relevant data requirements.
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 9.9-1: Endpoints and effect values relevant for the risk assessment for soil microorganisms

Endpoint	Substance	Exposure System	Results	Reference
N-mineralisation	dicamba	28 d	0 % effect atday 28 at **6.4** and 2.4 mg a.s./kg dw soil	EFSA Journal 2011;9(1):1965
N-mineralisation	Technical nicoslfuron	28 d	At 0.08 **& 0.8** mg a.s. /kg soil d.wt. < 25% deviation from control by study end	EFSA Scientific Report (2007) 120, 1-91
N-mineralisation	AUSN	28 d	**0.082 mg AUSN**/kg dry soil:. < 25% deviation from control by study end	EFSA Scientific Report (2007) 120, 1-91
	UCSN		**0.034mg UCSN**/kg dry soil:. < 25% deviation from control by study end	
	ASDM		**0.191mg** ASDM /kg dry soil:. < 25% deviation from control by study end	
N-mineralisation	Thifensulfuro-nmethyl	28 d	Rate at which there was less than 25% deviation from the control = **400 g/ha**	EFSA Journal 2015;13(7):4201
N-mineralisation	IN-A4098	28 d	Rate at which there was less than 25% deviation from the control = 0.125 mg/kg dw soil	EFSA Journal 2015;13(7):4201
N-mineralisation		42 d	Rate at which there was less than 25% deviation from the control = **0.204** mg/kg dw soil	EFSA Journal 2015;13(7):4201
N-mineralisation	IN-A5546	28 d	Rate at which there was less than 25% deviation from the control = **0.827 mg/kg dw soil**	EFSA Journal 2015;13(7):4201
N-mineralisation	IN-JZ789	28 d	51.6% deviation from the control at 0.1 mg/kg soil 48.8% deviation at 1.0 mg/kg soil (study not extended)	EFSA Journal 2015;13(7):4201
N-mineralisation	IN-L9223	28 d	Rate at which there was less than 25%	EFSA Journal 2015;13(7):4201

Endpoint	Substance	Exposure System	Results	Reference
			deviation from the control = **1.0 mg/kg dw soil**	
N-mineralisation		42 d	Rate at which there was less than 25% deviation from the control = 0.849 mg/kg dw soil	EFSA Journal 2015;13(7):4201
N-mineralisation	IN-L9225	28 d	Rate at which there was less than 25% deviation from the control = **0.42 mg/kg dw soil**	EFSA Journal 2015;13(7):4201
N-mineralisation		42 d	Rate at which there was less than 25% deviation from the control = 0.413 mg/kg dw soil	EFSA Journal 2015;13(7):4201
N-mineralisation	IN-L9226	28 d	Rate at which there was less than 25% deviation from the control = 0.39 mg/kg dw soil	EFSA Journal 2015;13(7):4201
N-mineralisation			Rate at which there was less than 25% deviation from the control = **0.827 mg/kg dw soil**	EFSA Journal 2015;13(7):4201
N-mineralisation	IN-V7160	28 d	Rate at which there was less than 25% deviation from the control **= 0.843 mg/kg dw soil**	EFSA Journal 2015;13(7):4201
N-mineralisation	IN-W8268	28 d	Rate at which there was less than 25% deviation from the control = 0.20 mg/kg dw soil	EFSA Journal 2015;13(7):4201
N-mineralisation		56 d	Rate at which there was less than 25% deviation from the control = **0.8 mg/kg dw soil**	EFSA Journal 2015;13(7):4201
N-mineralisation	2-acid-3-triuret	28 d	Rate at which there was less than 25% deviation from the control **= 1.0 mg/kg dw soil**	EFSA Journal 2015;13(7):4201

Endpoint	Substance	Exposure System	Results	Reference
N-mineralisation	EVRITELL 162 OD	42 d	Rate at which there was less than 25% deviation from the control = 1.35 mg/kg dw soil **and 6.74 mg/kg dw soil**	S. Sajdok-Czernecka, 2023, EMI/4/67/2022

9.9.1.1 Justification for new endpoints

No deviation from EU agreed endpoints.

9.9.2 Risk assessment

The evaluation of the risk for soil microorganisms was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev 2 (final), October 17, 2002).
The relevant PEC_{soil} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.2, Table 8.7-3 and were already used in the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) (see 9.8).

Table 9.9-2: Assessment of the risk for effects on soil micro-organisms due to the use of EVRITELL 162 OD in maize

Intended use			
N-mineralisation			
Product/active substance	**Max. conc. with effects ≤ 25 % (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**Risk acceptable?**
dicamba	6.4 (at 28 d)	0.110	yes
nicosulfuron	0.8	0.04	yes
UCSN	0.034	0.0061	yes
ASDM	0.191	0.0233	yes
AUSN	0.082	0.0119	yes
Thifensulfuron-methyl	400 g/ha	0.0120	yes
IN-A4098	0.204	0.0063	yes
IN-A5546	0.827	0.0020	yes
IN-L9223	1.0	0.0020	yes
IN-L9225	0.42	0.0135	yes
IN-L9226	0.827	0.0021	yes
IN-V7160	0.843	0.0008	yes
IN-W8268	0.8	0.0017	yes
2-acid-3-triuret (IN-U5F72)	1.0	0.0023	yes

EVRITELL 162 OD	6.74 (at 42 d)	1.02	yes

9.9.3 Overall conclusions

Risk assessments conducted with relevant PEC_{soil} for active substances dicamba, nicosulfuron, thifensulfuron-methyl and its relevant metabolites and in EVRITELL 162 OD indicate a low risk to soil microorganisms when applied to the proposed use rates.

zRMS comment: Agreed. The risk assessment for soil micro-organism after exposure of **EVRITELL 162 OD** has been accepted by the zRMS. The effects on the nitrogen transformations are acceptable (<25%) at concentration which is higher than the maximum relevant PECs for the maximum application rate of **EVRITELL 162 OD**. The results indicate no adverse effect on nitrogen transformation even at soil concentrations well higher than the ones expected following application of **EVRITELL 162 OD**.

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, nicosulfuron, thifensulfuron-methyl and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on non-target terrestrial plants of EVRITELL 162 OD were not evaluated as part of the EU assessment of dicamba, nicosulfuron, thifensulfuron-methyl. New data submitted with this application are listed in Appendix 1 summarised in Appendix 2.

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

Species	Substance	Exposure System	Results	Reference
Rice	SL-950 4% SC (nicosulfuron)	Post-emergence (Vegetative vigour)	ER_{50} = 0.47 g a.s./ha (based on % of plants showing visible adverse effects in glasshouse test)	EFSA Scientific Report (2007) 120, 1-91
Most sensitive species not ascertained (equivalent endpoint for six tested dicot / monocot crop species)		Pre-emergence	ER_{50} > 20 g a.s./ha (no adverse effects at 20 g a.s./ha)	
Beta vulgaris	Thifensulfuron methyl 50 SG	Vegetative vigour	ER_{50} = 0.606 g a.s./ha	EFSA Journal 2015;13(7):4201

Species	Substance	Exposure System	Results	Reference
Beta vulgaris	Thifensulfuron-methyl 50 SG	Seedling emergence	ER_{50} = 0.613 g a.s./ha	
Pea *(Pisum sativum)*	EVRITELL 162 OD	Seedling emergence	ER_{50} = 59.09 mL test item/ha	P. Pieczka, 2023, G-34-23
Oilseed rape *(Brassica napus)*	EVRITELL 162 OD	Vegetative vigour	ER_{50} = 27.85 mL test item/ha	P. Pieczka, 2023, G-33-23

m: monocotyledonous; d: dicotyledonous

Initial risk assessment is presented based on deterministic approach - the lowest ER_{50} for oilseed rape, which has been established estimated from the vegetative vigour study by Pieczka (2023).

In addition to the deterministic approach, a probabilistic data evaluation based on the individual effect data for vegetative vigour by Pieczka (2023) is also presented hereafter.
A Species Sensitivity Distribution (SSD) assessment based on the available ER_{50} estimates was performed by calculating normal distribution of the data sets and plotting 'Fraction affected' against "log10 Toxicity data" using ETX 2.3 software[1].

In accordance with the terrestrial guidance document (SANCO/10329/2002 rev.)[2], 'the risk for terrestrial plants is assumed to be acceptable', if 'the $ED[R]_{50}$ for less than 5% of the species is below the highest predicted exposure level' (i.e. TER trigger of 1 for acceptability of risk).

The following table presents the individual median effect rates (ER_{50}) for the most sensitive parameter – phytotoxicity- for EVRITELL 162 OD established in the vegetative vigour test. For more details reference is made to the study summary in Appendix 2.

Table 9.10-2: Median effect rates from the vegetative vigour study – phytotoxicity endpoint

Test plant		ER_{50} EVRITELL 162 OD (mL/ha)
Common name	**Scientific name (lat.)**	**Vegetative vigour test**
Pea	*Pisum sativum*	128.20
Lettuce	*Lactuca sativa*	80.01
Sugar beet	*Beta vulgaris*	112.32
Tomato	*Solanum lycopersicon*	42.01
Oilseed rape	***Brassica napus***	**27.85**
Carrot	*Daucus carota*	75.96
Cabbage	*Brassica olerace var. capitata*	153.19
Onion	*Allium cepa*	75.70
Perennial ryegrass	*Lolium perenne*	103.04

[1] ETX 2.3 – A Program to Calculate Hazardous Concentrations and Fraction Affected Based on Normally Distributed Toxicity Data, P.L.A. van Vlaardingen, T.P. Traas, A.M. Wintersen & T. Aldenberg, RIVM Report 601501028/2004

[2] European Commission. Health & Consumer Protection Directorat – General (2002). Draft Working Document. Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC. SANCO/10329/2002 rev.2

Oats	*Avena sativa*	410.92

Worst-case ER50 estimate is highlighted in **bold**

Thus, reliable ER_{50} values are available for all of the 10 tested plant species, without any clustering between mono- and dicotyledonous plants. According to GAP product EVRITELL 162 OD acts against both monocotyledonous plants and dicotyledonous plants. For that reason, endpoints from all tested species were used to derive the SSD value.

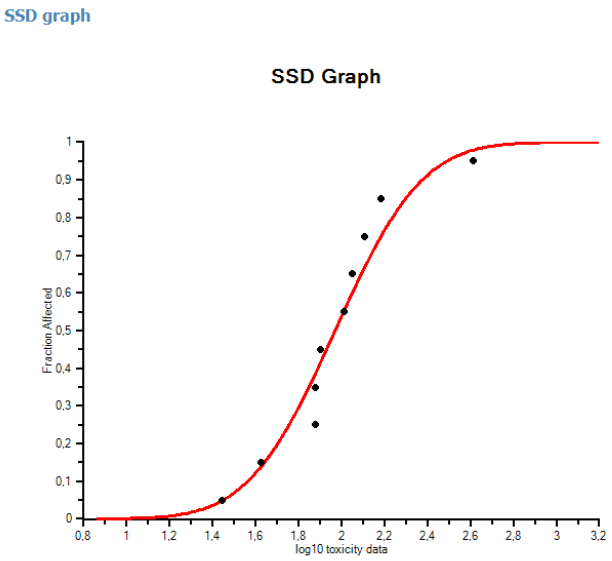
The results of the SSD and data distributions are presented in the following tables and graphs.

Table 9.10-3: SSD over ER_{50} from the relevant vegetative vigour data for EVRITELL 162 OD

Parameter:	**ER_{50} phytotoxicity (n = 10)**
Goodness of fit of toxicity data (normal distribution)	
Anderson-Darling test for normality	Accepted [a)]
Kolmogorov-Smirnov test for normality	Accepted [a)]
Cramer von Mises test for normality	Accepted [a)]
Median HC_5 mL/ha	26.95
95% confidence limits	11.15- 44.42

[a)] Acceptable normal distribution at all significance level

Thus, the data fulfil the criterion for normal distribution even at the lowest significance level and in accordance with all tests for normality.



Graph 1: SSD over ER_{50} from the relevant vegetative vigour data for EVRITELL 162 OD

Based on above and in addition to the deterministic approach with the overall lowest ER_{50}, the exposure estimates for EVRITELL 162 OD were further compared to the HC_5 of 26.95 mL product/ha. In this case an assessment factor of 1 was considered in the risk assessment.

zRMS comment: Accepted.

9.10.1.1 Justification for new endpoints

No deviation from EU agreed endpoints.

9.10.2 Risk assessment

9.10.2.1 Tier-1 risk assessment (based screening data)

Not relevant.

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

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

Table 9.10-2: Assessment of the risk for non-target plants due to the use of EVRITELL 162 od in maize

Intended use	maize			
Active substance/product	EVRITELL 162 OD			
Application rate (L/ha)	1 × 1			
MAF	1			
Test species	**ER_{50} (mL/ha)**	**Drift rate (%)**	**$PER_{off\text{-}field}$ (mL/ha)**	**TER criterion: TER ≥ 5**
Pea *(Pisum sativum)*	59.09	2.77	27.70	**2.13**
Oilseed rape *(Brassica napus)*	27.85			**1.01**
HC_5 (mL/ha)		**Drift rate (%)**	**$PER_{off\text{-}field}$ (mL/ha)**	**TER criterion: TER ≥ 1**
26.95		2.77	27.70	**0.97**

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

9.10.2.3 Higher-tier risk assessment

Not relevant.

9.10.2.4 Risk mitigation measures

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

Table 9.10-3: Risk assessment for non-target terrestrial plants due to the use of EVRITELL 162 OD in maize considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles)

Intended use		maize			
Active substance/product		EVRITELL 162 OD			
Application rate (L/ha)		1 × 1			
MAF		1			
Buffer strip (m)	**Drift rate (%)**	**$PER_{off-field}$ (mL/ha)**	**$PER_{off-field}$ 50 % drift red. (mL/ha)**	**$PER_{off-field}$ 75 % drift red. (mL/ha)**	**$PER_{off-field}$ 90 % drift red. (mL/ha)**
1	2.77	27.70	13.85	6.93	2.77
5	0.57	5.70	2.85	1.43	0.57
10	0.29	2.90	1.45	0.73	0.29
Toxicity value		**TER**			
ER_{50} = 27.85 mL/ha		**criterion: TER ≥ 5**			
1		**1.01**	**2.01**	**4.02**	10.05
5		**4.89**	9.77	19.54	-
10		9.60	-	-	-
Toxicity value		**TER**			
HC_5 = 26.55 mL/ha		**criterion: TER ≥ 1**			
1		**0.97**	1.95	-	-
5		2.84	-	-	-
10		-	-	-	-

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

9.10.3 Overall conclusions

For the proposed use of EVRITELL 162 OD, and depending on the approach considered, a safe risk for non-target plants is indicated when:

Deterministic approach: either 1 m buffer strip with 90% drift reduction or a 5 m buffer strip with 50% drift reduction, or 10 m buffer strip with no drift reduction is applied as risk mitigation measure.

Probabilistic approach: either 1 m buffer strip with 50% drift reduction or a 5 m buffer strip with no drift reduction is applied as risk mitigation measure.

reduction is applied as risk mitigation measure.

zRMS comment: Accepted.

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 risk indicated needs for further refinement.
For the proposed use of **EVRITELL 162 OD**, and depending on the approach considered, a safe risk for

non-target plants is indicated when:

Deterministic approach: either 1 m buffer strip with 90% drift reduction or a 5 m buffer strip with 50% drift reduction, or 10 m buffer strip with no drift reduction is applied as risk mitigation measure.

Probabilistic approach: either 1 m buffer strip with 50% drift reduction or a 5 m buffer strip with no drift reduction is applied as risk mitigation measure.

The risk assessment for non-target plants with considering the appropriate mitigation measures should be chosen by MSs considering their national conditions.

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

Additional tests on other non-target species are not required.

9.12 Monitoring data (KCP 10.8)

Not required.

9.13 Classification and Labelling

Formulation EVRITELL 162 OD was classified and labeled according to REGULATION (EC) No 1272/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006.

Ingredients classified as hazardous to the aquatic environment are:
- active substance DICAMBA in the form of sodium salt in a concentration of 12.2 % classified as Aquatic Chronic Category 3.,
- active substance Nicosulfuron a concentration of 4.1 % classified as Aquatic Chronic Category 1 and Aquatic Acute Category 1. Based on the lowest E_rC_{50} = 0.00139 mg a.s./L mm, multiplying factors of M=100 according to Table 4.1.3 was chosen and used in classification.
- active substance Thifensulfuron-methyl a concentration of 1.2 % classified as Aquatic Chronic Category 1 and Aquatic Acute Category 1. Based on the lowest ErC50 = 0.00023 mg a.s./L mm, multiplying factors of M=1000 according to Table 4.1.3 was chosen and used in classification.
- “Ingredient 2” in a concentration of 4.9 % classified as Aquatic Chronic Category 2.

According to point 4.1.3.1 of Regulation (EC) No 1272/2008 concerning the ‘relevant components’ “Ingredient 3” is not relevant components of a mixture. Therefore it is not taken into consideration in the classification of the product.
To see more details regarding confidential information please refer to Part C of dRR.
For classification of EVRITELL 162 OD summation method was used.

According to above information and Table 4.1.1 it was assumed that EVRITELL 162 OD is classified as Aquatic Acute Category 1 (concentration of Nicosulfuron and Thifensulfuron-methyl multiplied by its corresponding M-factor of 100 is equal 530 % thus it is higher value than 25 %).

According to above information and Table 4.1.2 it was assumed that EVRITELL 162 OD is classified

as Aquatic Chronic Category 1 (concentration of Nicosulfuron and Thifensulfuron-methyl by its corresponding M-factor of 1000 is equal 5300 % thus it is higher value than 25 %).

Summarizing formulation EVRITELL 162 OD should be classify and labelled as follows:

CLASSIFICATION	
Hazard class(es), categories:	Aquatic Acute 1, H400 Aquatic Chronic 1, H410
LABELLING	
Hazard pictograms:	 GHS09
Signal word:	Warning
Hazard statement(s):	H410 - Very toxic to aquatic life with long lasting effects.
Precautionary statement(s):	P273 - Avoid release to the environment. P391 - Collect spillage.
Other phrases:	EUH401 — To avoid risks to human health and the environment, comply with the instructions for use

zRMS comment: Accepted.

Appendix 1 Lists of data considered in support of the evaluation

Tables considered not relevant can be deleted as appropriate.

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

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
KCP 10.2.1/01	Kacperek-Karetta Z.	2023	DNT-162OD-R-CPd Navicula pelliculosa SAG 1050-3, Growth inhibition test W-11-23 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 10.2.1/02	Szlauer S.	2022	DNT-162OD-R-CPd Daphnia sp., Acute Immobilisation Test EMI/4/70/2022 Ecomelius Institute Sp. z o. o. GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 10.2.1/03	Szlauer S.	2023	DNT-162OD-R-CPd Freshwater Alga and Cyanobacteria, Growth Inhibition Test EMI/4/71/2022 Ecomelius Institute Sp. z o. o. GLP Unpublished	N	CIECH Sarzyna S.A.
KCP	Kacperek-Karetta Z.	2023	DNT-162OD-R-CPd Lemna gibba CPCC 310, Growth inhibition test	N	CIECH

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
10.2.1/04			W-12-23 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna GLP Unpublished		Sarzyna S.A.
KCP 10.2.1/05	Brzozowska – Wojoczek K.	2024	DNT-162OD-R-CPd Water-sediment Myriophyllum spicatum toxicity test ETOX-2024-39 EcoTox Alliance Sp. z o. o. GLP Unpublished	N	QEMETICA Agricultural Solutions Poland S.A.
KCP 10.3.1/01	Parma P.	2022	Honeybees (*Apis mellifera* L.), Acute oral toxicity test, DNT-162OD-R-CPd EMI/4/64/2022 Ecomelius Institute sp.z o.o. GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 10.3.1/02	Parma P.	2022	Honeybees (*Apis mellifera* L.), Acute contact toxicity test, DNT-162OD-R-CPd EMI/4/65/2022 Ecomelius Institute sp.z o.o. GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 10.3.1/03	Parma P.	2023	Honeybees (*Apis mellifera* L.),Chronic Oral Toxicity Test, DNT-162OD-R-CPd EMI/4/62/2022 Ecomelius Institute sp.z o.o. GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 10.3.1/04	Parma P.	2023	Honeybees (*Apis mellifera* L.), Larval Toxicity Test, Repeated Exposure, DNT-162OD-R-CPd EMI/4/63/2022 Ecomelius Institute sp.z o.o.	N	CIECH Sarzyna S.A.

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
			GLP Unpublished		
KCP 10.3.1/05	Wojciech A.	2023	DNT-162OD-R-CPd Bumblebees (*Bombus* spp.), Acute Oral Toxicity Test B-56-23 Łukasiewicz Research Network –Institute of Industrial Organic Chemistry, Branch Pszczyna GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 10.3.1/06	Wojciech A.	2023	DNT-162OD-R-CPd Bumblebees (Bombus spp.), Acute Contact Toxicity Test B-57-23 Łukasiewicz Research Network –Institute of Industrial Organic Chemistry, Branch Pszczyna GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 10.3.2/01	Kręglewska M.	2023	Extended laboratory test to evaluate effects on *Aphidius rhophalosiphi* (DeStephani-Perez) of the test item DNT-162OD-R-CPd according to IOBC, BART and EPPO Joint Initiative. M.P. Candolfi, et al. (2000) and Mead-Briggs M.A. et al. (2010) 0016/0178/E SORBOLAB Research Laboratory LLC GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 10.3.2/02	Kręglewska M.	2022	Extended laboratory test to evaluate effects on *Typhlodromus pyri* (Scheuten) of the test item DNT-162OD-R-CPd according to IOBC, BART and EPPO Joint Initiative, M.P. Candolfi, et al. (2000) and S. Blümel, et al. (2000) 0016/0175/E SORBOLAB Research Laboratory LLC GLP Unpublished	N	CIECH Sarzyna S.A.
KCP	Domagała J.	2023	Extended laboratory test to determine the effects of the test item DNT-162OD-R-CPd on the ladybird	N	CIECH

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Verte-brate study Y/N**	**Owner**
10.3.2/03			beetle (*Coccinella septempunctata*) 0016/0177/E SORBOLAB Research Laboratory LLC GLP Unpublished		Sarzyna S.A.
KCP 10.3.2/04	Kubisiak K.	2023	Extended laboratory test to determine the effects of the test item DNT-162OD-R-CPd on the green lacewing (*Chrysoperla carnea*) 0016/0176/E SORBOLAB Research Laboratory LLC GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 10.3.2/05	Domagała J.	2023	Aged-residue test to determine the effects of the test item DNT-162OD-R-CPd on the green lacewing (*Chrysoperla carnea*) according to IOBC, BART, EPPO Joint Initiative, Vogt et al., 2000 and ESCORT 2, Candolfi et al. 2001 0016/0224/E SORBOLAB Research Laboratory LLC GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 10.3.2/06	Domagała J.	2024	Aged-residue extended laboratory test to determine the effects of the test item DNT-162OD-R-CPd on the ladybird beetle (*Coccinella septempunctata*) according to IOBC, BART and EPPO Joint Initiative. Schmuck R. et al. (2000), ESCORT 2, Candolfi M.P. et al. (2001) 0016/0244/E SORBOLAB Research Laboratory LLC GLP Unpublished	N	QEMETICA Agricultural Solutions Poland S.A.*
KCP 10.4.1.1	Pieczka P.	2023	DNT-162OD-R-CPd Earthworm reproduction test (Eisenia andrei) G-24-23 Łukasiewicz Research Network –Institute of Industrial Organic Chemistry, Branch Pszczyna	N	CIECH Sarzyna S.A.

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Verte-brate study Y/N**	**Owner**
			GLP Unpublished		
KCP 10.4.2.1-01	Pieczka P.	2023	DNT-162OD-R-CPd Collembolan (Folsomia candida) Reproduction Test G-31-23 Łukasiewicz Research Network –Institute of Industrial Organic Chemistry, Branch Pszczyna GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 10.4.2.1-02	Pieczka P.	2023	DNT-162OD-R-CPd Predatory mite (Hypoaspis (Geolaelaps) aculeifer) reproduction test in soil G-32-23 Łukasiewicz Research Network –Institute of Industrial Organic Chemistry, Branch Pszczyna GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 10.5	Sajdok-Czernecka S.	2023	DNT-162OD-R-CPd Soil Microorganisms: Nitrogen Transformation Test EMI/4/67/2022 Ecomelius Institute Sp. z o. o. GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 10.6.2-01	P. Pieczka	2023	DNT-162OD-R-CPd Terrestial Plant Test: Seedling Emergence and Seedling Growth Test G-34-23 Łukasiewicz Research Network –Institute of Industrial Organic Chemistry, Branch Pszczyna GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 10.6.2-02	P. Pieczka	2023	DNT-162OD-R-CPd Terrestial Plant Test: Vegetative Vigour Test G-33-23 Łukasiewicz Research Network –Institute of Industrial Organic Chemistry, Branch Pszczyna GLP Unpublished	N	CIECH Sarzyna S.A.

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
*CIECH Sarzyna S.A. change its company name ito Qemetica Agricultural Solutions Poland S.A.					

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	Verte-brate study Y/N	Owner

The following tables are to be completed by MS

List of data submitted by the applicant and not relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
-	-	-	-	-	-

List of data relied on not submitted by the applicant but necessary for evaluation

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
-	-	-	-	-	-

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 10.1.1.2 Higher tier data on birds

A 2.1.2 KCP 10.1.2 Effects on terrestrial vertebrates other than birds

A 2.1.2.1 KCP 10.1.2.1 Acute oral toxicity to mammals

A 2.1.2.2 KCP 10.1.2.2 Higher tier data on mammals

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

A 2.2 KCP 10.2 Effects on aquatic organisms

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

Study 1

Comments of zRMS:	The study is acceptable. The validity criteria according OECD 201 of the test were met. Validity criteria: - the biomass in the control increased by a factor of 29.6 within the 72-hour test period (criterion: at least a 16-fold growth), - the coefficient of variation of the mean specific growth rate after the 72-hour test period (exposure initiation – exposure termination) in the control culture was 4.7% (criterion: it must not exceed 10%), - the mean coefficient of variation for the section-by-section growth rate in the control culture was 34.2% (criterion: it must not exceed 35.0%). Deviation of the study: none **Agreed toxicity endpoints:**

	The endpoint values based on nominal test item concentrations are given below: The E_rC_{50}/72 h value is 42.80 mg/L (95% confidence interval: 41.17 – 45.13). The LOEC/72 h value for growth rate is 40.0 mg/L. The NOEC/72 h value for growth rate is 20.0 mg/L. The E_yC_{50}/72 h value is 28.09 mg/L (95% confidence interval: 26.07 – 30.29). The LOEC/72 h value for yield is 40.0 mg/L. The NOEC/72 h value for yield is 20.0 mg/L. The endpoint values based on nominal concentrations of dicamba are given below: The E_rC_{50}/72 h value is 4.78 mg/L (95% confidence interval: 4.60 – 5.04). The LOEC/72 h value for growth rate is 4.47 mg/L. The NOEC/72 h value for growth rate is 2.23 mg/L. The E_yC_{50}/72 h value is 3.14 mg/L (95% confidence interval: 2.91 – 3.38). The LOEC/72 h value for yield is 4.47 mg/L. The NOEC/72 h value for yield is 2.23 mg/L. The endpoint values based on nominal concentrations of nicosulfuron are given below: The E_rC_{50}/72 h value is 1.66 mg/L (95% confidence interval: 1.60 – 1.76). The LOEC/72 h value for growth rate is 1.56 mg/L. The NOEC/72 h value for growth rate is 0.78 mg/L. The E_yC_{50}/72 h value is 1.09 mg/L (95% confidence interval: 1.01 – 1.18). The LOEC/72 h value for yield is 1.56 mg/L. The NOEC/72 h value for yield is 0.78 mg/L. The endpoint values based on the geometric means of determined concentrations of thifensulfuron-methyl are given below: The E_rC_{50}/72 h value is 0.52 mg/L (95% confidence interval: n.d. – n.d.). The LOEC/72 h value for growth rate is 0.50 mg/L. The NOEC/72 h value for growth rate is 0.25 mg/L. The E_yC_{50}/72 h value is 0.35 mg/L (95% confidence interval: 0.32 – 0.37). The LOEC/72 h value for yield is 0.50 mg/L. The NOEC/72 h value for yield is 0.25 mg/L.

Reference:	KCP 10.2.1/01
Report	DNT-162OD-R-CPd, Navicula pelliculosa SAG 1050-3, Growth inhibition test, Zuzanna Kacperek-Karetta, MSc, STUDY CODE: W-11-23, 2023 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna Ecotoxicology Research Group, Poland
Guideline(s):	Yes. According to the OECD Guideline No. 201 (2004)/EU method C.2.
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Test item:	DNT-162OD-R-CPd; batch no. 1/23, the content of dicamba: 112 g/L, nicosulfuron 39 g/L, thifensulfuron-methyl 12.4 g/L density: 1.003 g/cm3; manufacturing date: February 09, 2023, expiry date: February 09, 2025.
Test system:	The freshwater diatoms, *Navicula pelliculosa* SAG 1050-3 cultivated at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna, Ecotoxicology Research Group, Laboratory of Aquatic Organisms Toxicology. The diatoms were obtained from the Culture Collection of Algae at Göttingen University, Germany.
Test design:	72 hours of exposure; three replicates per each test item concentration; six replicates per control; initial diatoms cell density: 1 x 10^4 cells/mL.
Test conditions:	- temperature: 22.1 – 22.5°C - ph of the control: 7.56 – 7.61 - mean light intensiy: 6195 – 6420 lux
Tested concentrations:	80, 40, 20, 10 and 5 mg/L plus the control.
Nominal concentration of dicamba	8.94, 4.47, 2.23, 1.12 and 0.56 mg/L plus the control.
Nominal concentration of nicosulfuron:	3.11, 1.56, 0.78, 0.39 and 0.19 mg/L plus the control.
Geometric means of determined concentrations of thifensulfuron-methyl:	0.89, 0.50, 0.25, 0.12 and 0.06 mg/L plus the control.
Study duration:	72 h
Endpoints:	E_rC_{50}/72 h, E_rC_{20}/72 h, E_rC_{10}/72 h, E_yC_{50}/72 h, E_yC_{20}/72 h, E_yC_{10}/72 h, NOEC/72 h, LOEC/72 h.

The aim of the study was to determine the test item concentrations causing 50% inhibition of growth rate and yield of the diatoms, *Navicula pelliculosa* SAG 1050 -3 (E_rC_{50} E_rC_{20}, E_rC_{10} and E_yC_{50}, E_yC_{20}, E_yC_{10} after 72 hours of exposure, respectively). The LOEC and NOEC values were also determined.

Results and discussions

The effect of the test item on diatoms growth was assessed. The range of the test item concentrations used in the definitive test were determined on the basis of the preliminary tests results. The growth inhibition was estimated on the basis of the density of the diatoms cells determined in the definitive test.

Validity criteria:
The results are considered valid because the following criteria were met according to OECD Guideline No. 2 01 (2006) and EU Method C.3 were met:
- the biomass in the control increased by a factor of 29.6 within the 72-hour test period (criterion: at least a 16-fold growth),
- the coefficient of variation of the mean specific growth rate after the 72-hour test period (exposure initiation – exposure termination) in the control culture was 4.7% (criterion: it must not exceed 10%),
- the mean coefficient of variation for the section-by-section growth rate in the control culture was 34.2% (criterion: it must not exceed 35.0%).

Conclusion:
The endpoint values based on nominal test item concentrations are given below:

The E_rC_{50}/72 h value is 42.80 mg/L (95% confidence interval: 41.17 – 45.13).
The LOEC/72 h value for growth rate is 40.0 mg/L.
The NOEC/72 h value for growth rate is 20.0 mg/L.
The E_yC_{50}/72 h value is 28.09 mg/L (95% confidence interval: 26.07 – 30.29).
The LOEC/72 h value for yield is 40.0 mg/L.
The NOEC/72 h value for yield is 20.0 mg/L.

The endpoint values based on nominal concentrations of dicamba are given below:

The E_rC_{50}/72 h value is 4.78 mg/L (95% confidence interval: 4.60 – 5.04).
The LOEC/72 h value for growth rate is 4.47 mg/L.
The NOEC/72 h value for growth rate is 2.23 mg/L.
The E_yC_{50}/72 h value is 3.14 mg/L (95% confidence interval: 2.91 – 3.38).
The LOEC/72 h value for yield is 4.47 mg/L.
The NOEC/72 h value for yield is 2.23 mg/L.

The endpoint values based on nominal concentrations of nicosulfuron are given below:

The E_rC_{50}/72 h value is 1.66 mg/L (95% confidence interval: 1.60 – 1.76).
The LOEC/72 h value for growth rate is 1.56 mg/L.
The NOEC/72 h value for growth rate is 0.78 mg/L.
The E_yC_{50}/72 h value is 1.09 mg/L (95% confidence interval: 1.01 – 1.18).
The LOEC/72 h value for yield is 1.56 mg/L.
The NOEC/72 h value for yield is 0.78 mg/L.

The endpoint values based on the geometric means of determined concentrations of thifensulfuron-methyl are given below:

The E_rC_{50}/72 h value is 0.52 mg/L (95% confidence interval: n.d. – n.d.).
The LOEC/72 h value for growth rate is 0.50 mg/L.
The NOEC/72 h value for growth rate is 0.25 mg/L.
The E_yC_{50}/72 h value is 0.35 mg/L (95% confidence interval: 0.32 – 0.37).
The LOEC/72 h value for yield is 0.50 mg/L.
The NOEC/72 h value for yield is 0.25 mg/L.

Study 2

Comments of zRMS:	The study is acceptable. The validity criteria according OECD 202 of the test were met. Validity criteria:

- In the control 0% of daphnids were immobilized (criterium: not more than 10%);
- The concentration of dissolved oxygen in the test and control vessels was ≥ 3 mg/L at the end of the test.

Deviation of the study: during the study, the temperature should be within the range of 18°C and 22°C, and for each single test it should be constant within ±1°C. In this test, assumed temperature should be in the range 19.5±1°C (18.5°C – 20.5°C). Temperature fluctuations did not affect the test results. It was maximum a 0.6°C from the lower limit (18.5°C) and the validity criteria were met..

Agreed toxicity endpoints:

Calculations made on the basis of the nominal test item concentrations [mg/L] with 95% confidence limits.

Endpoint		Endpoint value [mg/L] after 24 h	Endpoint value [mg/L] after 48 h
EC_{10}	Test item	349.783 (199.146 – 489.686)	133.663 (76.842 – 177.417)
	Dicamba	39.787 (22.653 – 55.701)	15.204 (8.741 – 20.181)
	Nicosulfuron	13.632 (7.761 – 19.084)	5.209 (2.995 – 6.914)
	Thifensulfuron-methyl	4.162 (2.370 – 5.827)	1.590 (0.914 – 2.111)
EC_{20}	Test item	549.040 (365.040 – 711.530)	182.630 (122.763 – 227.118)
	Dicamba	62.453 (41.609 – 80.936)	20.774 (13.964 – 25.834)
	Nicosulfuron	21.397 (14.256 – 27.729)	7.117 (4.784 – 8.851)
	Thifensulfuron-methyl	6.533 (4.353 – 8.467)	2.173 (1.461 – 2.703)
EC_{50}	Test item	1084.786 (862.895 – 1328.659)	292.631 (237.717 – 345.600)
	Dicamba	123.393 (98.153 – 151.133)	33.286 (27.040 – 39.312)
	Nicosulfuron	42.276 (33.628 – 51.780)	11.404 (9.264 – 13.468)
	Thifensulfuron-methyl	12.908 (10.268 – 15.810)	3.482 (2.829 – 4.112)
LOEC	Test item	320.000	180.000
	Dicamba	36.400	20.470
	Nicosulfuron	12.471	7.010
	Thifensulfuron-methyl	3.808	2.140
NOEC	Test item	180.000	100.000
	Dicamba	20.475	11.370
	Nicosulfuron	7.015	3.900
	Thifensulfuron-methyl	2.142	1.190

Reference: KCP 10.2.1/02

Report: DNT-162OD-R-CPd, Daphnia sp., Acute Immobilisation Test, Sonia Szlauer, MSc, STUDY CODE: EMI/4/70/2022, Ecomelius Institute sp. z o. o. 2022

Guideline(s):	Yes. According to the OECD Guideline No. 202 (2004)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

For analytical method validation see selection B5 of dRR

Materials and methods

Materials and methods

Test item:	DNT-162OD-R-CPd; batch no. 13/22, the content of dicamba: 115 g/L, nicosulfuron 39.40 g/L, thifensulfuron-methyl 12.03 g/L density: 1.011 g/cm3; manufacturing date: July 13, 2022, expiry date: July 13, 2024.
Test system:	*Daphnia magna* cultivated at the Test Facility. The original source of the test system: Toxkit ephippia, Microbiotest, Kleimoer 15, 9030 Gent, Belgium. At the start of the test, the animals were less than 24 hours old and, to reduce variability, they were not first brood progeny
Test design:	48 hours of exposure; four replicates per each test item concentration; four replicates per control;
Test conditions:	- temperature: 17.9 – 20.4°C - ph of the control: 7.56 – 7.61 - average light intensity: 1408.35 lux
Tested concentrations:	100.0; 180.0; 320.0; 560.0; 1000.0; 1800.0; 3200.0 mg/L plus the control.
Test type:	Static test
Study duration:	48 h
Endpoints:	EC_{50}/48 h, EC_{20}/48 h, EC_{10}/48 h, NOEC/48 h, LOEC/48 h.

The study aimed at detecting adverse effects of the test item, DNT-162OD-R-CPd towards daphnids during 48 h of exposure.

Results and discussions
The immobility of Daphnia magna was assessed. The range of the test item concentrations used in the definitive test were determined on the basis of the preliminary tests results. Observations were carried out daily. Each test vessel was checked for immobilized daphnids at 24 and 48 hours after the beginning of the test. In addition to immobility, any abnormal behaviour or appearance were noted.

Validity criteria:
The results are considered valid because the following criteria were met according to OECD Guideline No. 202 (2004) were met:
- In the control 0% of daphnids were immobilized (criterium: not more than 10%),
- The concentration of dissolved oxygen in the test and control vessels was ≥ 3 mg/L at the end of the test.

RESULTS AND DISCUSSIONS

A. IMMOBILISATION

The percentage of immobilisation was calculated with the following formula:
%I = (number of immobilised organisms / number of exposed organisms)×100

Code	Nominal test item conc. (µg/L)	Number of exposed daphnids	Response at 24 h		Response at 48 h	
			Number of immobilised	%I	Number of immobilised	%I
CTRL	0.00	20	0	0.00	0	0.00
R1	100.0	20	0	0.00	0	0.00
R2	180.0	20	1	5	5	25
R3	320.0	20	3	15	12	60
R4	560.0	20	6	30	19	95
R5	1000.0	20	4	20	20	100
R6	1800.0	20	17	85	20	100
R7	3200.0	20	20	100	20	100

B. OBSERVATIONS

After 24 hours:
- In the control and test item concentrations R1 (100.0 mg/L), R2 (180.0 mg/L) and R3 (320.0 mg/L) daphnids had normal behavior and appearance.
- In the test item concentrations R4 (560.0 mg/L) and R5 (1800.0 mg/L) daphnids were whiter and slower.
- In the test item concentration R6 (1800.0 mg/L) and R7 (3200.0 mg/L) daphnids were white.

After 48 hours:
- In the control and test item concentrations R1 (100.0 mg/L), R2 (180.0 mg/L) and R3 (320.0 mg/L) daphnids had normal behavior and appearance.
- In the test item concentrations R4 (560.0 mg/L), R5 (1800.0 mg/L), R6 (1800.0 mg/L) and R7 (3200.0 mg/L) daphnids were white

C. ANALYTICAL DETERMINATIONS

The determination of the content of active ingredients:
Dicamba was maintained between 81.8% and 90.8% at the test initiation and between 84.2% and 104.0% at the test termination.
Nicosulfuron was maintained between 101.0% and 113.2% at the test initiation and between 92.0% and 112.0% at the test termination
Thifensulfuron methyl was maintained between 93.1% and 116.5% at the test initiation and between 97.8% and 118.2% at the test termination

III. CONCLUSIONS

The organisms were exposed to seven concentrations of test item DNT-162OD-R-CPd for 48 hours, under static conditions.

<u>The endpoint values based on nominal test item concentrations after 24h are given below:</u>

The EC_{50}/24 h value is 1084.786 mg/L (95% confidence interval: (862.895 – 1328.659).
The EC_{20}/24 h value is 549.040 mg/L (95% confidence interval: 365.040 – 711.530).
The EC_{10}/24 h value is 349.783 mg/L (95% confidence interval: 199.146 – 489.686).
The LOEC/24 h value for growth rate is 320.0 mg/L.
The NOEC/24 h value for growth rate is 180.0 mg/L.

<u>The endpoint values based on nominal test item concentrations after 48h are given below:</u>

The EC_{50}/48 h value is 292.631 mg/L (95% confidence interval: ((237.717 – 345.600).
The EC_{20}/48 h value is 182.630 mg/L (95% confidence interval: (122.763 – 227.118).
The EC_{10}/48 h value is 133.663 mg/L (95% confidence interval: 237.717 – 345.600).
The LOEC/48 h value for growth rate is 180.0 mg/L.
The NOEC/48 h value for growth rate is 100.0 mg/L.

Comments of zRMS:	The study is acceptable. The validity criteria according OECD 201 of the test were met. Validity criteria: - The biomass in the control increased by a factor of 130.3 within the 72–hour test period (criterion: at least a 16–fold growth). - The mean coefficient of variation for the section–by–section growth rate in the control culture was 9.0% (criterion: it must not exceed 35%). - The coefficient of variation of the mean specific growth rate after the 72–hour test period (exposure initiation – exposure termination) in the control culture was 1.2% (criterion: it must not exceed 7%). Deviation of the study: the temperature will be constant at 21°C to 24°C, controlled at ±2°C, by means of a phytotrone chamber. Instead of it, the temperature was controlled by the temperature recorder. Temperature in the experiment was in the range 23.4°C to 25.7°C. Temperature above 25.4°C was in 30.11.2022 at 15:26,01.12.2022 at 03:19 – 03:41 and 02.12.2022 at 03:34. Highest temperature was 25.7°C. Above deviations did not affect the study results, because all validity criteria were met. Agreed toxicity endpoints:

Calculations made on the basis of the nominal test item concentrations [mg/L] at the end of the experiment (72 h) with 95% confidence limits

Endpoint	mg/L	Growth rate	Yield
EC_{10}	DNT–162OD–R–CP–d	2.066 (1.611 – 2.505)	0.847 (0.729 – 0.958)
	Dicamba	0.235 (0.183 – 0.285)	0.096 (0.083 – 0.109)
	Nicosulfuron	0.081 (0.063 – 0.098)	0.033 (0.028 – 0.037)
	Thifensulfuron–methyl	0.025 (0.019 – 0.030)	0.010 (0.009 – 0.011)
EC_{20}	DNT–162OD–R–CP–d	3.264 (2.717 – 3.782)	1.209 (1.081 – 1.328)
	Dicamba	0.371 (0.309 – 0.430)	0.138 (0.123 – 0.151)
	Nicosulfuron	0.127 (0.106 – 0.147)	0.047 (0.042 – 0.052)
	Thifensulfuron–methyl	0.039 (0.032 – 0.045)	0.014 (0.013 – 0.016)
EC_{50}	DNT–162OD–R–CP–d	7.836 (7.048 – 8.713)	2.390 (2.239 – 2.552)
	Dicamba	0.891 (0.802 – 0.991)	0.272 (0.255 – 0.290)
	Nicosulfuron	0.305 (0.275 – 0.340)	0.093 (0.087 – 0.099)
	Thifensulfuron–methyl	0.093 (0.084 – 0.104)	0.028 (0.027 – 0.030)
NOEC	DNT–162OD–R–CP–d	≥56.0	≥56.0
	Dicamba	n.d	n.d
	Nicosulfuron	n.d	n.d
	Thifensulfuron–methyl	n.d	n.d
LOEC	DNT–162OD–R–CP–d	>56.0	>56.0
	Dicamba	n.d	n.d
	Nicosulfuron	n.d	n.d
	Thifensulfuron–methyl	n.d	n.d

n.d – not determined

Reference:	KCP 10.2.1
Report	DNT–162OD–R–CPd – *Freshwater Alga and Cyanobacetria*, Growth Inhibition Test, Sonia Szlauer, 2022, STUDY CODE: EMI/4/71/2022, Ecomelius Institute sp. z o. o.
Guideline(s):	Yes. According to the OECD Guideline No. 201 (2011f)
GLP:	Yes
Acceptability:	Yes

*

For analytical method validation see section B5 of dRR

Materials and methods

A. MATERIALS

1. Test material

Lot/Batch #	13/22
Appearance:	White to beige viscous liquid

Content of active substance	Dicamba: 115 g/L, Nicosulfuron: 39.40 g/L, Thifensulfuron–methyl: 12.03 g/L
Density:	1.011 g/mL
Expiry date	13 July 2024

2. Test organism

Species	Green alga *Raphidocelis subcapitata*
Source	Georg-August-Universität Göttingen Stiftungöffentlichen Rechts
Culturing	At 24°C ± 2°C under constant illumination (4400 – 8800 lux) in AAP medium.
Acclimation period	Culturing was done under test conditions.
Test units	100 mL conical glass flasks

3. Environmental conditions

Test water

Water temperature	Temperature in incubator was maintained between: 23.4 and 25.7°C, not varied more than ±2°C
Lighting	5945.5 – 6029 lux (not varied more than 15 %)

B. STUDY DESIGNS AND METHODS

1. Experimental conditions

Test design

The impact of DNT–162OD–R–CPd on Green freshwater alga species *Raphidocelis subcapitata* was investigated during a 72-hour toxicity study. There were prepared 12 solutions and 3 replicates for each concentrations. The control samples were prepared in 6 test vessels. Algae from the culture were added to vessels in quantity of 1×10^4 cells/mL. During the test, the cell concentrations were determined after 24, 48 and 72 h in samples taken directly form the test vessels.

Concentrations tested

Twelve exposure concentrations of 0.1; 0.18; 0.32; 0.56; 1.0; 1.8; 3.2; 5.6; 10.0; 18.0; 32.0 and 56.0 mg/L in three replicates and one control in six replicates.

Analytics

The aim of the analytical part of the experiment was to determine the concentrations of the test item with a liquid chromatographic method with mass spectroscopy. The analytical method was validated according to SANTE/2020/12830, Rev. 1

2. Sampling and measurements

The pH was measured on days 0 and 3 in each concentration and control.
The light intensity was measured on days 0 and 3, in different parts of the chamber.
Morphology observations of algae cells were performed at each day of experiment. Any changes in the appearance of the concentrations or morphological changes in the cells of the organisms tested were noticed

3. Calculation of toxicity

The test endpoint is inhibition of growth, expressed as the logarithmic increase in biomass (average specific growth rate) during the exposure period. From the average specific growth rates recorded in a series of test solutions, the concentration bringing about a specified x% inhibition of growth rate (e.g. 50%) is determined and expressed as the E_rC_x (e.g. E_rC_{50}), the toxicity of the test item from algae causing 10% and 20% inhibition of growth rate is determined as the E_rC_{10} and E_rC_{20}. The 50% inhibition of yield is determined and expressed as the E_YC_{50} of an algae culture in relation to the control. The ToxRat Professional commercial software was used to make calculations and to conduct statistical analyses.

Results and discussions

A. ANALYTICAL RESULTS

The aim of the analytical part of the experiment was to determine the concentrations of the test item with a liquid chromatographic method with mass spectroscopy.
The analytical method has been validated during the study. A Standard Operating Procedure for the analytical test method has been developed based on the validation carried out during the study. The determination of concentration was performed by the analytical personnel.
Based on the chemical determination it can be concluded, that the concentrations of the test item were prepared correctly (mean recovery at the beginning of the experiment was in the range: Dicamba – 98.8% – 117.6%; Nicosulfuron – 87.8% – 109.1%; Thifensulfuron–methyl – 95.9% – 113.3%). At the end of the experiment mean recovery was in the range: Dicamba – 94.9% – 112.3%; Nicosulfuron – 86.2% – 106.9%; Thifensulfuron–methyl – 92.3% – 116.9%

B. BIOLOGICAL RESULTS

Calculations made on the basis of the geometric mean test item concentrations [mg/L] at the end of the experiment.

Endpoint	mg/L	Growth rate	Yield
EC_{50}	DNT–162OD–R–CP–d	7.836 (7.048 – 8.713)	2.390 (2.239 – 2.552)
	Dicamba	0.891 (0.802 – 0.991)	0.272 (0.255 – 0.290)
	Nicosulfuron	0.305 (0.275 – 0.340)	0.093 (0.087 – 0.099)
	Thifensulfuron–methyl	0.093 (0.084 – 0.104)	0.028 (0.027 – 0.030)
NOEC	DNT–162OD–R–CP–d	≥56.0	≥56.0
	Dicamba	n.d	n.d
	Nicosulfuron	n.d	n.d
	Thifensulfuron–methyl	n.d	n.d
LOEC	DNT–162OD–R–CP–d	>56.0	>56.0
	Dicamba	n.d	n.d
	Nicosulfuron	n.d	n.d
	Thifensulfuron–methyl	n.d	n.d

C. VALIDITY CRITERIA

The following validity criteria specified in OECD Guideline No. 201 (2006) were met:

- the biomass in the control increased by a factor of 130.3 within the 72-hour test period (criterion: at least a 16-fold growth).
- the coefficient of variation of the mean specific growth rate after the 72-hour test period (exposure initiation - exposure termination) in the control culture was 1.2% (criterion: it must not exceed 7%).
- the mean coefficient of variation for the section-by-section growth rate in the control culture was 9.00% (criterion: it must not exceed 35%).

Comments of zRMS:	The study is acceptable. The validity criteria according OECD 221 of the test were met. Validity criteria: - the doubling time of frond number in the control was 1.9 days, criterion: less than 2.5 days (the factor of frond number in the control between 0 and 7 day was 12.2), - the average specific growth rate in the control between day 0 and day 7 was 0.357 d^{-1} (minimum requirement: higher than 0.275 d^{-1}). Deviation of the study: none. **Agreed toxicity endpoints:** The endpoint values based on the nominal test item concentrations: *Endpoints based on the frond number:* The E_rC_{50}/7 d value is 0.155 mg/L (95% confidence interval 0.107 – 0.222). The E_rC_{20}/7 d value is 0.018 mg/L (95% confidence interval 0.009 – 0.029). The E_rC_{10}/7 d value is 0.006 mg/L (95% confidence interval <0.006 – 0.011). The E_YC_{50}/7 d value is 0.037 mg/L (95% confidence interval 0.031 – 0.045). The E_YC_{20}/7 d value is 0.014 mg/L (95% confidence interval 0.010 – 0.017). The E_YC_{10}/7 d value is 0.008 mg/L (95% confidence interval <0.006 – 0.011). For growth rate and yield, the NOEC/7 d values are 0.006 mg/L, whereas the LOEC/7 d values are 0.019 mg/L. *Endpoints based on the dry weight:* The E_rC_{50}/7 d value is 1.663 mg/L (95% confidence interval 1.071 – 2.728). The E_rC_{20}/7 d value is 0.057 mg/L (95% confidence interval 0.024 – 0.106). The E_rC_{10}/7 d value is 0.010 mg/L (95% confidence interval <0.006 – 0.023). For growth rate, the NOEC/7 d value is 0.006 mg/L, whereas the LOEC/7 d value is 0.019 mg/L The E_YC_{50}/7 d value is 0.083 mg/L (95% confidence interval 0.054 – 0.125). The E_YC_{20}/7 d value is <0.006 mg/L (95% confidence interval <0.006 – 0.007). The E_YC_{10}/7 d value was not determined. For yield, the NOEC/7 d value is <0.006 mg/L, whereas the LOEC/7 d value is ≤0.006 mg/L. The endpoint values based on the nominal concentrations of dicamba: *Endpoints based on the frond number:* The E_rC_{50}/7 d value is 17.265 µg/L (95% confidence interval 11.945 – 24.818). The E_rC_{20}/7 d value is 1.984 µg/L (95% confidence interval 0.979 – 3.289). The E_rC_{10}/7 d value is <0.670 µg/L (95% confidence interval <0.670 – 1.244). The E_YC_{50}/7 d value is 4.176 µg/L (95% confidence interval 3.496 – 4.987). The E_YC_{20}/7 d value is 1.533 µg/L (95% confidence interval 1.106 – 1.941). The E_YC_{10}/7 d value is 0.908 µg/L (95% confidence interval <0.670 – 1.234). For growth rate and yield, the NOEC/7 d values are 0.670 µg/L, whereas LOEC/7 d values are 2.120 µg/L.

Endpoints based on the dry weight:
The E_rC_{50}/7 d value is 185.669 µg/L (95% confidence interval 119.541 – 304.585).
The E_rC_{20}/7 d value is 6.406 µg/L (95% confidence interval 2.721 – 11.874).
The E_rC_{10}/7 d value is 1.102 µg/L (95% confidence interval <0.670 – 2.613).
For growth rate, the NOEC/7 d value is 0.670 µg/L, whereas LOEC/7 d value is 2.120 µg/L.

The E_yC_{50}/7 d value is 9.315 µg/L (95% confidence interval 5.990 – 13.957).
The E_yC_{20}/7 d value is <0.670 µg/L (95% confidence interval <0.670 – 0.801).
The E_yC_{10}/7 d value was not determined.
For yield, the NOEC/7 d value is <0.670 µg/L, whereas LOEC/7 d value is ≤0.670 µg/L.

The endpoint values based on the nominal concentrations of nicosulfuron:
Endpoints based on the frond number:
The E_rC_{50}/7 d value is 6.011 µg/L (95% confidence interval 4.159 – 8.641).
The E_rC_{20}/7 d value is 0.691 µg/L (95% confidence interval 0.341 – 1.145).
The E_rC_{10}/7 d value is <0.233 µg/L (95% confidence interval <0.233 – 0.433).

The E_yC_{50}/7 d value is 1.454 µg/L (95% confidence interval 1.217 – 1.736).
The E_yC_{20}/7 d value is 0.534 µg/L (95% confidence interval 0.385 – 0.676).
The E_yC_{10}/7 d value is 0.316 µg/L (95% confidence interval <0.233 – 0.430).
For growth rate and yield, the NOEC/7 d values are 0.233 µg/L, whereas LOEC/7 d values are 0.739 µg/L.

Endpoints based on the dry weight:
The E_rC_{50}/7 d value is 64.667 µg/L (95% confidence interval 41.639 – 106.071).
The E_rC_{20}/7 d value is 2.230 µg/L (95% confidence interval 0.948 – 4.133).
The E_rC_{10}/7 d value is 0.384 µg/L (95% confidence interval <0.233 – 0.909).
For growth rate, the NOEC/7 d value is 0.233 µg/L, whereas LOEC/7 d value is 0.739 µg/L.

The E_yC_{50}/7 d value is 3.243 µg/L (95% confidence interval 2.086 – 4.859).
The E_yC_{20}/7 d value is <0.233 µg/L (95% confidence interval <0.233 – 0.279).
The E_yC_{10}/7 d value was not determined.
For yield, the NOEC/7 d value is <0.233 µg/L, whereas LOEC/7 d value is ≤0.233 µg/L.

The endpoint values based on the nominal concentrations of thifensulfuron-methyl:
Endpoints based on the frond number:
The E_rC_{50}/7 d value is 1.918 µg/L (95% confidence interval 1.327 – 2.757).
The E_rC_{20}/7 d value is 0.220 µg/L (95% confidence interval 0.109 – 0.365).
The E_rC_{10}/7 d value is <0.074 µg/L (95% confidence interval <0.074 – 0.138).

The E_yC_{50}/7 d value is 0.464 µg/L (95% confidence interval 0.388 – 0.554).
The E_yC_{20}/7 d value is 0.170 µg/L (95% confidence interval 0.123 – 0.216).
The E_yC_{10}/7 d value is 0.101 µg/L (95% confidence interval <0.074 – 0.137).
For growth rate and yield, the NOEC/7 d values are 0.074 µg/L, whereas LOEC/7 d values are 0.236 µg/L.

Endpoints based on the dry weight:
The E_rC_{50}/7 d value is 20.626 µg/L (95% confidence interval 13.282 – 33.830).
The E_rC_{20}/7 d value is 0.712 µg/L (95% confidence interval 0.302 – 1.319).
The E_rC_{10}/7 d value is 0.122 µg/L (95% confidence interval <0.074 – 0.290).
For growth rate the NOEC/7 d value is 0.074 µg/L, whereas LOEC/7 d value is 0.236 µg/L.

The E_yC_{50}/7 d value is 1.034 µg/L (95% confidence interval 0.665 – 1.550).
The E_yC_{20}/7 d value is <0.074 µg/L (95% confidence interval <0.074 – 0.089).
The E_yC_{10}/7 d value was not determined.
For yield, the NOEC/7 d value is <0.074 µg/L, whereas LOEC/7 d value is ≤0.074 µg/L.

Reference:	KCP 10.2.1
Report	DNT-162OD-R-CPd – *Lemna sp.*, Growth Inhibition Test, Zuzanna Kacperek-Karetta, 2023, STUDY CODE: W-12-23, Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna
Guideline(s):	Yes. According to the OECD Guideline No. 221 (2006)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

For analytical method validation see section B5 of dRR

Materials and methods

A. MATERIALS

1. Test material

Lot/Batch #	1/23
Appearance:	White to beige colour oil suspension
Content of active substance	dicamba: 112 g/L nicosulfuron: 39.00 g/L thifensulfuron-methyl: 12.40 g/L
Density:	1.003 g/mL
Expiry date	09.02.2025

2. Test organism

Species	*Lemna gibba*
Source	The fronds *Lemna gibba* were obtained from the Landolt Duckweed Collection
Culturing	At 24 ± 2 °C under constant illumination in 20X AAP medium.
Acclimation period	Culturing was done under test conditions.
Test units	150 mL glass beakers

3. Environmental conditions

Water temperature	23.0-23.3°C
pH	pH of the control not warried more than 1.5 unit during the test; pH of the control was in the range 7.44 – 7.86
Lighting	Constant illumination in the range of 7208 to 7232 lux
Test vessel	Glass crystallizers containing 150 mL of a given test item concentration or control

B. STUDY DESIGNS AND METHODS

1. Experimental conditions

Test design

Semi-static; 7 days of exposure; three replicates of each test item concentration; six replicates of control.

Concentrations tested

Nine test item concentrations: 20, 6.25, 1.95, 0.61, 0.19, 0.06, 0.019 and 0.006 mg/L plus the control

Analytics

The concentrations of active substance in the test item were chemically determined using the high performance liquid chromatography (HPLC) with MS/MS detection. The analytical method was validated according to SANTE/2020/12830, Rev. 2.

2. Sampling and measurements

In order to quantify the test item-related effects on vegetative growth over a period of 7 days, the number of fronds in each replicate was counted twice during exposure (days 3 and 5) and at exposure termination. At the same time observations of plant development were performed, i.e. size of fronds, necroses, chloroses, colony break up, gibbosity, changes in appearance of roots.
The dry weight of the representative sample of the duckweed culture used as the inoculum was measured at exposure initiation. The dry weight of all plants from each test vessel was measured after exposure termination. All colonies (with roots) were transferred onto previously weighed microscopic slides and dried at 60°C in a laboratory oven until constant weight.

3. Calculation of toxicity

The endpoint values were determined on the basis of the nominal test item concentrations and the nominal concentrations of dicamba, nicosulfuron and thifensulfuron-methyl. The endpoint values were calculated with a probit analysis. The lowest observed effect concentration (LOEC) and the no observed effect concentration (NOEC) were estimated on the basis of statistical analysis. ToxRat Professional commercial software Version 3.3.0 was used to conduct statistical analyses

Results and discussions

A. ANALYTICAL RESULTS

The aim of the analytical part of the experiment was to determine the concentrations of the test item with a liquid chromatographic method with mass spectroscopy.
The analytical method has been validated during the study. A Standard Operating Procedure for the analytical test method has been developed based on the validation carried out during the study. The determination of concentration was performed by the analytical personnel.
In fresh samples at exposure initiation and at renewals, the determined concentrations of dicamba were in the range of 80.5 – 102.2%, the determined concentrations of nicosulfuron were in the range of 99.6 – 118.9% and the determined concentrations of thifensulfuron-methyl were in the range of 81.5 – 119.0% of the nominal concentration. The results confirm that the test item concentrations were prepared correctly. In spent samples at renewals and at exposure termination, the determined concentrations of dicamba were in the range of 80.5 – 105.4%, the determined concentrations of nicosulfuron were in the range of 87.1 – 118.9% and the determined concentrations of thifensulfuron-methyl were in the range of 81.0 – 119.5% of the nominal concentration. Therefore, the concentrations of dicamba, nicosulfuron and thifensulfuron-methyl were stable under test conditions.

B. BIOLOGICAL RESULTS

At exposure termination, in the test item concentrations in the range of 0.006 – 0.61 mg/L, no distinctive changes from the normal development of plants in the control were observed. In the test item concentrations in the range of 1.95 – 20 mg/L, shorter roots and smaller new fronds were observed

Results of inhibition of growth rate and yield:

Nominal test item concentration [mg/L]	Based on frond number		Based on dry weight	
	[%] inhibition at exposure termination of growth rate	[%] inhibition at exposure termination of yield	[%] inhibition at exposure termination of growth rate	[%] inhibition at exposure termination of yield
Control	0.0	0.0	0.0	0.0
0.006	0.4	1.5	7.5	20.7
0.019	11.6	27.2	17.9	42.1
0.06	41.4	70.0	16.7	39.0
0.19	63.7	86.8	30.1	60.6
0.61	73.9	91.8	43.4	74.6
1.95	78.7	93.7	48.8	78.7
6.25	81.5	94.7	55.0	83.4
20	89.7	97.4	85.9	95.5

Time of exposure: 11.10 – 18.10.2023

The endpoint values based on the nominal test item concentrations:

Endpoints based on the frond number:

The E_rC_{50}/7 d value is 0.155 mg/L (95% confidence interval 0.107 – 0.222).

The E_rC_{20}/7 d value is 0.018 mg/L (95% confidence interval 0.009 – 0.029).

The E_rC_{10}/7 d value is 0.006 mg/L (95% confidence interval <0.006 – 0.011).

The E_yC_{50}/7 d value is 0.037 mg/L (95% confidence interval 0.031 – 0.045).

The E_yC_{20}/7 d value is 0.014 mg/L (95% confidence interval 0.010 – 0.017).

The E_yC_{10}/7 d value is 0.008 mg/L (95% confidence interval <0.006 – 0.011).

For growth rate and yield, the NOEC/7 d values are 0.006 mg/L, whereas the LOEC/7 d values are 0.019 mg/L.

Endpoints based on the dry weight:

The E_rC_{50}/7 d value is 1.663 mg/L (95% confidence interval 1.071 – 2.728).

The E_rC_{20}/7 d value is 0.057 mg/L (95% confidence interval 0.024 – 0.106).

The E_rC_{10}/7 d value is 0.010 mg/L (95% confidence interval <0.006 – 0.023).

For growth rate, the NOEC/7 d value is 0.006 mg/L, whereas the LOEC/7 d value is 0.019 mg/L.

The E_yC_{50}/7 d value is 0.083 mg/L (95% confidence interval 0.054 – 0.125).

The E_yC_{20}/7 d value is <0.006 mg/L (95% confidence interval <0.006 – 0.007). The E_yC_{10}/7 d value was not determined.

For yield, the NOEC/7 d value is <0.006 mg/L, whereas the LOEC/7 d value is ≤0.006 mg/L.

C. VALIDITY CRITERIA

For the test to be valid, the following performance criteria specified in OECD Guideline No. 221 were met for both experimental parts:

- the doubling time of frond number in the control was 1.9 days, criterion: less than 2.5 days (the factor of frond number in the control between 0 and 7 day was 12.2)
- the average specific growth rate in the control between day 0 and day 7 was 0.357 d -1 (minimum requirement: higher than 0.275 d-1)

Study 5

Comments of zRMS:	The study is acceptable. The validity criteria according OECD 239 of the test were met. Validity criteria: • The mean total shoot length in the control increased by a factor of 3.0 within the 14 days of exposure (criterion: at least a 2-fold growth). • The mean total shoot fresh weight in control plants increased by a factor of 6.0 within the 14 days of exposure (criterion: at least a 2-fold growth). • Control plants did not show any 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 test medium. • The mean coefficient of variation for yield based on measurements of shoot fresh weight (i.e. from test initiation to test termination) in the control cultures was 23.4 % (criterion: it must not exceed 35%). **Agreed toxicity endpoints:** **Endpoint values [mg/L] for growth rate based on nominal test item concentrations**

Endpoint	Total shoot length	Fresh weight	Dry weight
	day 14	day 14	day 14
E_rC_{50}	0.1060 (0.0745 – 0.1507)	4.3494 (2.2624 – 10.9947)	10.0649 (7.1156 – 16.8818)
E_rC_{20}	0.0232 (0.0118 – 0.0361)	0.0965 (0.0310 – 0.2020)	1.6560 (0.8989 – 2.4245)
E_rC_{10}	0.0105 (0.0041 – 0.0186)	0.0132 (0.0021 – 0.0389)	0.6447 (0.2391 – 1.1207)
LOEC	0.08	0.08	2.0
NOEC	0.016	0.016	0.4

(-) - 95% confidence limits

Endpoint values [mg/L] for yield based on nominal test item concentrations

Endpoint	Total shoot length	Fresh weight	Dry weight
	day 14	day 14	day 14
E_yC_{50}	0.0736 (0.0576 – 0.0937)	0.4328 (0.2406 – 0.8079)	3.5170 (2.1630 – 6.4498)
E_yC_{20}	0.0288 (0.0171 – 0.0391)	0.0211 (0.0062 – 0.0465)	0.4941 (0.1678 – 0.9001)
E_yC_{10}	0.0176 (0.0085 – 0.0263)	0.0043 (0.0008 – 0.0126)	0.1771 (0.0356 – 0.3985)
LOEC	0.08	0.08	2.0
NOEC	0.016	0.016	0.4

(-) - 95% confidence limits

Reference:	KCP 10.2.1
Report	DNT-162OD-R-CPd Water-sediment *Myriophyllum spicatum* toxicity test according to OECD 239, Katarzyna Brzozowska - Wojoczek, 2024, STUDY CODE: ETOX-2024-39, EcoTox Alliance Sp. z o. o., Poland
Guideline(s):	Yes. According to the OECD Guideline No. 239
Deviations:	Yes
GLP:	Yes
Acceptability:	Yes

Materials and methods

A. MATERIALS

1. Test material

Lot/Batch #	1/24
Content of active substance	113 g/L of dicamba 41 g/L of nicosulfuron 12.1 g/L thifensulfuron-methyl
Density:	1.018 g/mL
Expiry date	12.03.2026

2. Test organism

Species	aquatic submersed dicotyledonous macrophyte plant, watermilfoil *Myriophyllum spicatum*
Source	laboratory culture obtained from a commercial supplier: F.P.-H. „Szuwarek'' Przeskok 14, 63-400 Ostrów Wielkopolski, Poland.
Culturing	At 20 ± 2 °C under constant illumination in Smart & Barko medium.

Acclimation period	Culturing was done under test conditions.
Test units	The test was conducted using 2 L glass beakers. Plastic pots (9 cm diameter and 6 cm high and 300 mL volume) were used as containers for potting the plants into the sediment.

3. Environmental conditions

Test water	Smart & Barko medium containing the following constituents:

Ingredient	Concentration in the medium [mg/L]
calcium chloride dihydrate $CaCl_2 \cdot 2H_2O$	91.7
magnesium sulphate heptahydrate $MgSO_4 \cdot 7H_2O$	69.0
sodium hydrogen carbonate $NaHCO_3$	58.4
potassium hydrogen carbonate $KHCO_3$	15.4

Temperature	minimal temperature 18.0°C, maximal temperature 21.7°C
pH	The pH value in the experimental vessels was maintained at 7.73-7.80 at the start, 8.82-9.13 at the end and did not fluctuate by more than 1.5 units during the experiment (OECD 211 requirements: pH 6-9)
Lighting	Photoperiod 16 h day/8 h night with a light intensity 9075-9100 lux

B. STUDY DESIGNS AND METHODS

The aim of the study was to determine the effect of the test item DNT-162OD-R-CPd on the vegetative growth of spiked water-milfoil Myriophyllum spicatum. Effects on growth were determined from quantitative assessments of shoot length, fresh weight and dry weight, as well as qualitative observations of symptoms such as chlorosis, necrosis or growth deformities. To quantify substance-related effects, growth in the test solutions was compared with that of the controls and the concentration bringing about a specified 50% inhibition of growth was determined and expressed as the EC50. The EC10 and EC20 values were also determined. The lowest observed effect concentration (LOEC) and the no observed effect concentration (NOEC) were statistically determined from estimates of average specific growth rates and yield

1. Experimental conditions

Test design

All concentrations of the test item were prepared in four replicates and control in six. Plastic pots with sediment were placed in bakers in which three shoot apices were planted and the baker was filled with Smart&Barko medium. Rooting phase lasted 7 days. Exposition phase to tested item lasted 14 days.

Concentrations tested

Product was tested at concentrations of 0.0032, 0.016, 0.08, 0.4, 2 and 10 mg/L in four repetitions. Definitive test concentrations were chosen based on range finding test results. A control group with untreated test medium was used. The reference item was tested in a separate study. According to OECD 239 Guideline the separation factor between test concentrations should not exceed 3.2. However, in the preliminary test, a flat concentration-response curve occurred. Due to mathematical reasons, to calculate all endpoint values with 95% confidence intervals a larger factor was used. In the main test, all endpoints were calculated and all validity criteria were met. Therefore, a larger factor did not impact the validity of the test.

Treatment/Application

A stock solution was prepared by weighing 200 mg of the test item and filling it up to 2000 mL test medium. The test solutions were prepared by dilution of the respective amount of the stock solution with test medium.

Analytics

The actual content of test item measured as dicamba, nicosulfuron and thifensulfuron-methyl was determined using ultrahigh performance liquid chromatographic method with tandem mass spectrometry detection (UHPLC-MS/MS). At exposure initiation, the chemical determinations in aqueous phase of the water-sediment system of each test item concentration and the control was performed. the chemical determinations in sediment and sediment pore-water of the highest test item concentration and the control was performed. Additionally chemical analysis in aqueous phase of the water-sediment system of the highest and the lowest test item concentration and the control was performed at each renewal (i.e. day 4, day 7 and day 11) and exposure termination The method was validated according to SANTE/2020/12830, Rev. 2

2. Sampling and measurements

Test was conducted in semi-static system; test item solution were renewal after 4, 7 and 11 days of exposure.

3. Calculation of toxicity

The end points of the experiment are ECx, NOEC and LOEC values.

4. Statistics

Based on the obtained data, a statistical analysis was carried out using the ToxRat Professional software.

Results and discussions

The endpoint values determined on the basis of the nominal test item concentration are given below:

Endpoint values [mg/L] for growth rate based on nominal test item concentrations

Endpoint	Total shoot length	Fresh weight	Dry weight
	day 14	day 14	day 14
E_rC_{50}	0.1060 (0.0745 – 0.1507)	4.3494 (2.2624 – 10.9947)	10.0649 (7.1156 – 16.8818)
E_rC_{20}	0.0232 (0.0118 – 0.0361)	0.0965 (0.0310 – 0.2020)	1.6560 (0.8989 – 2.4245)
E_rC_{10}	0.0105 (0.0041 – 0.0186)	0.0132 (0.0021 – 0.0389)	0.6447 (0.2391 – 1.1207)
LOEC	0.08	0.08	2.0
NOEC	0.016	0.016	0.4

(-) - 95% confidence limits

Endpoint values [mg/L] for yield based on nominal test item concentrations

Endpoint	Total shoot length	Fresh weight	Dry weight
	day 14	day 14	day 14
E_yC_{50}	0.0736 (0.0576 – 0.0937)	0.4328 (0.2406 – 0.8079)	3.5170 (2.1630 – 6.4498)
E_yC_{20}	0.0288 (0.0171 – 0.0391)	0.0211 (0.0062 – 0.0465)	0.4941 (0.1678 – 0.9001)
E_yC_{10}	0.0176 (0.0085 – 0.0263)	0.0043 (0.0008 – 0.0126)	0.1771 (0.0356 – 0.3985)
LOEC	0.08	0.08	2.0
NOEC	0.016	0.016	0.4

(-) - 95% confidence limits

C. VALIDITY CRITERIA

- The mean total shoot length in the control increased by a factor of 3.0 within the 14 days of exposure (criterion: at least a 2-fold growth).
- The mean total shoot fresh weight in control plants increased by a factor of 6.0 within the 14 days of exposure (criterion: at least a 2-fold growth).
- Control plants did not show any 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 test medium.
- The mean coefficient of variation for yield based on measurements of shoot fresh weight (i.e. from test initiation to test termination) in the control cultures was 23.4 % (criterion: it must not exceed 35%).

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 is acceptable. The validity criteria according OECD 213 of the test were met.

Validity criteria:

- the average mortality for the total number of controls was 0.0% at the end of the experiment (criterion: it must not exceed 10%).
- the LD_{50} after 24 h for the reference item (dimethoate) was 0.105 µg a.i./bee (criterion: 0.10 - 0.35 µg a.i./bee).

Deviation of the study: Deviations concerning short term decreased relative air humidity in the preliminary and the main experiment occurred. According to the documents mentioned above, the air humidity should vary from 50 to 70%. Deviations did not have any influence on the results of the study. No other deviation occurred. Despite of deviations from the study plan validity criterion of the study were met.

Agreed toxicity endpoints:

Dose		Number of tested bees [no.]	Mortality after 48 h		LD_{50} after 48 h	
			Total			
[µg t.i./bee]	[µg a.i./bee]		[no.]	[%]	[µg t.i./bee]	[µg a.i./bee]
0.0 (Control)		30	0	0.0	above 200	above $22.7^a + 7.8^b + 2.4^c$
12.5	$1.4^a + 0.5^b + 0.1^c$	30	0	0.0		
25.0	$2.8^a + 1.0^b + 0.3^c$	30	0	0.0		
50.0	$5.7^a + 1.9^b + 0.6^c$	30	0	0.0		
100.0	$11.4^a + 3.9^b + 1.2^c$	30	1	3.3		
200.0	$22.7^a + 7.8^b + 2.4^c$	30	1	3.3		

a.i.: active ingredient:
[a]: dicamba
[b]: nicosulfuron
[c]: thifensulfuron-methyl
t.i.: test item

Reference:	KCP 10.3.1_01
Report	Honeybees (*Apis mellifera* L.), Acute Oral Toxicity Test, DNT-162OD-R-CPd, Parma P., 2022, EMI/4/64/2022
Guideline(s):	OECD Guideline for the Testing of Chemicals No. 213 (1998)
Deviations:	No
GLP:	Yes
Acceptability:	yes
Duplication (if vertebrate study)	-

Materials and methods

Test item:

Name:	DNT-162OD-R-CPd
Sample number:	13/22
Production date:	2022-07-13
Expiry date:	2024-07-13
Active substances:	Dicamba: 115 g/L
Nicosulfuron:	39.40 g/L
Thifensulfuron-methyl:	12.03 g/L
Active substances CAS No.:	Dicamba: 1918-00-9 [5] Nicosulfuron: 111991-09-4 [6] Thifensulfuron-methyl: 79277-27-3 [7]
Appearance:	Gray to beige viscous liquid
Density:	1.011 g/cm3

Reference item:

Dimethoate technical was used to verify the precision of the test procedure and the sensitivity of the biological test system. It is in the form of colourless crystals. The Certificate of Analysis provides the following data: sample number: 2L/21; expiry date: 31 December 2022; purity: 97.3 ±0.6% (CAS number: 60-51-5).

Test system:

A biological test system was the young adult worker honeybee, *Apis mellifera* L. (strain: carnica), approximately 3-week-old worker honeybees from healthy, queen-right families with known history and physiological status were collected from honeycombs before the treatment. These colonies were not treated with any chemicals, such as antibiotics or anti-varroa for four weeks preceding the study.
Before the experiment, the honeycomb with the bees was transferred from the apiary to an experimental room. The honeybees were removed from the comb and starved for up to two hours before the initiation of the treatment.

Test item and reference item preparation:

In the preliminary and in the main experiment, the test item was mixed with the 50% saccharose solution. In the preliminary experiment the highest dose of the test item of 200.0 µg/bee was prepared.
In the main experiment the highest dose of the test item of 200.0 µg/bee was prepared. For this purpose 200 mg of the test item was weighed into a glass flask with a capacity of 10 mL. Then, the flask was filled up to the total volume of 10 mL with the saccharose solution. The remaining suspensions of the test item at the doses of 100.0, 50.0, 25.0, and 12.5 µg/bee were prepared by making sequential 1:1 dilutions with the 50% saccharose solution (v/v). In the main experiment dimethoate was used as the reference item in order to verify the sensitivity of the test system and the susceptibility of the bees.

Preliminary test:
Mortality of the control group after 48 hours of exposure was 0.0%. The percentages of mortality of the bees treated with the test item at the doses of 8.0, 40.0, and 200.0 µg/honeybee were 0.0, 0.0 and 0.0%, respectively. No abnormal behavioural effects were observed during the experiment.

Main test:
Mortality of the bees in the control group after 24 and 48 hours was 0.0%. After 24 and 48 hours of exposure to the test item the percentages of mortality of the bees at the doses of 12.5, 25.0, 50.0, 100.0, and 200.0 µg/bee were 0.0, 0.0, 0.0, 3.3, and 3.3%, respectively. No sublethal toxicity effects (behavioural abnormalities) such as excitement (uncoordinated movement, increased activity, intensive cleaning) or any signs of paralysis with respect to the test item and the control were observed over the 48 hours exposure

Study results:
The acute oral toxicity study of the test item, DNT-162OD-R-CPd on honeybees (*Apis mellifera* L.) in the main experiment are summarized below.

Dose		Number of tested bees [no.]	Mortality after 48 h		LD_{50} after 48 h	
			Total			
[µg t.i./bee]	[µg a.i./bee]		[no.]	[%]	[µg t.i./bee]	[µg a.i./bee]
0.0 (Control)		30	0	0.0	above 200	above $22.7^a + 7.8^b + 2.4^c$
12.5	$1.4^a + 0.5^b + 0.1^c$	30	0	0.0		
25.0	$2.8^a + 1.0^b + 0.3^c$	30	0	0.0		
50.0	$5.7^a + 1.9^b + 0.6^c$	30	0	0.0		
100.0	$11.4^a + 3.9^b + 1.2^c$	30	1	3.3		
200.0	$22.7^a + 7.8^b + 2.4^c$	30	1	3.3		

a.i.: active ingredient:
[a]: dicamba
[b]: nicosulfuron
[c]: thifensulfuron-methyl
t.i.: test item

The median lethal doses for the test item (LD_{50}/24 h, LD_{50}/48 h) are higher than the highest dose used in the test, i.e. > 200.0 µg test item/bee.

A 2.3.1.1.2 KCP 10.3.1.1.2 Acute contact toxicity to bees

Comments of zRMS:	The study is acceptable. The validity criteria according OECD 214 of the test were met. Validity criteria:

- the average mortality for the total number of controls was 0.0% at the end of the experiment (criterion: it must not exceed 10%).
- the LD_{50} after 24 h for the reference item (dimethoate) was 0.148 µg a.i./bee (criterion: 0.10 - 0.30 µg a.i./bee).

Deviation of the study:
Deviations concerning short term decreased relative air humidity in the preliminary and the main experiment occurred. According to the documents mentioned above, the air humidity should vary from 50 to 70%. A deviation from the Study Plan concerning a study completion date occurred. However deviations did not have any influence on the results of the study. No other deviation occurred. Despite of deviations from the study plan validity criterion of the study were met.

Agreed toxicity endpoints:

Dose		Number of tested bees [no.]	Mortality after 48 h		LD_{50} after 48 h	
			Total			
[µg t.i. /bee]	[µg a.i. /bee]		[no.]	[%]	[µg t.i. /bee]	[µg a.i. /bee]
0.0 (Control)		30	0	0.0	above 200.0	above $22.7^a + 7.8^b + 2.4^c$
12.5	$1.4^a + 0.5^b + 0.1^c$	30	1	3.3		
25.0	$2.8^a + 1.0^b + 0.3^c$	30	0	0.0		
50.0	$5.7^a + 1.9^b + 0.6^c$	30	1	3.3		
100.0	$11.4^a + 3.9^b + 1.2^c$	30	2	6.7		
200.0	$22.7^a + 7.8^b + 2.4^c$	30	4	13.3		

a.i.: active ingredient:
[a]: dicamba
[b]: nicosulfuron
[c]: thifensulfuron-methyl
t.i.: test item

Reference:	KCP 10.3.1_02
Report	Honeybees (*Apis mellifera* L.), Acute Contact Toxicity Test, DNT-162OD-R-CPd, Parma P., 2022, EMI/4/65/2022
Guideline(s):	OECD Guideline for the Testing of Chemicals No. 214 (1998) and EU Method C.17. (2018)
Deviations:	No
GLP:	Yes
Acceptability:	yes
Duplication (if vertebrate study)	-

Materials and methods
Test item:

Name:	DNT-162OD-R-CPd
Sample number:	13/22
Production date:	2022-07-13
Expiry date:	2024-07-13
Active substances:	Dicamba: 115 g/L Nicosulfuron: 39.40 g/L Thifensulfuron-methyl: 12.03 g/L
Active substances IUPAC:	3,6-dichloro-2-methoxybenzoic acid 2-[(4,6-dimethoxypyrimidin-2-ylcarbamoyl)sulfamoyl]- N,N-dimethylnicotinamide methyl 3-(4-methoxy-6-methyl-1,3,5-triazin-2-ylcabamoylsulfamoyl)thiophene-2-carboxylate
Active substances CAS No.:	Dicamba: 1918-00-9 [5] Nicosulfuron: 111991-09-4 [6] Thifensulfuron-methyl: 79277-27-3 [7]
Appearance:	Gray to beige viscous liquid
Density:	1.011 g/cm3

Reference item:
Dimethoate technical was used to verify the precision of the test procedure and the sensitivity of the biological test system. It is in the form of colourless crystals. The Certificate of Analysis provides the following data: sample number: 2L/21; expiry date: 31 December 2022; purity: 97.3 ±0.6% (CAS number: 60-51-5).

Test system:
A biological test system was the young adult worker honeybee, *Apis mellifera* L. (strain: carnica), approximately 3-week-old worker honeybees from healthy, queen-right families with known history and physiological status were collected from honeycombs before the treatment. These colonies were not treated with any chemicals, such as antibiotics or anti-varroa for four weeks preceding the study.
Before the experiment, the honeycomb with the bees was transferred from the apiary to an experimental room. The honeybees were removed from the comb and starved for up to two hours before the treatment.

Test item and reference item preparation:
In the preliminary and in the main experiment, the test item was diluted in ultra-pure water. In the preliminary experiment the highest dose of the test item of 200.0 µg/bee was prepared.
In the main experiment the highest dose of the test item of 200.0 µg/bee was prepared. For this purpose 200 mg of the test item were weighed into a glass flask with a capacity of 10 mL. Then, the flask was filled up to the total volume of 10 mL with the ultra-pure water. The remaining suspensions of the test item at the doses of 100.0, 50.0, 25.0, and 12.5 µg/bee were prepared by making sequential 1:1 dilutions with ultra-pure water (v/v). In the main experiment dimethoate was used as the reference item in order to verify the sensitivity of the test system and the susceptibility of the bees.

Preliminary test:
Mortality of the control group after 48 hours of exposure was 0.0%. The percentages of mortality of the bees treated with the test item at the doses of 8.0, 40.0, and 200.0 µg/honeybee were 0.0, 10.0, and 20.0%, respectively. No abnormal behavioural effects were observed during the test.

Main test:
The percentages of mortality of the honeybees treated with the test item at the doses of 12.5, 25.0, 50.0, 100.0, and 200.0 µg/honeybee were 3.3, 0.0, 3.3, 6.7, and 13.3%, respectively. The median lethal doses

(LD50/24 h and LD50/48 h) are higher than the maximum dose used in the test, i.e. 200.0 µg test item/bee. No signs of toxicity (behavioural abnormalities) such as excitement (uncoordinated movement. increased activity or intensive cleaning) or paralysis were observed during the 48-hour exposure.

Study results:
The acute contact toxicity study of the test item, DNT-162OD-R-CPd on honeybees (*Apis mellifera* L.) in the laboratory test are summarized below.

Dose		Number of tested bees [no.]	Mortality after 48 h		LD_{50} after 48 h	
			Total			
[µg t.i. /bee]	[µg a.i. /bee]		[no.]	[%]	[µg t.i. /bee]	[µg a.i. /bee]
0.0 (Control)		30	0	0.0	above 200.0	above $22.7^a + 7.8^b + 2.4^c$
12.5	$1.4^a + 0.5^b + 0.1^c$	30	1	3.3		
25.0	$2.8^a + 1.0^b + 0.3^c$	30	0	0.0		
50.0	$5.7^a + 1.9^b + 0.6^c$	30	1	3.3		
100.0	$11.4^a + 3.9^b + 1.2^c$	30	2	6.7		
200.0	$22.7^a + 7.8^b + 2.4^c$	30	4	13.3		

a.i.: active ingredient
[a]: dicamba
[b]: nicosulfuron
[c]: thifensulfuron-methyl
t.i.: test item

The median lethal doses (LD_{50}/24h and LD_{50}/48h) are higher than the maximum used dose, i.e. 200 µg test item/honeybee.

A 2.3.1.2 KCP 10.3.1.2. Chronic toxicity to bees

Comments of zRMS:	The study is acceptable. The validity criteria according OECD 245 of the test were met. **Validity criteria:** - at the end of the experiment average mortality of the control groups was 0.0% (criterion: it must not exceed 15%), - after 10 days of exposure corrected mortality of the honeybees exposed to the reference item at the concentration of 1 mg/kg (0.03 µg/bee/day) was 90.0%. Deviation of the study: none **Agreed toxicity endpoints:**

Initial		Consumed*	Number of tested bees [no]	Mortality		LC_{50}	LDD_{50}
Dose [µg t.i./bee/day]	Concentration [mg t.i./kg]	Dose [µg t.i./bee/day]		Total No.	Total [%]		
DNT-162OD-R-CPd							
0.0 (Control)			30	0	0.0	> 1000 mg t.i./kg	> 113.7[a] + 39[b] + 11.9[c] mg/kg
0.77	25.6	0.80	30	0	0.0		
1.92	64	1.86	30	0	0.0		
4.8	160	4.98	30	4	13.3+		
12.0	400	11.41	30	6	20.0+		
30.0	1000	29.83	30	10	33.3+		
NOEC				64 mg t.i./kg 7.3[a] + 2.5[b] + 0.8[c] mg/kg			
NOEDD				1.86 µg t.i./bee/day 0.21[a] + 0.07[b] + 0.02[c] µg/ bee/day			
Initial		Consumed*	Dimethoate				
Dose [µg/bee/day]	Concentration [mg/kg]	Dose [µg/bee/day]					
0.03	1.0	0.02	30	27	90.0	not determined	

*: consumed doses were calculated on the basis of the initial doses of the test item and average saccharose solution consumption
t.i..: test item
a: dicamba content
b: nicosulfuron content
c: thifensulfuron-methyl content
+: Significant difference to the control ($p \leq 0.05$)
The main experiment was performed between 21.09 - 01.10.2022.

Reference:	KCP 10.3.1_03
Report	Honeybees (*Apis mellifera* L.), Chronic Oral Toxicity Test, DNT-162OD-R-CPd, Parma P., 2023, EMI/4/62/2022
Guideline(s):	OECD Guideline for the Testing of Chemicals No. 245 (2017)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Materials and methods

Test item:

Name:	DNT-162OD-R-CPd
Sample number:	13/22
Production date:	2022-07-13
Expiry date:	2024-07-13
Active substances:	Dicamba: 115 g/L

	Nicosulfuron: 39.40 g/L Thifensulfuron-methyl: 12.03 g/L
Active substances IUPAC:	3,6-dichloro-2-methoxybenzoic acid 2-[(4,6-dimethoxypyrimidin-2-ylcarbamoyl)sulfamoyl]- N,N-dimethylnicotinamide methyl 3-(4-methoxy-6-methyl-1,3,5-triazin-2- ylcarbamoylsulfamoyl)thiophene-2-carboxylate
Active substances CAS No.:	Dicamba: 1918-00-9 [4] Nicosulfuron: 111991-09-4 [5] Thifensulfuron-methyl: 79277-27-3 [6]
Appearance:	Gray to beige viscous liquid
Density:	1.011 g/cm3

Reference item:
Dimethoate technical was used to verify the precision of the test procedure and the sensitivity of the biological test system. It is in the form of colourless crystals. The Certificate of Analysis provides the following data: sample number: 2L/21; expiry date: 31 December 2022; purity: 97.3 ±0.6% (CAS number: 60-51-5).

Test system:
The freshly emerged young (max. 2 days old) worker honeybees, *Apis mellifera* L. (strain: carnica, line: Prima) from healthy, queen-right families with known history and physiological status were collected before the treatment. These colonies were not treated with any chemicals, such as antibiotics or anti-varroa for four weeks preceding the study.

Study design:
Group of young honeybees (3 replicates/group; 10 bees/replicate) after about one day of adaptation were fed with 2 mL of a 50% saccharose solution containing the test item, the reference item or 50% saccharose solution alone at the concentrations mentioned previously for 10 days. Syringe were used as a feeders. The tip of each syringe was removed that the bees had access to the feeding solutions. All syringes were replaced daily by the new ones containing appropriate feeding solution. Dimethoate, which is a recommended reference item, was used to verify the sensitivity of the bees and the precision of the test procedure. The chronic oral toxicity test finished after 10 days of exposure.
The preliminary experiment was conducted to determine the number and the range of concentrations to be used in the main experiment. The main experiment was done on one untreated control group, five test item groups, and one reference item group. There were three replicates of each of them (10 bees/replicate).

Results:
The chronic oral toxicity study of the test item DNT-162OD-R-CPd, on honeybees (*Apis mellifera* L.) in the laboratory test is summarized below.:

Initial		Consumed*	Number of tested bees [no]	Mortality		LC_{50}		LDD_{50}	
Dose [µg t.i./bee/day]	Concentration [mg t.i./kg]	Dose [µg t.i./bee/day]		Total No.	Total [%]				
DNT-162OD-R-CPd									
0.0 (Control)			30	0	0.0	**> 1000 mg t.i./kg**	> 113.7[a] + 39[b] + 11.9[c] mg/kg	**> 29.83 µg t.i./bee/day**	> 3.39[a] + 1.16[b] + 0.35[c] µg/bee/day
0.77	25.6	0.80	30	0	0.0				
1.92	64	1.86	30	0	0.0				
4.8	160	4.98	30	4	13.3+				
12.0	400	11.41	30	6	20.0+				
30.0	1000	29.83	30	10	33.3+				
NOEC				64 mg t.i./kg 7.3[a] + 2.5[b] + 0.8[c] mg/kg					
NOEDD				1.86 µg t.i./bee/day 0.21[a] + 0.07[b] + 0.02[c] µg/ bee/day					
Initial		Consumed*	Dimethoate						
Dose [µg/bee/day]	Concentration [mg/kg]	Dose [µg/bee/day]							
0.03	1.0	0.02	30	27	90.0	not determined			

*: consumed doses were calculated on the basis of the initial doses of the test item and average saccharose solution consumption
t.i..: test item
a: dicamba content
b: nicosulfuron content
c: thifensulfuron-methyl content
+: Significant difference to the control ($p \leq 0.05$)

The percentages of mortality of the honeybees exposed to the test item at the concentrations: 25.6, 64, 160, 400, and 1000 mg t.i./kg (i.e. 0.77, 1.92, 4.8, 12.0, and 30.0 µg t.i./bee/day) were 0.0, 0.0, 13.3, 20.0, and 33.3% respectively.
On the basis of the obtained mortality results, the LDD_{50} value is higher than 29.83µgt.i./bee/day(>3.39 µg dicamba./bee/day + 1.16 µg nicosulfuron./bee/day + 0.35 µg thifensulfuron-methyl./bee/day).

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

Comments of zRMS:	The study is acceptable. The validity criteria according OECD 239 of the test were met. **Validity criteria:** - cumulative larval mortality in the control group was 8.3% at day 8 (D8) (criterion: ≤ 15%). - Abbott corrected mortality of the larvae treated with the reference item at day 8 (D8) (dimethoate) was 83.3% (criterion: ≥ 50%). - emergence rate in the control group on D22 was 83.3% (criterion: ≥ 70%). Deviation of the study: Deviations concerning short term variation of relative air humidity and temperature in the preliminary and temperature in the main experiment occurred. Variations occurred on the following days: • 26.07.2022 at 11:02 (the air humidity was equal to 69.6%), and at 14:32 (the air humidity was equal to 68.9% and the temperature was equal to 33.6°C), (RH required: 90 ± 5%, temp. required: 34-35°C), • 31.07.2022 at 12:02 (the air humidity was equal to 70.2% and the temperature was equal to 33.8°C), (RH required: 80 ± 5%, temp. required: 34-35°C), • 04.08.2022 at 09:32 (the air humidity was equal to 59.4% and the temperature was equal to 33.8°C), (RH required: 80 ± 5%, temp. required: 34-35°C), • 05.08.2022 at 18:02 to 06.08.2022 at 02:32 (the temperature was between 35.1-35.6°C), (temp. required: 34-35°C), • 07.08.2022 from 10:02 to 14:02 (the air humidity was between 80.3-81.0%) and from 16:02 to 08.08.2022 at 01:32 (the air humidity was between 80.1-81.5%) (RH required: 50-80%), • 26.09.2022 from 12:02 to 12:32 (the air humidity was between 81.6-81.8%) (RH required: 90 ± 5%). Above deviations did not have any impact on the study results, as the validity criteria were met. Despite of deviations from the study plan validity criterion of the study were met. **Agreed toxicity endpoints:**

Cumulative dose [µg test item/larva]	Concentration [mg test item/kg food]*	Number of tested larvae [no.]	Total mortality (larval and pupal) on day 22 (D22)			Number of emerged adults [No.]	Emergence rate [%]
			Number [no.]	[%]	[%]C		
Test item: DNT-162OD-R-CPd							
Control (0.0)		36	6	**16.7**	–	30	**83.3**
6.25	**40.6**	36	4	**11.1**	**0.0**	32	**88.9**
12.5	**81.2**	36	7	**19.4**	**3.3**	29	**80.6**
25.0	**162.3**	36	6	**16.7**	**0.0**	30	**83.3**
50.0	**324.7**	36	8	22.2	**6.7**	28	**77.8**
100.0	**649.4**	36	11	**30.6**	**16.7**	25	**69.4**
ED_{50} [µg test item/larva]			**> 100.0** ($11.37^{d} + 3.9^{n} + 1.19^{t}$ µg /larva)				
NOED [µg test item/larva]			**> 100.0** ($11.37^{d} + 3.9^{n} + 1.19^{t}$ µg /larva)				
EC_{50} [mg test item/kg food]			**> 649.4** ($73.9^{d} + 25.3^{n} + 7.7^{t}$ mg/kg food)				
NOEC [mg test item/kg food]			**> 649.4** ($73.9^{d} + 25.3^{n} + 7.7^{t}$ mg/kg food)				
Reference item: Technical dimethoate mortality on day 8 (D8)							
7.39	**48.0**	**36**	**30**		**83.3**	**not determined**	

+: statistically significant difference [ToxRat 3.3.0.]
*: calculations performed taking into account density of diet C which is according to OECD GD 239 equal to 1.1 mg/µL [1]
C: mortality corrected according to the Abbott formula
d: Dicamba content
n: Nicosulfuron content
t: Thifensulfuron-methyl content
The main experiment was performed between 19.09 - 10.10.2022.

Reference:	KCP 10.3.1_04
Report	Honeybees (Apis mellifera L.) Larval Toxicity Test, Repeated Exposure, DNT-162OD-R-CPd, Parma P., 2023, EMI/4/63/2022
Guideline(s):	OECD Guideline for the Testing of Chemicals No. 239 (2016)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Materials and methods

Test item:

Name:	DNT-162OD-R-CPd
Sample number:	13/22
Production date:	2022-07-13
Expiry date:	2024-07-13
Active substances:	Dicamba: 115 g/L Nicosulfuron: 39.40 g/L Thifensulfuron-methyl: 12.03 g/L
Active substances IUPAC:	3,6-dichloro-2-methoxybenzoic acid

	2-[(4,6-dimethoxypyrimidin-2-ylcarbamoyl)sulfamoyl]- N,N-dimethylnicotinamide methyl 3-(4-methoxy-6-methyl-1,3,5-triazin-2- ylcarbamoylsulfamoyl)thiophene-2-carboxylate
Active substances CAS No.:	Dicamba: 1918-00-9 Nicosulfuron: 111991-09-4 Thifensulfuron-methyl: 79277-27-3
Appearance:	Gray to beige viscous liquid
Density:	1.011 g/cm3

Reference item:
Dimethoate technical was used to verify the precision of the test procedure and the sensitivity of the biological test system. It is in the form of colourless crystals. The Certificate of Analysis provides the following data: sample number: 2L/21; expiry date: 31 December 2022; purity: 97.3 ± 0.6% (CAS number: 60-51-5).

Test system:
A biological test system were one-day-old *Apis mellifera* L. (strain: carnica, line: prima) larvae (first instar). Larvae were taken from healthy, queen-right families (3 replicates) with known history and physiological status. The colonies were not be treated with chemical substances, such as antibiotics, anti-varroa, etc. for at least four weeks before the experiment.
On day 1 of the test combs with larvae were transferred from the apiary to the experimental room. Next, each one-day-old larva, which had not formed a C shape yet, was transferred to a grafting cell, on the surface of diet A (20 µL), using a grafting tool. When a plate was filled with larvae, plates were placed into an incubator.
Study design:
The study was divided into a preliminary experiment and a main experiment. The preliminary experiment was conducted to determine the number and the range of concentrations to be used in the main experiment. The preliminary experiment was done on one untreated control group and three test item groups. The reference item was not used. There was one replicate of each group (12 larva/replicate).
The main experiment was done on one untreated control group, five test item groups, and one reference item group. There were three replicates of each of them (12 larva/replicate).
At D1, the comb from three colonies containing first instars larvae were carried from the hive to the laboratory room where newly hatched larvae that have not yet formed a “C” shape were transferred to the cells on the 48-well plate. Each cell contained 20 µL of Diet A. The cells were placed into the incubator. From day 3 (D3) until day 6 (D6) of the experiment, the test and reference item were administered daily to the larvae. Mortality and other observations/abnormal effects were recorded daily from D3 to D8 and on D15 of the experiment, and emergence rate on D22.

Results:
The larval toxicity test – repeated exposure, of the test item, DNT-162OD-R-CPd, on honeybees (*Apis mellifera* L.) in the laboratory test is summarized below.

Cumulative dose [µg test item/larva]	Concentration [mg test item/kg food]*	Number of tested larvae [no.]	Total mortality (larval and pupal) on day 22 (D22)			Number of emerged adults [No.]	Emergence rate [%]
			Number [no.]	[%]	[%]C		
Test item: DNT-162OD-R-CPd							
Control (0.0)		36	6	16.7	–	30	83.3
6.25	40.6	36	4	11.1	0.0	32	88.9
12.5	81.2	36	7	19.4	3.3	29	80.6
25.0	162.3	36	6	16.7	0.0	30	83.3
50.0	324.7	36	8	22.2	6.7	28	77.8
100.0	649.4	36	11	30.6	16.7	25	69.4
ED_{50} [µg test item/larva]			> 100.0 (11.37^{d} + 3.9^{n} + 1.19^{t} µg /larva)				
NOED [µg test item/larva]			> 100.0 (11.37^{d} + 3.9^{n} + 1.19^{t} µg /larva)				
EC_{50} [mg test item/kg food]			> 649.4 (73.9^{d} + 25.3^{n} + 7.7^{t} mg/kg food)				
NOEC [mg test item/kg food]			> 649.4 (73.9^{d} + 25.3^{n} + 7.7^{t} mg/kg food)				
Reference item: Technical dimethoate mortality on day 8 (D8)							
7.39	48.0	36	30	83.3		not determined	

+: statistically significant difference [ToxRat 3.3.0.]
*: calculations performed taking into account density of diet C which is according to OECD GD 239 equal to 1.1 mg/µL [1]
C: mortality corrected according to the Abbott formula
d: Dicamba content
n: Nicosulfuron content
t: Thifensulfuron-methyl content

Total mortality (D3 – D22) of the control group at test termination (day 22) was 16.7%. The percentages of corrected mortality of the honeybee larvae and pupae, exposed to the test item, DNT-162OD-R-CPd at the cumulative doses of 6.25, 12.5, 25.0, 50.0 and 100.0 µg/larva at the end of the test (D22) were: 0.0, 3.3, 0.0, 6.7 and 16.7%, respectively. The emergence of adults (emergence rate) at the end of the test (on D22) in the control group was 83.3%. In the groups treated with the test item at the cumulative doses of 6.25, 12.5, 25.0, 50.0 and 100.0 µg test item/larva the adult emergence rates were: 88.9, 80.6, 83.3, 77.8 and 69.4%, respectively.

ED_{50} value is higher than 100.0 µg test item/larva corresponding to EC_{50} value is higher than 649.4 mg test item/kg food.

− NOED value is higher than 100.0 µg test item/larva, corresponding to NOEC is higher than 649.4 mg test item/kg food.

A 2.3.1.4 Effect on bumble bees

A 2.3.1.4.1 Acute oral toxicity to bumble bees

Comments of zRMS:

The study is acceptable. The validity criteria according OECD 247 of the test were met.

Validity criteria:

- Mortality of control group was 0.0% at the end of the test (criterion: ≤ 10%).
- Mortality in the toxic reference item group (dimethoate) at the end of the test was 100.0% (criterion: ≥ 50%).

Deviation of the study: According to the OECD Guideline No. 247 it is recommended to use plastic syringes for the test item administration with consumption controlled by weight. In the experiment they was replaced by glass calibrated pipettes. The use of glass pipettes provides a more precise administration of the contaminated diet in a given volume and allows for visual real-time control of consumption during exposure. This deviations had no impact on the quality, integrity and final results of the study.

Agreed toxicity endpoints:

Results obtained at the end of the definitive test (after 48 hours):

Dose				Number of tested bumble-bees [no.]	Mortality after 48 h		LD_{50}			
Test item [µg/ bumble-bee]	Dicamba [µg of dicamba/ bumblebee]	Nicosulfuron [µg of nicosulfuron/ bumblebee]	Thifensulfuron-methyl [µg of thifensulfuron-methyl/ bumblebee]		[no.]	[%]	Test item [µg/ bumble-bee]	Dicamba [µg of dicamba/ bumblebee]	Nicosulfuron [µg of nicosulfuron / bumblebee]	Thifensulfuron-methyl [µg of thifensulfuron-methyl/ bumblebee]
Control (50% (*w/v*) aqueous sucrose solution)				50	0	0.0	> 400.0	> 44.67	> 15.55	> 4.95
400.0	44.67	15.55	4.95	50	1	2.0				
Reference item: dimethoate										
Dose [µg/bumblebee]		4.0		30	30	100.0	–			

Reference:	KCP 10.3.1_05
Report	DNT-162OD-R-CPd Bumblebees (*Bombus* spp.), Acute Oral Toxicity Test, Wojciech A., 2023, B-56-23
Guideline(s):	OECD Guideline for Testing of Chemicals No. 247 (2017)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Materials and methods

Test item:

Name:	DNT-162OD-R-CPd
Batch number:	1/23

Production date:	09.02.2023
Expiry date:	09.02.2025
Active substances:	112.0 g/L of dicamba (CAS No. 1918-00-9), 39.0 g/L of nicosulfuron (CAS No. 111991-09-4), 12.4 g/L of thifensulfuron-methyl (CAS No. 79277-27-3)
Appearance:	Color white to beige
Density at 20°C:	1.003 g/mL

The study was conducted to determine the acute oral toxicity of DNT-162OD-R-CPd to bumblebees (Bombus spp.) with a laboratory method and to demonstrate, that the median lethal dose, i.e. the LD_{50} at the end of exposure, is higher than the dose used in the test (limit test).
One dose of the test item, i.e. 400.0 µg test item/bumblebee, plus the control and one dose of the reference item were used. The design of the definitive test was selected on the basis of non-GLP preliminary range-finding test results.

Reference item:
Dimethoate technical was used to verify the precision of the test procedure and the sensitivity of the biological test system. It is in the form of crystalline solid. The Certificate of Analysis provides the following data: sample number: IPO 146; serial number: 3L/22; expiry date: June 2024; purity: 97.3 ± 0.6% (m/m), (CAS number: 60-51-5)

Test system:
A biological test system was the adult worker bumblebees (*Bombus* spp.) coming from 3 different families used for the definitive test. Bumblebees were taken from three healthy families consisting of 60 – 70 individuals.

Preparation of the medium
A 50% (w/v) solution of sucrose in water recommended by the OECD Guideline for the Testing of Chemicals No. 247 was used as a solvent of the test and reference item. To prepare the solution, 250 g of sucrose were weighed and dissolved in distilled water. The volume was made up to 500 mL with distilled water.

Test item and reference item preparation:
In the first preliminary test, an emulsion of the test item at the highest concentration, i.e. of 200.0 µg/40 µL (corresponding to 200.0 µg/bumblebee) was prepared. First, 50.1 mg of the test item were weighed into a glass cylinder with a capacity of 10 mL. The volume was made up to 10 mL with 50% (w/v) aqueous sucrose solution. The remaining emulsions of the test item at the concentrations of 40.0 and 8.0 µg/40 µL (corresponding to 40.0 and 8.0 µg/bumblebee) were prepared by making 1:4 dilutions with 50% (w/v) aqueous sucrose solution (volume per volume).
In the second preliminary test, an emulsion of the test item at the concentration, i.e. of 400.0 µg/40 µL (corresponding to 400.0 µg/bumblebee) was prepared. First, 100.1 mg of the test item were weighed into a glass cylinder with a capacity of 10 mL. The volume was made up to 10 mL with 50% (w/v) aqueous sucrose solution.
In the definitive test, an emulsion of the test item at the concentration, i.e. 400.0 µg/40 µL (corresponding to 400.0 µg/bumblebee), was prepared. First, 300.1 mg of the test item were weighed into a glass cylinder with a capacity of 50 mL. The volume was made up to 30 mL with 50% (w/v) aqueous sucrose solution (volume per volume)

Preliminary test:
In the preliminary tests, there were 15 bumblebees prepared for each dose and the control having regard 'non-feeders' (for each group 10 bumblebees which ate whole diet, i.e. 40 µL, was included in the test). All bumblebees were acclimatized to the test conditions for 24 hours before the experiment. All bumblebees were taken directly from a hive using red light. Too big or too small individuals were not

be used in the experiment. All the bumblebees were individually placed in plastic isolators. For two hours before starting the exposure the bumblebees were starved. In preliminary test insects were not weighted.
The bumblebees were exposed to the test item distributed in a 50% (w/v) aqueous sucrose solution. The treated diet was provided in calibrated pipettes (0.2 mL). Each pipette contained 40 µL of the sucrose solution with the test item at a suitable dose. Once consumed, the pipettes were removed and replaced with syringes containing a sucrose solution alone. After the treatment, the bumblebees were kept individually in isolators. The isolators are well-ventilated and provide enough space. After the treatment, the insects had continuous access to food, i.e. a 50% (w/v) aqueous sucrose solution.

Main test conditions:
In the definitive test for the acclimatization, 168 bumblebees were taken including 30 'non-feeders' (10 bumblebees for each treatment) and additional 5% of the total number of bumblebees, i.e. 8 bumblebees, were taken, in case of occuring mortality before start the treatment. These additional bumblebees were marked and discarded before treatment. After that, they were acclimatized to the test conditions for about 24 hours before starting the experiment. Food, i.e. 50% (w/v) aqueous sucrose solution was provided. For 2 hours before starting the exposure the bumblebees were starved. The bumblebees were exposed to the test item (60 bumblebees) or reference item (40 bumblebees) distributed in the 50% (w/v) aqueous sucrose solution and to the 50% (w/v) aqueous sucrose solution for the control (60 bumblebees). The treated diet was provided in calibrated pipettes (0.2 mL). Each pipette contained 40 µL of the sucrose solution with the test item, reference item at a suitable dose or 50% (w/v) aqueous sucrose solution for the control.
Once consumed, the pipettes were removed and replaced with syringes containing a sucrose solution alone. After the treatment, the bumblebees were kept individually in isolators. The isolators were well-ventilated and provide enough space. After the treatment, the insects had continuous access to food, i.e. a 50% (w/v) aqueous sucrose solution in 2-mL syringes. Fresh food was added if necessary.

Study results:
The median lethal dose LD_{50}/24 h and LD_{50}/48 h, is higher than 400.0 µg/bumblebee (i.e. 44.67 µg dicamba/bumblebee, 15.55 µg nicosulfuron/bumblebee, 4.95 µg thifensulfuron-methyl/bumblebee). The NOED value after 48 hours is higher than or equal to 400.0 µg/bumblebee.

Results obtained at the end of the definitive test (after 48 hours):

Dose				Number of tested bumble-bees [no.]	Mortality after 48 h		LD_{50}			
Test item [µg/ bumble-bee]	Dicamba [µg of dicamba/ bumblebee]	Nicosulfuron [µg of nicosulfuron/ bumblebee]	Thifensulfuron-methyl [µg of thifensulfuron-methyl/ bumblebee]		[no.]	[%]	Test item [µg/ bumble-bee]	Dicamba [µg of dicamba/ bumblebee]	Nicosulfuron [µg of nicosulfuron/ bumblebee]	Thifensulfuron-methyl [µg of thifensulfuron-methyl/ bumblebee]
Control (50% (*w/v*) aqueous sucrose solution)				50	0	0.0	> 400.0	> 44.67	> 15.55	> 4.95
400.0	44.67	15.55	4.95	50	1	2.0				
Reference item: dimethoate										
Dose [µg/bumblebee]		4.0		30	30	100.0	–			

A 2.3.1.4.2 Acute contact toxicity to bumble bees

Comments of zRMS:

The study is acceptable. The validity criteria according OECD 246 of the test were met.

Validity criteria:

- Mortality of control group was 0.0% at the end of the test (criterion: ≤ 10%).
- Mortality in the toxic reference item group (dimethoate) at the end of the test was 56.7% (criterion: ≥ 50%).

Deviation of the study: According to the OECD Guideline No. 246 the bumblebees may be anesthetized with carbon dioxide or chilled for the application of the test item. Anesthesia with carbon dioxide or chilling was replaced with mechanical immobilisation. This deviation had no impact on the quality, integrity and final results of the study.

Agreed toxicity endpoints:

Dose				Number of tested bumble-bees [no.]	Mortality after 48 h		LD_{50}			
Test item [µg/ bumble-bee]	Dicamba [µg of dicamba/ bumblebee]	Nicosulfuron [µg of nicosulfuron/ bumblebee]	Thifensulfuron-methyl [µg of thifensulfuron-methyl / bumblebee]		[no.]	[%]	Test item [µg/ bumble-bee]	Dicamba [µg of dicamba/ bumblebee]	Nicosulfuron [µg of nicosulfuron/ bumblebee]	Thifensulfuron-methyl [µg of thifensulfuron-methyl / bumblebee]
Control + 1% surfactant				50	0	0.0	> 400.0	> 44.67	> 15.55	> 4.95
400.0	44.67	15.55	4.95	50	1	2.0				
Reference item: dimethoate + 1% surfactant										
Dose [µg/bumblebee]	10.0			30	17	56.7	–			

Reference:	KCP 10.3.1_06
Report	DNT-162OD-R-CPd Bumblebees (*Bombus* spp.), Acute Contact Toxicity Test, Wojciech A., 2023, B-57-23
Guideline(s):	OECD Guideline for Testing of Chemicals No. 246 (2017)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-
Reference:	KCP 10.3.1_06
Report	DNT-162OD-R-CPd Bumblebees (*Bombus* spp.), Acute Contact Toxicity Test, Wojciech A., 2023, B-57-23
Guideline(s):	OECD Guideline for Testing of Chemicals No. 246 (2017)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Duplication (if vertebrate study)	-

Materials and methods

Test item:

Name:	DNT-162OD-R-CPd
Batch number:	1/23
Production date:	09.02.2023
Expiry date:	09.02.2025
Active substances:	112.0 g/L of dicamba (CAS No. 1918-00-9), 39.0 g/L of nicosulfuron (CAS No. 111991-09-4), 12.4 g/L of thifensulfuron-methyl (CAS No. 79277-27-3)
Appearance:	Color white to beige
Density at 20°C:	1.003 g/mL

The study was conducted to determine the acute contact toxicity of DNT-162OD-R-CPd to bumblebees (*Bombus* spp.) with a laboratory method and to demonstrate, that the median lethal dose, i.e. the LD50 at the end of exposure, is higher than the dose used in the test (limit test). One dose of the test item, i.e. 400.0 µg test item/bumblebee, plus the control with surfactant 1% Triton(R) X-100 and one dose of the reference item with surfactant were used. The design of the definitive test was selected on the basis of the non-GLP preliminary range- finding tests results.

Reference item:

Dimethoate technical was used to verify the precision of the test procedure and the sensitivity of the biological test system. It is in the form of crystalline solid. The Certificate of Analysis provides the following data: sample number: IPO 146; serial number: 3L/22; expiry date: June 2024; purity: 97.3 ± 0.6% (m/m), (CAS number: 60-51-5)

Test system:

A biological test system was the adult worker bumblebees (*Bombus* spp.) coming from 3 different families used for the definitive test. Bumblebees were taken from three healthy families consisting of 60 – 70 individuals.

Preparation of the medium

As a solvent of the test and reference item in the definitive test, distilled water with 1% of surfactant, Triton(R) X-100, was used. Triton(R) X-100 is in the form of a liquid. To prepare the 1% water solution, 1.0005 g of surfactant, Triton(R) X-100 were weighed and dissolved in distilled water. The volume was made up to 100 mL with distilled water.

Test item and reference item preparation:

In the first preliminary non-GLP range-finding test, the doses of 8.0, 40.0 and 200.0 µg test item/bumblebee (with 1% surfactant for all doses) were used. It was divided into 10 replicates (1 bumblebee/replicate). There was also a concurrent control group with surfactant (10 bumblebees) treated with 1% water solution of surfactant, Triton(R) X-100 (w/v). In the preliminary test, reference item was not used.

In the second preliminary non-GLP range-finding test, the dose of 400.0 µg test item/bumblebee (with 1% surfactant with all doses) was used. It was divided into 10 replicates (1 bumblebee/replicate). There was also a concurrent control group (10 bumblebees) treated with 1% water solution of surfactant, Triton(R) X-100 (w/v). In the second preliminary test, reference item was not used.

In the definitive test, one dose, i.e. 400.0 µg test item/bumblebee (with 1% surfactant with all doses) was used (limit test). There were 50 replicates with 1 bumblebee per replicate. There was also a concurrent control group with 1% surfactant, with 50 bumblebees each.

In the definitive test with range of doses, dimethoate was used as a reference item in order to verify the

sensitivity of the bumblebees.
There was one dose of the reference item, i.e. 10.0 µg/bumblebee. In the reference item group there were 30 replicates with one bumblebee per replicate.
Each insect was treated with 2 µL of the test item emulsion or reference item solution at the respective concentration. The surfactant control was treated with 2 µL of 1% water solution of surfactant, Triton(R) X-100.

Preliminary test:
In the first preliminary test, an emulsion of the test item at the highest concentration, i.e. of 200.0 µg/2 µL (corresponding to 200.0 µg/bumblebee) was prepared. First, 1.0007 g of the test item were weighed into a glass cylinder with a capacity of 10 mL. The volume was made up to 10 mL with 1% water solution of surfactant (Triton (R)X-100). The remaining emulsions of the test item at the concentrations of 40.0 and 8.0 µg/2 µL (with 1% surfactant) were prepared by making sequential 1:4 dilutions with 1% water solution of surfactant (v/v).
In the second preliminary test, an emulsion of the test item at the highest concentration, i.e. of 400.0 µg/2 µL (corresponding to 400.0 µg/bumblebee) was prepared. First, 2.0009 g of the test item were weighed into a glass cylinder with a capacity of 10 mL. The volume was made up to 10 mL with 1% water solution of surfactant (Triton (R)X-100).

Main test conditions:
In the definitive test, an emulsion of the test item at the concentration, i.e. 400.0 µg/2 µL, was prepared. First, 6.0078 g of the test item were weighed into a glass flask with a capacity of 50 mL. The volume was made up to 30 mL with 1% water solution of surfactant (Triton(R) X-100).
A solution of the reference item at the concentration of 10 µg/2 µL was prepared. First, 25.0 mg of dimethoate was weighed in a glass cylinder and filled up to a total volume of 5.0 mL with distilled water with 1% water solution of surfactant.
From the freshly prepared test item concentration of 400 µg/2 µL (with 1% surfactant) and the control with 1% surfactant, samples in a volume of 10 mL were collected and transferred for chemical determinations.

In the first preliminary test for the acclimatization, 40 bumblebees were taken. In the second preliminary test for the acclimatization, 20 bumblebees were taken. In the definitive test for the acclimatization, 130 bumblebees were taken including additional 5% of the total number of bumblebees, i.e. 7 bumblebees, were taken, in case of occuring mortality before start the treatment. These additional bumblebees were marked and discarded before treatment.
For two preliminary as well as for definitive test the adult bumblebees were collected from the hives under red light and individually placed in plastic isolators of known weight. There was 1 bumblebee in each isolator. The insects were selected for the exposure in terms of their sizes. Very small or very large individuals were excluded by visual inspection. After that, they were acclimatized to the test conditions for about 24 hours before starting the experiment. Food, i.e. 50% sucrose solution was provided. To determine the weight of each bumblebee, the isolator was weighed.
After acclimatization period, each bumblebee was introduced into a glass probe (15 cm long and 2.5 cm wide) plugged with a plastic stopper. There was a plunger inside each probe to immobilize a bumblebee during the application of the test item. Then, 2 µL of the test item emulsion, reference item solution or distilled water with 1% surfactant were applied to the dorsal part of the thorax with a microapplicator. After the application, the bumblebees were allocated back to isolators. The isolators were well-ventilated and provide enough space. During the whole experiment, the insects had continuous access to food, i.e. a 50% sucrose solution (w/v) in 2-mL syringes. Fresh food was added if necessary. During the experiment, the bumblebees were kept in constant darkness

Study results:
The median lethal dose LD_{50}/24 h, LD_{50}/48 h is higher than 400.0 µg/bumblebee (i.e. 44.67 µg dicamba/bumblebee, 15.55 µg nicosulfuron/bumblebee, 4.95 µg thifensulfuron-methyl/bumblebee).

The NOED value after 24 hours is higher than or equal to 400.0 µg/bumblebee. The NOED value after 48 hours is higher than or equal to 400.0 µg/bumblebee (i.e. 44.67 µg dicamba/bumblebee, 15.55 µg nicosulfuron/bumblebee, 4.95 µg thifensulfuron-methyl/bumblebee).

Results obtained at the end of the definitive test (after 48 hours):

Dose				Number of tested bumble-bees [no.]	Mortality after 48 h		LD_{50}			
Test item [µg/ bumble -bee]	Dicamba [µg of dicamba/ bumblebee]	Nicosulfuron [µg of nicosulfuron/ bumblebee]	Thifensulfuron-methyl [µg of thifensulfuron-methyl / bumblebee]		[no.]	[%]	Test item [µg/ bumble-bee]	Dicamba [µg of dicamba/ bumblebee]	Nicosulfuron [µg of nicosulfuron/ bumblebee]	Thifensulfuron-methyl [µg of thifensulfuron-methyl / bumblebee]
Control + 1% surfactant				50	0	0.0	> 400.0	> 44.67	> 15.55	> 4.95
400.0	44.67	15.55	4.95	50	1	2.0				
Reference item: dimethoate + 1% surfactant										
Dose [µg/bumblebee]		10.0		30	17	56.7	–			

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 Effect on arthropods other than bees

A 2.3.2.1 KCP 10.3.2.1 Extended laboratory testing and aged residue studies

A 2.3.2.1.1 Study 1: toxicity to *Aphidius rhophalosiphi*

Comments of zRMS:	The study is acceptable. The validity criteria of the test according IOBC, BART and EPPO Joint Initiative. M.P. Candolfi, et al. (2000) and Mead-Briggs M.A. et al. (2010) were met. **Validity criteria:** • mortality of organisms in control group after 48 h should be <10%, mortality in definitive test was 6.7%. • mortality of organism in reference group after 48 h should be >50 %; mortality in reference test was 100.0% • reproduction in control group should be >5 mummies/female (female should be alive after 24 h parasitisation period) and should be no more than two wasps producing no mummies) • the number of mummies per female was 13.5

- none of the surviving wasps in the negative control produced zero mummies (for the test to be considered valid, no more than two of the surviving wasps should produce zero values).

Deviation of the study: Deviations from the Study plan were found concerning changes in humidity during range finding test. During the range-finding test, the humidity lowered to 41.4% (requirements: 60-90%). The above deviations were temporary and did not affect the condition of the test system. These deviations did not affect the results of the study. The validity criteria of the test have been met.

Agreed toxicity endpoints:

Impact of test item on survival					
Dose [mL test item /400 L water/ha]	Mortality [%]	Abbott corrected mortality [%]	LR_{50}	NOER	LOER
			[mL test item/400 L water/ha]		
Control	6.7	not applicable	> 2000	≥ 2000	> 2000
125	6.7	0.0	Dicamba > 230.0 g a.s.	Dicamba ≥ 230.0 g a.s.	Dicamba > 230.0 g a.s.
250	0.0	0.0			
500	0.0	0.0	Nicosulfuron > 78.8 g a.s.	Nicosulfuron ≥ 78.8 g a.s.	Nicosulfuron > 78.8 g a.s.
1000	3.3	0.0	Thifensulfuron-methyl > 24.1 g a.s.	Thifensulfuron-methyl ≥ 24.1 g a.s.	Thifensulfuron-methyl > 24.1 g a.s.
2000	6.7	0.0			
Reference item - 4 g dimethoate /400 L water/ha	100.0	100.0			

Impact of test item on reproduction					
Dose [mL test item /400 L water/ha]	Average number of offspring per female [pcs.]	Reduction of offspring [%]	LR_{50}	NOER	LOER
			[mL test item/400 L water/ha]		
Control	13.5	not applicable	> 2000	≥ 2000	> 2000
125	13.2	2.5	Dicamba > 230.0 g a.s.	Dicamba ≥ 230.0 g a.s.	Dicamba > 230.0 g a.s.
250	12.1	10.3			
500	12.1	10.8	Nicosulfuron > 78.8 g a.s.	Nicosulfuron ≥ 78.8 g a.s.	Nicosulfuron > 78.8 g a.s.
1000	10.8	20.2	Thifensulfuron-methyl > 24.1 g a.s.	Thifensulfuron-methyl ≥ 24.1 g a.s.	Thifensulfuron-methyl > 24.1 g a.s.
2000	10.9	19.2			

Repellency assessment						
Time	Dose [mL of the test item/400L water/ha]					
	Control	125	250	500	1000	2000
0.5 h	56.667	63.333	63.333	66.667	66.667	70.000
1 h	60.000	70.000	66.667	66.667	66.667	70.000
1.5 h	56.667	66.667	63.333	56.667	66.667	70.000
2 h	66.667	63.333	63.333	70.000	63.333	66.667
2.5 h	66.667	70.000	73.333	66.667	66.667	70.000
24 h	60.000	73.333	66.667	72.500	66.667	76.667
48 h	70.833	70.833	60.000	73.333	69.167	63.333

Reference:	KCP 10.3.2_01
Report	Extended laboratory test to evaluate effects on *Aphidius rhophalosiphi* (DeStephani-Perez) of the test item DNT-162OD-R-CPd, Kręglewska M., 2023, 0016/0178/E
Guideline(s):	IOBC, BART and EPPO Joint Initiative. M.P. Candolfi, et al. (2000) and Mead-Briggs M.A. et al. (2010)
Deviations:	Yes
GLP:	Yes

Acceptability:	Yes
Duplication (if vertebrate study)	-

Test item:

Name	DNT-162OD-R-CPd
Type of packaging material	HDPE
Test item appearance	white to beige color
Date of receipt	22.07.2022
Batch No.	13/22
Production date	13.07.2022
Expire date	13.07.2024
Water solubility, other solvents solubility, ability to form emulsion, solution, dispersion	product in the form of an oil suspension
Name of active substance	Dicamba
IUPAC nomenclature of active substance	2,5-dichloro-6-methoxybenzoic acid
Molecular weight	221.03 g/mol
Molecular formula	C8H6Cl2O3
Content of active substance	115 g/L
CAS of active substance	1918-00-9
Name of active substance	Nicosulfuron
IUPAC nomenclature of active substance	2-[(4,6-dimethoxypyrimidin-2-ylcarbamoyl)sulfamoyl]-N,N-dimethylnicotinamide
Molecular weight	410.4 g/mol
Molecular formula	C15H18N6O6S
Content of active substance	39.40 g/L
CAS of active substance	111991-09-4
Name of active substance	Thifensulfuron-methyl
IUPAC nomenclature of active substance	methyl 3-(4-methoxy-6-methyl-1,3,5-triazin-2-ylcarbamoylsulfamoyl)thiophene-2-carboxylate
Molecular weight	387.4 g/mol
Molecular formula	C12H13N5O6S2
Content of active substance	12.03 g/L
CAS of active substance	79277-27-3

Reference item:

Parallel to the definitive test (mortality phase), the reference tests were conducted with using a reference item: dimethoate. The reference item dose used in the study: 4 g dimethoate/400 L water/ha.

Name	Dimethoate HPC Standards GmbH
Batch No.	806992
CAS of reference item	60-51-5
Purity	99.29 % (w/w)
IUPAC nomenclature	2-Dimethoxy-phosphinothioylthio-N-methylacetamide
Molecular weight	229.30 g/mol
Molecular formula	C5H12NO3PS2
Expiry date	01.01.2027

Materials and methods

Tested species:

The study was conducted on the parasitic wasp *Aphidius rhopalosiphi* (Hymenoptera: Braconidae), which is one of the most sensitive standard indicator species for non-target arthropod regulatory testing for plant protection products. *Aphidius rhopalosiphi* were purchased as synchronized aphid mummies *Rhopalosiphum padi* from breeder which has a certificate confirming their species, ie. Katz Biotech AG, An der Birkenpfuhlheide 10, D-15837 Baruth, Germany. In the study were used adults, 1-2 days old *Aphidius rhopalosiphi* individuals.

Test design:

The study was conducted on the basis of the to IOBC, BART and EPPO Joint Initiative guideline and Mead-Briggs et al. (2010), using barley seedlings (*Hordeum vulgare*) as a test unit.

The study was conducted in two stages: a range-finding test and definitive test.
In order to determine the concentration range of the test item solutions for the definitive test, a range-finding test was performed in which a mortality and a repellency were assessed.
In the definitive test, the mortality, repellency and the reproduction of the *Aphidius rhopalosiphi* were assessed.

Results:
The test item in extended laboratory test did not show the statistically significant impact on survival and reproduction of wasps *Aphidius rhophalosiphi* from dose 125 mL of the test item/400L water/ha to dose 2000 mL of the test item/400L water/ha and repellence assessment did not show repellency properties of the test item. The end points of the test causing 50% mortality of the population in the test (LR50) and 50% decrease in the reproduction of the test organisms (ER50) were determined. The NOER and LOER values were also determined.

Impact of test item on survival					
Dose [mL test item /400 L water/ha]	Mortality [%]	Abbott corrected mortality [%]	LR_{50}	NOER	LOER
			[mL test item/400 L water/ha]		
Control	6.7	not applicable	> 2000 Dicamba > 230.0 g a.s. Nicosulfuron > 78.8 g a.s. Thifensulfuron-methyl > 24.1 g a.s.	≥ 2000 Dicamba ≥ 230.0 g a.s. Nicosulfuron ≥ 78.8 g a.s. Thifensulfuron-methyl ≥ 24.1 g a.s.	> 2000 Dicamba > 230.0 g a.s. Nicosulfuron > 78.8 g a.s. Thifensulfuron-methyl > 24.1 g a.s.
125	6.7	0.0			
250	0.0	0.0			
500	0.0	0.0			
1000	3.3	0.0			
2000	6.7	0.0			
Reference item - 4 g dimethoate /400 L water/ha	100.0	100.0			
Impact of test item on reproduction					
Dose [mL test item /400 L water/ha]	Average number of offspring per female [pcs.]	Reduction of offspring [%]	LR_{50}	NOER	LOER
			[mL test item/400 L water/ha]		
Control	13.5	not applicable	> 2000 Dicamba > 230.0 g a.s. Nicosulfuron > 78.8 g a.s. Thifensulfuron-methyl > 24.1 g a.s.	≥ 2000 Dicamba ≥ 230.0 g a.s. Nicosulfuron ≥ 78.8 g a.s. Thifensulfuron-methyl ≥ 24.1 g a.s.	> 2000 Dicamba > 230.0 g a.s. Nicosulfuron > 78.8 g a.s. Thifensulfuron-methyl > 24.1 g a.s.
125	13.2	2.5			
250	12.1	10.3			
500	12.1	10.8			
1000	10.8	20.2			
2000	10.9	19.2			

A 2.3.2.1.1 Study 2: toxicity to *Typhlodromus pyri*

Comments of zRMS:	The study is acceptable. The validity criteria of the test according IOBC, BART and EPPO Joint Initiative, M.P. Candolfi, et al. (2000) and S. Blümel, et al. (2000) were met. **Validity criteria:**

– mortality of juveniles for control group on day 7 should be <20%; mortality in control in definitive test was 9.0%
– mortality of juveniles for reference group on day 7 should be >50 %; mortality in reference test was 96.3%.

Deviation of the study: During the range-finding, definitive and reference test changes in humidity took place. They resulted from everyday activities and observations and were recorded and corrected on an ongoing basis. These were short-term changes which did not affect the condition of the test system. The above deviations did not affect the test result. The study met the validity criteria.

Agreed toxicity endpoints:

Dose [mL test item /200 L water/ha]	Impact of test item on survival				
	Mortality [%]	Abbott corrected mortality [%]	LR_{50}	NOER	LOER
			[mL test item /200 L water/ha]		
Control	9.0	not applicable	1403.1 (1270.1 – 1569.4)*	500.0	1000.0
125.0	7.0	0.0	Dicamba 161.4 g a.s. (146.1 – 180.5) Nicosulfuron 55.3 g a.s. (50.0 – 61.8) Thifensulfuron-methyl 16.9 g a.s. (15.3 – 18.9)	Dicamba 57.5 g a.s. Nicosulfuron 19.7 g a.s. Thifensulfuron-methyl 6.0 g a.s.	Dicamba 115.0 g a.s. Nicosulfuron 39.4 g a.s. Thifensulfuron-methyl 12.0 g a.s.
250.0	9.0	0.0			
500.0	11.0	2.2			
1000.0	39.0	33.0			
2000.0	73.0	70.3			
Reference item 6.8 g dimethoate /200L water/ha	96.3	95.9			

Dose [mL test item/200 L water/ha]	Impact of test item on reproduction				
	Average number of offspring per female [psc.]	Reproduction reduction [%]**	ER_{50}	NOER	LOER
			[mL test item /200 L water/ha]		
Control	6.0	not applicable	>1000.0*** (n.d. – n.d.)	250.0	500.0
125.0	5.6	6.6	Dicamba > 115.0 g a.s. Nicosulfuron > 39.4 g a.s. Thifensulfuron-methyl > 12.0 g a.s.	Dicamba 28.8 g a.s. Nicosulfuron 9.9 g a.s. Thifensulfuron-methyl 3.0 g a.s.	Dicamba 57.5 g a.s. Nicosulfuron 19.7 g a.s. Thifensulfuron-methyl 6.0 g a.s.
250.0	5.6	6.1			
500.0	5.5	8.0			
1000.0	5.2	13.1			

LR_{50} statistically calculated dose of the test item which caused 50% mortality of treated population after 7 days; the value is calculated by using ToxRat Professional with Probit Analysis with at the significance level p>0.05
ER_{50} statistically calculated dose of the test item which caused an occurrence of observed effect of 50% population treated by test item after 14 days; the value was calculated by the 3-parameter normal cumulative distribution function
NOER the highest dose of test item cause no statistically significant differences compared to control
LOER the lowest dose of test item cause statistically significant differences compared to control
a.s. active substance (content of active substance in 200 L water per ha)
* the lower and upper 95% confidence limits are given in brackets
** calculated according to formula presented in point 4.9.3
*** values determined on the basis of ToxRat results analysis

Reference: KCP 10.3.2_02

Report Extended laboratory test to evaluate effects on *Typhlodromus pyri* (Scheuten)

	of the test item DNT-162OD-R-CPd, Kręglewska M., 2023, 0016/0175/E
Guideline(s):	IOBC, BART and EPPO Joint Initiative, M.P. Candolfi, et al. (2000) and S. Blümel, et al. (2000)
Deviations:	Yes
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Test item:

Name	DNT-162OD-R-CPd
Type of packaging material	HDPE
Test item appearance	white to beige color
Date of receipt	22.07.2022
Batch No.	13/22
Production date	13.07.2022
Expire date	13.07.2024
Water solubility, other solvents solubility, ability to form emulsion, solution, dispersion	product in the form of an oil suspension
Name of active substance	Dicamba
IUPAC nomenclature of active substance	2,5-dichloro-6-methoxybenzoic acid
Molecular weight	221.03 g/mol
Molecular formula	C8H6Cl2O3
Content of active substance	115 g/L
CAS of active substance	1918-00-9
Name of active substance	Nicosulfuron
IUPAC nomenclature of active substance	2-[(4,6-dimethoxypyrimidin-2-ylcarbamoyl)sulfamoyl]-N,N-dimethylnicotinamide
Molecular weight	410.4 g/mol
Molecular formula	C15H18N6O6S
Content of active substance	39.40 g/L
CAS of active substance	111991-09-4
Name of active substance	Thifensulfuron-methyl
IUPAC nomenclature of active substance	methyl 3-(4-methoxy-6-methyl-1,3,5-triazin-2-ylcarbamoylsulfamoyl)thiophene-2-carboxylate
Molecular weight	387.4 g/mol
Molecular formula	C12H13N5O6S2
Content of active substance	12.03 g/L
CAS of active substance	79277-27-3

Reference item:

Parallel to the definitive test (mortality phase), the reference test was conducted with using a reference item: dimethoate. The aim of the study was a determination of physiological condition of predatory mites which were used in the study and an evaluation of a reliability of the study.
A single dose of the reference item was use: 6.8 g dimethoate/200L water/ha.

Name	Dimethoate HPC Standards GmbH
Batch No.	806992
CAS of reference item	60-51-5
Purity	99.29 % (w/w)
IUPAC nomenclature	2-Dimethoxy-phosphinothioylthio-N-methylacetamide
Molecular weight	229.30 g/mol
Molecular formula	C5H12NO3PS2
Expiry date	01.01.2027

Materials and methods

Tested species:
The study was conducted on the predatory mite *Typhlodromus pyri*, which is of the most sensitive standard indicator species for non-target arthropod regulatory testing for plant protection products. Mites for range-finding test, definitive test and reference test were purchased as synchronized eggs from breeder which has a certificate confirming their species.

Test design:
The study consisted in two steps: the assessment of the mortality of young mites and evaluation of reproductive ability of organisms. The first step involved the exposure of young mites to dose range of the test for 7 days. The test item was applied on the bean leaf surface. In the second part of the study, in reproductive phase, reproductive ability were observed for a further 7 days on dosage levels in which min. 50% of the initial number of organisms survived. At that time, the adult mites were laying eggs. After 10, 12, 14 days from the beginning of the study, the number of eggs and larvae in specified doses of the test item was checked, as well as the number of females.
In order to determine the dose range of the test item in the definitive test, a range-finding test was performed.

Results:
The test item in extended laboratory test showed the statistically significant impact on survival of predatory mites *Typhlodromus pyri* from dose 1000.0 mL of the test item/200 L water/ha to dose 2000.0 mL of the test item/200 L water/ha. The test item showed significant impact on the reproduction of predatory mites *Typhlodromus pyri* from dose 500 mL of the test item/200 L water/ha to dose 1000.0 mL of the test item/200 L water/ha (in dose 2000.0 mL of the test item/200 L water/ha mortality after 7 days was 73.0% and it caused disqualification for reproduction phase; additionally, at dose 2000.0 mL of the test item/200 L water/ha the sex ratio was insufficient).

Dose [mL test item /200 L water/ha]	Impact of test item on survival				
	Mortality [%]	Abbott corrected mortality [%]	LR_{50}	NOER	LOER
			[mL test item /200 L water/ha]		
Control	9.0	not applicable	1403.1 (1270.1 – 1569.4)*	500.0	1000.0
125.0	7.0	0.0	Dicamba 161.4 g a.s. (146.1 – 180.5) Nicosulfuron 55.3 g a.s. (50.0 – 61.8) Thifensulfuron-methyl 16.9 g a.s. (15.3 – 18.9)	Dicamba 57.5 g a.s. Nicosulfuron 19.7 g a.s. Thifensulfuron-methyl 6.0 g a.s.	Dicamba 115.0 g a.s. Nicosulfuron 39.4 g a.s. Thifensulfuron-methyl 12.0 g a.s.
250.0	9.0	0.0			
500.0	11.0	2.2			
1000.0	39.0	33.0			
2000.0	73.0	70.3			
Reference item 6.8 g dimethoate /200L water/ha	96.3	95.9			
Dose [mL test item/200 L water/ha]	Impact of test item on reproduction				
	Average number of offspring per female [psc.]	Reproduc-tion reduction [%]**	ER_{50}	NOER	LOER
			[mL test item /200 L water/ha]		
Control	6.0	not applicable	>1000.0*** (n.d. – n.d.)	250.0	500.0
125.0	5.6	6.6	Dicamba > 115.0 g a.s. Nicosulfuron > 39.4 g a.s. Thifensulfuron-methyl > 12.0 g a.s.	Dicamba 28.8 g a.s. Nicosulfuron 9.9 g a.s. Thifensulfuron-methyl 3.0 g a.s.	Dicamba 57.5 g a.s. Nicosulfuron 19.7 g a.s. Thifensulfuron-methyl 6.0 g a.s.
250.0	5.6	6.1			
500.0	5.5	8.0			
1000.0	5.2	13.1			

A 2.3.2.1.3 Study 3: toxicity to *Coccinella septempunctata*

Comments of zRMS:	The study is acceptable. The validity criteria of the test according SETAC; ESCORT I, II; IOBC/BART/EPPO were met. **Validity criteria:** The study met the reliability criteria in accordance with the

guideline:

- control larvae mortality: 6.7% (requirements <30%).

Deviation of the study: Deviations from the Study plan were found, concerning minimum and maximum temperature during range-finding test, definitive test, reference test and minimum humidity during definitive test, reference test. They resulted from everyday activities and observations and were recorded and corrected on an ongoing basis. These were short-term changes which did not affect the condition of the test system. The experimental part of the study was completed in December 2022, not as planned in November 2022.These deviations did not affect the results of the study. The validity criteria of the test have been met.

Agreed toxicity endpoints:

Dose [mL of the test item /200 L water/ha]	Impact of the test item on survival						
	Mortality [%]	Abbott corected mortality [%]	LR_{10}	LR_{20}	LR_{50}	NOER	LOER
			[mL of the test item/200 L water/ha]				
Control	13.3	n.a.	n.d.	21.406	283.137	<31.250	≤31.250
31.25	33.3	23.1	[g of active substance/200 L water/ha]				
62.5	40.0	30.8	Dicamba: n.d. Nicosulfuron: n.d. Thifensulfuron-methyl: n.d.	Dicamba: 2.462 Nicosulfuron: 0.843 Thifensulfuron-methyl: 0.258	Dicamba: 32.561 Nicosulfuron: 11.156 Thifensulfuron-methyl: 3.406	Dicamba: <3.594 Nicosulfuron: <1.231 Thifensulfuron-methyl: <0.376	Dicamba: ≤3.594 Nicosulfuron: ≤1.231 Thifensulfuron-methyl: ≤0.376
125	50.0	42.3					
250	53.3	46.1					
500	63.3	57.7					

Reference test	Mortality [%]	Abbott corected mortality [%]
Control	0.0	n.a.
3.2 g of dimethoate/ 200 L water /ha	100.00	100.00

Dose [mL of the test item /200 L water /ha]	Impact of the test item on reproduction			
	Average number of eggs/female/day [pcs.]	Average hatching [%]	Average number of hatchable eggs/female/day [pcs.]	Significance*)
Control	7.9	76.5	6.1	n.a.
31.25	7.1	66.4	4.7	-
62.5	6.6	62.9	4.2	-
125	6.1	60.3	3.7	-

LR_{10} dose of the test item resulting in a reduction of 10%
LR_{20} dose of the test item resulting in a reduction of 20%
LR_{50} dose of the test item resulting in a reduction of 50%
NOER the highest dose of the test item did not cause statistically significant differences compared to the control
LOER the lowest dose of the test item causing statistically significant differences compared to the control
*) the average number of hattched egg / female / day is > 2.0 for control, which means that the test item has no effect on reproductive ability
n.a. not applicable
n.d. not determined due to mathematical reasons or value is beyond the tested rates
- insignificant

Reference:	KCP 10.3.2_03
Report	Extended laboratory test to determine the effects of the test item DNT-162OD-R-

	CPd on the ladybird beetle (*Coccinella septempunctata*), Domagała J., 2023, 0016/0177/E
Guideline(s):	SETAC; ESCORT I, II; IOBC/BART/EPPO
Deviations:	Yes
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Test item:

Name	DNT-162OD-R-CPd
Type of packaging material	HDPE
Test item appearance	white to beige color
Date of receipt	22.07.2022
Batch No.	13/22
Production date	13.07.2022
Expire date	13.07.2024
Water solubility, other solvents solubility, ability to form emulsion, solution, dispersion	product in the form of an oil suspension
Name of active substance	Dicamba
IUPAC nomenclature of active substance	2,5-dichloro-6-methoxybenzoic acid
Molecular weight	221.03 g/mol
Molecular formula	C8H6Cl2O3
Content of active substance	115 g/L
CAS of active substance	1918-00-9
Name of active substance	Nicosulfuron
IUPAC nomenclature of active substance	2-[(4,6-dimethoxypyrimidin-2-ylcarbamoyl)sulfamoyl]-N,N-dimethylnicotinamide
Molecular weight	410.4 g/mol
Molecular formula	C15H18N6O6S
Content of active substance	39.40 g/L
CAS of active substance	111991-09-4
Name of active substance	Thifensulfuron-methyl
IUPAC nomenclature of active substance	methyl 3-(4-methoxy-6-methyl-1,3,5-triazin-2-ylcarbamoylsulfamoyl)thiophene-2-carboxylate
Molecular weight	387.4 g/mol
Molecular formula	C12H13N5O6S2
Content of active substance	12.03 g/L
CAS of active substance	79277-27-3

Reference item:

In order to determine the condition of the test system, a test with the use of the reference item - dimethoate was carried out in parallel with the definitive test. Reference test involved the mortality phase. One reference item concentration of 3.2 g dimethoate/200 L of water/ha and control was used in the experiment in 30 replicates, one larva per replicate.

Name	Dimethoate HPC Standards GmbH
Batch No.	806992
CAS of reference item	60-51-5
Purity	99.29 % (w/w)
IUPAC nomenclature	2-Dimethoxy-phosphinothioylthio-N-methylacetamide
Molecular weight	229.30 g/mol
Molecular formula	C5H12NO3PS2
Expiry date	01.01.2027

Materials and methods

Tested species:

The test organisms used for the study were the ladybird beetle (*Coccinella septempunctata*) larvae, which are an indicator species for assessing the effects of plant protection products on non-target arthropods. The organisms for the experiments were purchased in the form of synchronized eggs from the breeder having a certificate confirming their species.

Test design:
The study consisted of two stages. Larvae and pupae mortality assessment: the test item was sprayed onto the test leaf surface of bean. After drying, ladybird beetle larvae were introduced on the test surface and left there until pupated. The mortality of the organisms was assessed during this stage.
Reproduction assessment: if 50% of the larvae exposed to a given dose of test item survived and were able to pupate, the organisms were observed for reproduction. The reproduction assessment was carried out one week after the start of egg laying in the control (the number of eggs is stabilized during this time). The number of eggs laid by females and the ability to hatch for the were assessed daily (on working days) during 14 days.

Results:
The test item in the conditions of an extended laboratory test showed a statistically significant effect on the survival of the larvae and pupae of the ladybird beetle (*Coccinella septempunctata*) in doses of 31.25 mL of the test item/200 L of water/ha, 62.5 mL of the test item/200 L of water/ha, 125 mL of the test item/200 L of water/ha, 250 mL of the test item/200 L of water/ha and 500 mL of the test item/ 200 L of water/ha.
The test item did not affect the reproductive abilities of the ladybird beetle (*Coccinella septempunctata*) in dose of 31.25 mL of the test item/200 L of water/ha, 62.5 mL of the test item/200 L of water/ha and 125 mL of the test item/200 L of water/ha. Individuals from the dose of 250 mL of the test item/200 L of water/ha and 500 mL of the test item/200 L of water/ha after the mortality assessment phase were not assessed for reproduction. This was due to a mortality of >50% of the introduced organisms. Average number of laid eggs/female/day for test dose 31.25 mL of the test item/200 L of water/ha, 62.5 mL of the test item/200 L of water/ha and 125 mL of the test item/200 L of water/ha was >2.0, which, in accordance with the adopted method of assessment (based on historical data), means no effect of the test item on reproductive ability.

Dose [mL of the test item /200 L water/ha]	Impact of the test item on survival						
	Mortality [%]	Abbott corected mortality [%]	LR_{10}	LR_{20}	LR_{50}	NOER	LOER
			[mL of the test item/200 L water/ha]				
Control	13.3	n.a.	n.d.	21.406	283.137	<31.250	≤31.250
31.25	33.3	23.1	[g of active substance/200 L water/ha]				
62.5	40.0	30.8	Dicamba: n.d. Nicosulfuron: n.d. Thifensulfuron-methyl: n.d.	Dicamba: 2.462 Nicosulfuron: 0.843 Thifensulfuron-methyl: 0.258	Dicamba: 32.561 Nicosulfuron: 11.156 Thifensulfuron-methyl: 3.406	Dicamba: <3.594 Nicosulfuron: <1.231 Thifensulfuron-methyl: <0.376	Dicamba: ≤3.594 Nicosulfuron: ≤1.231 Thifensulfuron-methyl: ≤0.376
125	50.0	42.3					
250	53.3	46.1					
500	63.3	57.7					
Reference test	**Mortality [%]**	**Abbott corected mortality [%]**					
Control	0.0	n.a.					
3.2 g of dimethoate/ 200 L water /ha	100.00	100.00					

Dose [mL of the test item /200 L water /ha]	Impact of the test item on reproduction			
	Average number of eggs/female/day [pcs.]	Average hatching [%]	Average number of hatchable eggs/female/day [pcs.]	Significance*)
Control	7.9	76.5	6.1	n.a.
31.25	7.1	66.4	4.7	-
62.5	6.6	62.9	4.2	-
125	6.1	60.3	3.7	-

A 2.3.2.1.4 Study 4: toxicity to *Chrysoperla carnea*

Comments of zRMS:	The study is acceptable. The validity criteria of the test according IOBC, BART, EPPO Joint Initiative and Vogt et al., 2000 were met. **Validity criteria:** The study met the reliability criteria in accordance with the guideline: • control cumulative mortality — dead larvae, pupae and adults died during emergence or not successfully moulted: 0.00% (requirements <20%). **Deviation of the study:** During the range-finding test, the temperature lowered to minimum of 20.9°C (requirements: 25±2°C). During the definitive test, the temperature increased to maximum of 27.9°C and lowered to minimum of 19.9°C (requirements: 25±2°C) and relative humidity was lowered to minimum of 48.4% (requirements: 60-90%). During the reference test, minimum temperature was increased to

maximum of 27.9°C and lowered to minimum of 21.5°C (requirements: 25±2°C) and relative humidity was lowered to minimum of 48.4%. Experimental part ended in December instead of planned November in Study plan. The above deviations were temporary and did not affect the condition of the test system. These deviations did not affect the results of the study. The validity criteria of the study have been met.

Agreed toxicity endpoints:

Dose [mL of the test item/200 L water/ha]	Impact of the test item on survival						
	Mortality [%]	Abbott corected mortality [%]	LR_{10}	LR_{20}	LR_{50}	NOER	LOER
Control	0.00	n.a.	[mL of the test item/200 L water/ha]				
31.25	13.33	n.a.	19.399	49.622	205.006	<31.250	≤31.250
62.5	23.33	n.a.	[g of active substance/200 l water/ha]				
125	36.67	n.a.	Dicamba 2.231 g Nicosulfuron 0.764 g Thifensulfuron -methyl 0.233 g	Dicamba 5.707 g Nicosulfuron 1.955 g Thifensulfuron -methyl 0.597 g	Dicamba 23.576 g Nicosulfuron 8.077 g Thifensulfuron -methyl 2.466 g	Dicamba <3.594 g Nicosulfuron <1.231 g Thifensulfuron -methyl <0.376 g	Dicamba ≤3.594 g Nicosulfuron ≤1.231 g Thifensulfuron -methyl ≤0.376 g
250	60.00	n.a.					
500	73.33	n.a.					
Reference item [15 g of dimethoate/ 200 L water/ha]	93.33	n.a.					

Dose [mL of the test item/ 200 L water/ha]	Impact of the test item on reproduction		
	Average number of eggs per female per day [psc.]	Hatching rate [%]	Significance*
Control	19.82	89.29	not applicable
31.25	19.04	91.89	-
62.5	18.82	80.80	-
125	19.28	89.94	-

LR_{10} dose of the test item resulting in a reduction of 10%
LR_{20} dose of the test item resulting in a reduction of 20%
LR_{50} dose of the test item resulting in a reduction of 50%
NOER the highest dose of the test item did not cause statistically significant differences compared to the control
LOER the lowest dose of the test item causing statistically significant differences compared to the control
n.a. not applicable because the pre-selected Abbott-correction is not needed since the control response is 0.00%
* the average number of eggs/female /day is ≥15 and hatching rate is ≥70%, which means that the test item has no effect on reproductive ability

Reference:	KCP 10.3.2_04
Report	Extended laboratory test to determine the effects of the test item DNT-162OD-R-CPd on the green lacewing (*Chrysoperla carnea*), Kubisiak K., 2023, 0016/0176/E
Guideline(s):	IOBC, BART, EPPO Joint Initiative and Vogt et al., 2000.
Deviations:	Yes
GLP:	Yes
Acceptability:	Yes

Duplication -
(if vertebrate study)

Test item:

Name	DNT-162OD-R-CPd
Type of packaging material	HDPE
Test item appearance	white to beige color
Date of receipt	22.07.2022
Batch No.	13/22
Production date	13.07.2022
Expire date	13.07.2024
Water solubility, other solvents solubility, ability to form emulsion, solution, dispersion	product in the form of an oil suspension
Name of active substance	Dicamba
IUPAC nomenclature of active substance	2,5-dichloro-6-methoxybenzoic acid
Molecular weight	221.03 g/mol
Molecular formula	C8H6Cl2O3
Content of active substance	115 g/L
CAS of active substance	1918-00-9
Name of active substance	Nicosulfuron
IUPAC nomenclature of active substance	2-[(4,6-dimethoxypyrimidin-2-ylcarbamoyl)sulfamoyl]-N,N-dimethylnicotinamide
Molecular weight	410.4 g/mol
Molecular formula	C15H18N6O6S
Content of active substance	39.40 g/L
CAS of active substance	111991-09-4
Name of active substance	Thifensulfuron-methyl
IUPAC nomenclature of active substance	methyl 3-(4-methoxy-6-methyl-1,3,5-triazin-2-ylcarbamoylsulfamoyl)thiophene-2-carboxylate
Molecular weight	387.4 g/mol
Molecular formula	C12H13N5O6S2
Content of active substance	12.03 g/L
CAS of active substance	79277-27-3

Reference item:

In order to determine the condition of the test system, a test with the use of the reference item - dimethoate was carried out in parallel with the definitive test. Reference test involved the mortality phase. One reference item concentration of 15 g dimethoate/200 L of water/ha was used in the experiment in 30 replicates.

Name	Dimethoate HPC Standards GmbH
Batch No.	806992
CAS of reference item	60-51-5
Purity	99.29 % (w/w)
IUPAC nomenclature	2-Dimethoxy-phosphinothioylthio-N-methylacetamide
Molecular weight	229.30 g/mol
Molecular formula	C5H12NO3PS2
Expiry date	01.01.2027

Materials and methods

Tested species:

The test organisms used for the study were the green lacewings (*Chrysoperla carnea*) larvae, which are an indicator species for assessing the effects of plant protection products on non-target arthropods. The organisms for the experiments were purchased in the form of synchronized eggs from the breeder. Young, 3-day-old larvae were used in the test.

Test design:

The study was conducted on the basis of the IOBC, BART, EPPO Joint Initiative and Vogt et al., 2000 using bean leaves (*Phaseolus vulgaris*) as a test surface.
The study consisted of two stages. Larvae, pupae and adults during emergence mortality assessment:

the test item was sprayed onto the test leaf surface. After drying, 1 hour after spraying, green lacewing larvae were introduced on the test surface and left there up to the 5th day from the formation of pupae. Then pupae were transferred to larger containers, where adults hatching. The mortality of the organisms was assessed during this stage. Reproduction assessment : if 50% of the larvae exposed to a given dose of test item survived and were able to pupate, the organisms were observed for reproduction. The reproduction assessment was carried out one week after the start of egg laying. The number of eggs laid by females within 24 hours and the ability to hatch were assessed twice a week.

Results:
The test item in the conditions of an extended laboratory test showed a statistically significant effect on the survival of the larvae, pupae and adults during emergence of the green lacewing (*Chrysoperla carnea*) in all tested doses (from 31.25 mL of the test item/200 L of water /ha to 500 mL of the test item/200 L of water /ha). The test item did not affect the reproductive abilities of the green lacewing (*Chrysoperla carnea*). Average number of laid eggs/female/day for control and tested doses (31.25 mL of the test item/200 L of water/ha, 62.5 mL of the test item/ 200 L of water/ha and 125 mL of the test item/200 L of water/ha) is ≥15 and mean hatching rate is ≥70%, which, in accordance with the adopted method of assessment, means no effect of the tested item on reproductive ability. Individuals from the dose of 250 mL of the test item/200 L of water/ha and 500 mL of the test item/200 L of water/ha after the mortality assessment phase were not assessed for reproduction due to a mortality of >50% of the introduced organisms.

Dose [mL of the test item/200 L water/ha]	Impact of the test item on survival						
	Mortality [%]	Abbott corected mortality [%]	LR_{10}	LR_{20}	LR_{50}	NOER	LOER
Control	0.00	n.a.	[mL of the test item/200 L water/ha]				
31.25	13.33	n.a.	19.399	49.622	205.006	<31.250	≤31.250
62.5	23.33	n.a.	[g of active substance/200 l water/ha]				
125	36.67	n.a.	Dicamba 2.231 g Nicosulfuron 0.764 g Thifensulfuron -methyl 0.233 g	Dicamba 5.707 g Nicosulfuron 1.955 g Thifensulfuron -methyl 0.597 g	Dicamba 23.576 g Nicosulfuron 8.077 g Thifensulfuron -methyl 2.466 g	Dicamba <3.594 g Nicosulfuron <1.231 g Thifensulfuron -methyl <0.376 g	Dicamba ≤3.594 g Nicosulfuron ≤1.231 g Thifensulfuron -methyl ≤0.376 g
250	60.00	n.a.					
500	73.33	n.a.					
Reference item [15 g of dimethoate/ 200 L water/ha]	93.33	n.a.					

Dose [mL of the test item/ 200 L water/ha]	Impact of the test item on reproduction		
	Average number of eggs per female per day [psc.]	Hatching rate [%]	Significance*
Control	19.82	89.29	not applicable
31.25	19.04	91.89	-
62.5	18.82	80.80	-
125	19.28	89.94	-

A 2.3.2.1.5 Study 5: Age residue study on *Ch.carnea*

Comments of zRMS:	The study is acceptable. The validity criteria of the test according IOBC, BART, EPPO Joint Initiative, Vogt et al., 2000 and ESCORT 2, Candolfi et al. 2001 were met. **Validity criteria:** – mortality of larvae, pupae and adults during emergence in the control must not exceed 20% – mortality of larvae exposed to the reference item should be ≥50% – the number of eggs laid by a female per day must be at least 15 eggs in control – the hatching rate: ≥70% in control. **Deviation of the study:**

1. During study initiated at 0 DAT temperature lowered to 22.2°C, highered to 27.7°C, average temperature was 25.5°C (requirements: 25±2°C).
During the study initiated at 14 DAT temperature lowered to 22.1°C, highered to 27.7°C, average temperature was 25.2°C (requirements: 25±2°C).
During the study initiated at 28 DAT temperature lowered to 22.1°C, highered to 27.7°C, average temperature was 25.1°C (requirements: 25±2°C).
In all bioassays the average temperature and humidity during study was in the range of the requirements.
Deviation from Study plan was found:
2. Experimental part of the study ended in September 2023 instead of planned August 2023.
The above deviations did not affect the study results. The study met the validity criteria.

Agreed toxicity endpoints:

Treatment	DAT*	Mortality assessment		Reproduction assessment		
		Mortality [%]	Abbott corrected mortality [%]	Average number of eggs/ female/day [pcs.]	Average hatching rate [%]	Statistical significance**
control	0	2.50	0.00	not tested		
	14	0.00	n.a.	19.13	91.91	not applicable
	28	0.00	n.a.	19.89	93.45	not applicable
1000 mL of the test item/200 L of water/ha	0	65.00	64.10	not tested		
	14	37.50	n.a.	16.62	79.85	-
	28	12.50	n.a.	16.35	88.43	-
Reference item 15 g of dimethoate/ 200 L of water/ha	0	92.50	92.31	not tested		
	14	95.00	n.a.			
	28	85.00	n.a.			

n.a. not applicable, because the pre-selected Abbott-correction is not needed since the control response is 0.00%
* timing of bioassays in terms of days after application of test-item treatment (DAT)
** if the average number of eggs/female /day is ≥15 and hatching rate is ≥70%, which means that the test item has no effect on reproductive ability

Reference:	KCP 10.3.2_05
Report	Aged-residue test to determine the effects of the test item DNT-162OD-R-CPd on the green lacewing (*Chrysoperla carnea*) according to IOBC, BART, EPPO Joint Initiative, Vogt et al., 2000 and ESCORT 2, Candolfi et al. 2001, Domagała J., 0016/0224/E., 2023
Guideline(s):	IOBC, BART, EPPO Joint Initiative, Vogt et al., 2000 and ESCORT 2, Candolfi et al. 2001
Deviations:	No.
GLP:	Yes
Acceptability:	Yes
Reference:	KCP 10.3.2_05
Report	Aged-residue test to determine the effects of the test item DNT-162OD-R-CPd

	on the green lacewing (*Chrysoperla carnea*) according to IOBC, BART, EPPO Joint Initiative, Vogt et al., 2000 and ESCORT 2, Candolfi et al. 2001, Domagała J., 0016/0224/E., 2023
Guideline(s):	IOBC, BART, EPPO Joint Initiative, Vogt et al., 2000 and ESCORT 2, Candolfi et al. 2001
Deviations:	No.
GLP:	Yes
Acceptability:	Yes

Test item

Name	DNT-162OD-R-CPd
Type of packaging material	PE/PA
Test item	appearance white to beige color
Batch No.	1/23
Production date	09.02.2023
Expiry date	09.02.2025
Name of active substances	Dicamba, Nicosulfuron, Thifensulfuron-methyl
Dicamba:	
Content	112 g/L
CAS	1918-00-9
IUPAC nomenclature	3,6-dichloro-2-methoxybenzoic acid
Molecular weight	221.03 g/mol
Nicosulfuron:	
Content	39.00 g/L
CAS	111991-09-4
IUPAC nomenclature	1-(4,6-dimethoxypyrimidin-2-yl)-3-(3-dimethylcarbamoyl-2-pyridylsulfonyl)urea
Molecular weight	410.4 g/mol
Thifensulfuron-methyl:	
Content	12.40 g/L
CAS	79277-27-3
IUPAC nomenclature	methyl 3-(4-methoxy-6-methyl-1,3,5-triazin-2-ylcarbamoylsulfamoyl)thiophene-2-carboxylate
Molecular weight	387.4 g/mol

Reference item

Name	Dimethoate
IUPAC nomenclature	2-Dimethoxy-phosphinothioylthio-Nmethylacetamide
Batch No.	806992
Producer	HPC Standards GmbH
CAS	60-51-5
Purity	99.29 % (w/w)
Molecular weight	229.26 g/mol[[

Materials and methods

The study was conducted on the basis of the IOBC, BART, EPPO Joint Initiative, Vogt et al., 2000 and ESCORT 2, Candolfi et.al. 2001 using bean leaves as a test unit a certain number days after treatment (DAT).

The test item or control was sprayed onto the plants, adaxial surface at BBCH 12 growth stage at 0 DAT (for reference item in all observed DAT). Then at certain DAT sprayed leaves were removed

and placed into Petri dish for further assessments. Assessments were performed until no negative impact (<50%) of test item in all tested rates was recorded in 2 consecutive bioassays i.e. on 0 DAT, 14 DAT and 28 DAT.
The spray solutions were applied to the potted plants using a spray chamber T&B Masters s.c. The test item and reference item were diluted with deionized water prior to the spraying and the deionized water was used as the control. The test item and control was applied once in the study, at the date corresponding to the 0 DAT bioassay, the reference item was applied prior to each consecutive bioassay. The order of spray application at 0 DAT was as follows: control, 1000 mL test item/200 L of water/ha and the reference item and in between applications the nozzle was rinsed using deionized water.
During the study one rate of the test item was used: 1000 mL of the test item/200 L of water/ha, control and single rate of the reference item (15 g dimethoate/200L of water/ha). Laboratory bioassays (mortality assessment and if applicable reproductive performance) were performed on 0 DAT and every 14 days after 0 DAT until no negative impact (<50%) of test item were recorded in 2 consecutive bioassays. There were 40 replicates, 1 larva per replicate for test item treatment, reference item treatment and the control. Laboratory bioassays were carried out using leaves of treated plants at 0, 14 and 28 DAT.

Results:
The impact of the freshly-dried and field-aged residues of the test item on the *Chrysoperla carnea* L. were evaluated under laboratory test conditions on following bioassays: 0 DAT, 14 DAT and 28 DAT. Reproduction assessment on 0 DAT was not performed due to mortality ≥50% of introduced organisms. The test item achieved <50% mortality and <50% reduction in reproductive performance (relative to the control) in two subsequent bioassays at 14 DAT and 28 DAT (further assessments were not performed because the required parameters of mortality and reproductive performance were achieved at 14 DAT and 28 DAT).

Treatment	DAT	Mortality assessment		Reproduction assessment		
		Mortality [%]	Abbott corrected mortality [%]	Average number of eggs/ female/day [pcs.]	Average hatching rate [%]	Statistical significance*
control	0	2.50	0.00	not tested		
	14	0.00	n.a.	19.13	91.91	not applicable
	28	0.00	n.a.	19.89	93.45	not applicable
1000 mL of the test item/200 L of water/ha	0	65.00	64.10	not tested		
	14	37.50	n.a.	16.62	79.85	-
	28	12.50	n.a.	16.35	88.43	-
Reference item 15 g of dimethoate/ 200 L of water/ha	0	92.50	92.31	not tested		
	14	95.00	n.a.			
	28	85.00	n.a.			

n.a. not applicable, because the pre-selected Abbott-correction is not needed since the control response is 0.00%
* if the average number of eggs/female /day is ≥15 and hatching rate is ≥70%, which means that the test item has no effect on reproductive ability

A 2.3.2.1.6 Study 6: Age residue study on *Coccinella septempunctata*

Comments of zRMS:	The study is acceptable. The validity criteria of the test according to IOBC, BART, EPPO Joint Initiative, Vogt et al., 2000 and ESCORT 2, Candolfi et al. 2001 were met. **Validity criteria:** The study met the validity criteria for all bioassays. The validity criteria were: – control larvae mortality: requirements: <30% – number of hatchable eggs laid by a live female per day: requirements: >2 eggs/day – mortality of larvae exposed to the reference item at a rate of 3.2 g dimethoate/200 L of water/ha: requirements: >40%) **Deviation of the study:** Deviations from Study plan and Guideline were found: 1. The environmental conditions during ageing period were obtained from nearest weather station Poznań – Marcelin, those measurements were performed outside the GLP system. 2. During the study in bioassay at 0 DAT temperature highered to 28.5°C, average temperature was 26.8°C (requirements: 25±2°C). During the study in bioassay at 14 DAT temperature lowered to 22.5°C and highered to 30.6°C, average temperature was 25.2°C (requirements: 25±2°C). During the study in bioassay at 14 DAT humidity lowered to 55.5%, highered to 91.2%, average humidity was 78.6% (requirements: 60-90% RH). During the study in bioassay at 28 DAT temperature lowered to 22.5°C and highered to 30.6°C, average temperature was 24.9°C (requirements: 25±2°C). During the study in bioassay at 28 DAT humidity lowered to 52.2%, highered to 99.9%, average humidity was 77.4% (requirements: 60-90% RH). Deviation from Study plan was found: 3. Experimental part of the study ended in September 2024 instead of planned August 2024. The above deviations did not affect the study results. The study met the validity criteria. **Agreed toxicity endpoints:**

Treatment	DAT*	Mortality assessment		Reproduction assessment			
		Mortality [%]**	Abbott corrected mortality [%]	Average number of eggs/ female/day [pcs.]	Average hatching [%]	Average number of fertile eggs/ female/day [pcs.]	Statistical significance***
Control (0 mL of test item/ 200L of water/ha)	0	5.00		not tested			
	14	7.50		8.0	78.8	6.3	not applicable
	28	5.00		7.9	78.6	6.2	
1000 mL of the test item/200L of water/ha	0	87.50	86.84	not tested			
	14	50.00	45.95	6.6	66.0	4.3	-
	28	35.00	31.58	7.0	70.1	4.9	-
Reference item 3.2 g of dimethoate/ 200L of water/ha	0	100.00	100.00	not tested			
	14	100.00	100.00				
	28	100.00	100.00				

* timing of bioassays in terms of days after application of test-item treatment (DAT)
** until the end of reference item treatment, control mortality was 0.0%
*** value calculated using ToxRat using test with significance level p= 0.05
\- not statistically significant

Reference:	KCP 10.3.2_06
Report	Aged-residue extended laboratory test to determine the effects of the test item DNT-162OD-R-CPd on the ladybird beetle (*Coccinella septempunctata*) according to IOBC, BART and EPPO Joint Initiative. Schmuck R. et al. (2000), ESCORT 2, Candolfi M.P. et al. (2001), Domagała J., 0016/0244/E., 2024
Guideline(s):	IOBC, BART, EPPO Joint Initiative, Vogt et al., 2000 and ESCORT 2, Candolfi et al. 2001
Deviations:	No.
GLP:	Yes
Acceptability:	Yes

Test item

Name	DNT-162OD-R-CPd
Type of packaging material	HDPE
Test item	appearance homogeneous, creamy, opaque mixture
Batch No.	2/24
Production date	12.03.2024
Expiry date	12.03.2026
Name of active substances	Dicamba, Nicosulfuron, Thifensulfuron-methyl
Dicamba:	
Content	107 g/L
CAS	1918-00-9
IUPAC nomenclature	3,6-dichloro-2-methoxybenzoic acid

Molecular weight	243.02 g/mol
Nicosulfuron:	
Content	39.90 g/L
CAS	111991-09-4
IUPAC nomenclature	1-(4,6-dimethoxypyrimidin-2-yl)-3-(3-dimethylcarbamoyl-2-pyridylsulfonyl)urea
Molecular weight	410.4 g/mol
Thifensulfuron-methyl:	
Content	11.60 g/L
CAS	79277-27-3
IUPAC nomenclature	methyl 3-(4-methoxy-6-methyl-1,3,5-triazin-2-ylcarbamoylsulfamoyl)thiophene-2-carboxylate
Molecular weight	387.4 g/mol

Reference item

Name	Dimethoate
IUPAC nomenclature	2-Dimethoxy-phosphinothioylthio-Nmethyla-cetamide
Batch No.	1-CGS-8-1
Producer	TRC Canada
CAS	60-51-5
Purity	98%
Molecular weight	229.26 g/mol[[

Materials and methods

The study was conducted on the basis of the IOBC, BART, EPPO Joint Initiative, Vogt et al., 2000 and ESCORT 2, Candolfi et.al. 2001 using bean leaves as a test surface a certain number days after treatment (DAT).
The test item was sprayed onto the plants, adaxial surface at BBCH 12 growth stage at 0 DAT (for reference item in all observed DAT). Then at 0 DAT, 14 DAT, 28 DAT until no negative impact (<50%) of test item all tested rates was recorded in 2 consecutive bioassays. Sprayed leaves were removed and placed into Petri dish for further assessments.
The study started on each day after treatment consisted of two stages:

- Larvae and pupae and adults during emergence mortality assessment
- Reproduction assessment.

The spray solutions were applied to the potted plants using a spray chamber T&B Masters s.c. The test item and reference item were diluted with deionized water prior to the spraying and the deionized water was used as the control. The water volume was 200 L of water/ha for control, test item and reference item. The test item and control was applied once in the study, at the date corresponding to the 0 DAT bioassay, the reference item was applied prior to each consecutive bioassay. The order of spray application at 0 DAT was as follows: control, 1000 mL test item/200 L of water/ha and the reference item and in between applications the nozzle was rinsed using deionized water.

During the study one rate of the test item was used: 1000 mL of the test item/200 L of water/ha, control and single rate of the reference item (3.2g dimethoate/200L of water/ha). Laboratory bioassays (mortality assessment and if applicable reproductive performance) were performed on 0 DAT and every 14 days after 0 DAT until no negative impact (<50%) of test item were recorded in 2 consecutive bioassays. There were 40 replicates, 1 larva per replicate for each test item treatment and the control, and 30 replicates for the reference item treatment. Laboratory bioassays were carried out using leaves of treated plants at 0, 14 and 28 DAT.

Results:
The impact of the freshly-dried and field-aged residues of the test item on the *Coccinella septempunctata* were evaluated under extended laboratory conditions on following bioassays: 0 DAT, 14 DAT and 28 DAT. The test item achieved <50% mortality and <50% reduction in reproductive performance (relative to the control) in two subsequent bioassays at 14 DAT and 28 DAT for rate 1000 mL of the test item/200 L of water/ha.
Reference item at a rate of 3.2 g dimethoate/200L of water/ha shows a statistically significant effect on the mortality of ladybird beetle and caused 100% mortality in each bioassay of the introduced organisms. The obtained result complies with the adopted validity criterion (larval mortality ≥40%). The response of the test organisms to the reference item was correct.

Treatment	DAT*	Mortality assessment		Reproduction assessment			
		Mortality [%]**	Abbott corrected mortality [%]	Average number of eggs/ female/day [pcs.]	Average hatching [%]	Average number of fertile eggs/ female/day [pcs.]	Statistical significance***
Control (0 mL of test item/ 200L of water/ha)	0	5.00		not tested			
	14	7.50		8.0	78.8	6.3	not applicable
	28	5.00		7.9	78.6	6.2	
1000 mL of the test item/200L of water/ha	0	87.50	86.84	not tested			
	14	50.00	45.95	6.6	66.0	4.3	-
	28	35.00	31.58	7.0	70.1	4.9	-
Reference item 3.2 g of dimethoate/ 200L of water/ha	0	100.00	100.00	not tested			
	14	100.00	100.00				
	28	100.00	100.00				

* timing of bioassays in terms of days after application of test-item treatment (DAT)
** until the end of reference item treatment, control mortality was 0.0%
*** value calculated using ToxRat using test with significance level p= 0.05
\- not statistically significant

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 has been provisionally accepted. The validity criteria of the test according to OECD 222 (2016) were met. **Validity criteria:** The results are considered valid because the following criteria were satisfied in the controls: • each replicate produced from 95 to 127 juveniles (111.6 mean) at the end of

the exposure period (criterion: ≥ 30 juveniles by the end of the experiment),
- the coefficient of variation of reproduction was 10.8% (criterion: ≤ 30%),
- adult mortality over the initial 4 weeks of the experiment was 2.5% (criterion: ≤ 10%).

Agreed toxicity endpoints:

Parameter	Value [mg test item/kg dry weight of artificial soil]	Value [mg of dicamba/kg dry weight of artificial soil]	Value [mg of nicosulfuron/kg dry weight of artificial soil]	Value [mg of thifensulfuron-methyl/kg dry weight of artificial soil]
EC_{10}	586.8 (402.2 – 745.8)	65.53 (44.91 – 83.28)	22.82 (15.64 – 29.00)	7.25 (4.97 – 9.22)
EC_{20}	> 1000	> 111.67	> 38.88	> 12.36
EC_{50}	> 1000	> 111.67	> 38.88	> 12.36
NOEC (reproduction)	560	62.53	21.77	6.92
LOEC (reproduction)	1000	111.67	38.88	12.36
LC_{10}	> 1000	> 111.67	> 38.88	> 12.36
LC_{20}	> 1000	> 111.67	> 38.88	> 12.36
LC_{50}	> 1000	> 111.67	> 38.88	> 12.36
NOEC (survival)	≥ 1000	≥ 111.67	≥ 38.88	≥ 12.36
LOEC (survival)	> 1000	> 111.67	> 38.88	> 12.36

The studies for formulation of **EVRITELL 162 OD** for earthworms with risk assessment was accepted by zRMS only provisionally. The toxicity endpoints were based on nominal concentration. At the end on the studies concentration of substances active were not reported. The analytical measurements should be performed and reported at least at the start, middle, and end of the study. The intermediate measurements should be to capture the degradation of the substance (i.e., designed substance property dependent). The TWA or geometric mean measured concentration should be calculated over the duration of the test and used if the concentration falls under 80% of nominal. Please complete the information regarding the analytical measurements of active substances during the study.

It should be considered at MSs level.

April 2025 updated

The information regarding the analytical measurements of active substances during the soil studies for formulation of EVRITELL 162 OD (DNT-162OD-R-CPd) with earthworms and *Folsomia candida* and *Hypoapsis aculeifer* was accepted by zRMS. No additional risk assessment for earthworms and other soil macroorganism is required.

It should be considered at MSs level.

Report	DNT-162OD-R-CPd Earthworm Reproduction Test (*Eisenia andrei*) according to the OECD Guideline for the Testing of Chemicals No. 222 (2016), STUDY CODE: G-24-23, Łukasiewicz Research Network – Institute of Industrial Organic Chemistry; Pieczka P., 2023
Guideline(s):	OECD Guideline for the Testing of Chemicals No. 222 (2016): "Earthworm Reproduction Test *(Eisenia fetida/Eisenia andrei)"*.
Deviations:	No.
GLP:	Yes
Acceptability:	Yes
Reference:	KCP 10.4.1.1
Report	DNT-162OD-R-CPd Earthworm Reproduction Test (*Eisenia andrei*) according to the OECD Guideline for the Testing of Chemicals No. 222 (2016), STUDY CODE: G-24-23, Łukasiewicz Research Network – Institute of Industrial Organic Chemistry; Pieczka P., 2023
Guideline(s):	OECD Guideline for the Testing of Chemicals No. 222 (2016): "Earthworm Reproduction Test *(Eisenia fetida/Eisenia andrei)"*.
Deviations:	No.
GLP:	Yes
Acceptability:	Yes

Material and Methods

Test item:	name: DNT-162OD-R-CPd; active ingredients content (analysed): Dicamba: 112 g/L Nicosulfuron: 39.0 g/L Thifensulfuron-methyl: 12.4 g/L, batch number: 1/23; manufacturing date: 09.02.2023; expiry date: 09.02.2025
Test organism:	A biological systems used in the study were earthworms *Eisenia andrei*. The source of the test system was laboratory-bred synchronized culture cultivated at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna, Poland, Ecotoxicology Research Group, Laboratory of Soil Organisms Toxicology. Adult worms with clitella and with fresh weight between 303-484 mg were used in this study. Age of organisms used in the study was 6-7 months and didn't differ in age by more than 4 weeks. The animals selected for the experiment were acclimatized in untreated artificial soil for 24 hours prior to the start of the experiment.
Artificial soil	Components: - 10% sphagnum peat (pH = 5.87) - 20% kaolin clay (kaolinite content >30%) - 69.78% air-dried industrial sand (above 60% of particles between 50 and 200 μm) - 0.22% calcium carbonate

Test conditions:	– temperature: 20.3 – 22.0°C - controlled light – dark cycles (16h : 8h)
Tested concentrations:	Eight concentrations of the test item were used in the experiment (18, 32, 56, 100, 180, 320, 560 and 1000 mg test item/kg soil dry weight). There were four replicates of each test concentration. At the same time, an untreated control group (eight replicates) was introduced to the soil without the test item. The test item in the form of a aqueous suspension was mixed with a suitable amounts of the artificial soil.
Study duration:	8 weeks
Observations:	After 4 weeks: mortality, behavioral and morphological changes After 8 weeks: number of juveniles hatched from the cocoons
Endpoints:	NOEC, LOEC, EC_{10}, EC_{20}, EC_{50}, LC_{10}, LC_{20}, LC_{50}

Results
After 28 days of exposure to DNT-162OD-R-CPd, the percent mortalities of adult earthworms in the control and concentrations: 18, 32, 56, 100, 180, 320, 560 and 1000 mg test item/kg dry soil weight in treatment groups were 2.5, 0.0, 0.0, 0.0, 2.5, 0.0, 0.0, 0.0 and 0.0% respectively. Mortalities in the treatment groups of 18, 32, 56, 100, 180, 320, 560 and 1000 mg test item/kg dry soil were not statistically different from these in the control group. The treatment-related mortality of adult earthworm did not occurred and the LC_{10}, LC_{20} and LC_{50} values were not determined due to mathematical reasons. All surviving earthworms in the control and the treatment groups were normal in appearance and behavior.

After 28-day exposure period, the mean body weights change of the survived adult worms in the control and concentrations: 18, 32, 56, 100, 180, 320, 560 and 1000 mg test item/kg dry soil treatment groups were 29.1, 35.9, 41.5, 53.1, 53.3, 49.6, 45.8, 41.5 and 39.7 % respectively.

On Day 56, the impact of the test item on reproduction of the worms was assessed by counting the number of juveniles hatched from the cocoons in the test soils. The mean number of juveniles in the control and 18, 32, 56, 100, 180, 320, 560 and 1000 mg test item/kg dry soil treatment groups were 111.6, 112.8, 111.5, 108.0, 111.8, 105.0, 107.3, 102.3 and 92.3, respectively. The mean numbers of juveniles produced in 1000 mg test item/kg dry soil groups were significantly different when compared to the mean value of control group.

Evaluation of endpoints results are shown in below table:

Parameter	Value [mg test item/kg dry weight of artificial soil]	Value [mg of dicamba/kg dry weight of artificial soil]	Value [mg of nicosulfuron/kg dry weight of artificial soil]	Value [mg of thifensulfuron-methyl/kg dry weight of artificial soil]
EC_{10}	586.8 (402.2 – 745.8)	65.53 (44.91 – 83.28)	22.82 (15.64 – 29.00)	7.25 (4.97 – 9.22)
EC_{20}	> 1000	> 111.67	> 38.88	> 12.36
EC_{50}	> 1000	> 111.67	> 38.88	> 12.36
NOEC (reproduction)	560	62.53	21.77	6.92
LOEC (reproduction)	1000	111.67	38.88	12.36
LC_{10}	> 1000	> 111.67	> 38.88	> 12.36
LC_{20}	> 1000	> 111.67	> 38.88	> 12.36
LC_{50}	> 1000	> 111.67	> 38.88	> 12.36
NOEC (survival)	≥ 1000	≥ 111.67	≥ 38.88	≥ 12.36
LOEC (survival)	> 1000	> 111.67	> 38.88	> 12.36

Validity criteria:

The results are considered valid because the following criteria were satisfied in the controls:

- mortality of adult worms over initial 28 days of the experiment was 2.5% (criterion: it have not to exceed 10%);
- each replicate produced from 95 to 127 juveniles (111.6 mean) at the end of the exposure period (criterion: a minimum of 30 offspring are produced in each replicate containing 10 adults);
- the coefficient of variation of reproduction was equal to 10.8% (criterion: it should not exceed 30%).

Conclusions

In the 56 - day Earthworm reproduction study with DNT-162OD-R-CPd, the lowest endpoint (NOEC) of 560.0 mg of test item/kg dry soil was obtained and thus, it is proposed to be used in the risk assessment.

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 has been provisionally accepted. The validity criteria of the test according to OECD 232 were met.

Validity criteria:

The results are considered valid because the following criteria were satisfied in the controls:

• mean adult mortality: 6.3% (criterion: ≤ 20%),

• the mean number of juveniles per vessel at the end of the test: 898.0 (criterion: ≥100 juveniles at the end of the test),

• the coefficient of variation calculated for the number of juveniles: 7.1% (criterion: ≤ 30%).

Deviation of the study:

Deviation from the OECD Guideline No. 232 (2016):

- at the end of the test the soil moisture content was determined by drying small sample of the artificial soil in 105°C instead of weighing the test vessels as it is mentioned in OECD Guideline No. 232 (2016) (3.3.5.5.).

The deviation from the GLP principles:

Since the test Guideline No. 232 (2016) does not require the necessity of checking the concentration, homogeneity and stability of the test item, such analyses were not carried out. The waiver of these analyses constitutes a derogation from the principles of Good Laboratory Practice.

These deviations did not affect the results of the study.

Toxicity endpoints:

Survival

Endpoint	Value [mg test item/kg dry weight of the artificial soil]	Value [mg of dicamba/kg dry weight of the artificial soil]	Value [mg of nicosulfuron/kg dry weight of the artificial soil]	Value [mg of thifensulfuron-methyl/kg dry weight of the artificial soil]
LC_{10}	79.88 (39.98 – 159.61)	8.92 (4.46 – 17.82)	3.11 (1.55 – 6.21)	0.99 (0.49 – 1.97)
LC_{20}	392.18 (206.92 – 743.33)	43.79 (23.11 – 83.00)	15.25 (8.05 – 28.90)	4.85 (2.56 – 9.19)
LC_{50}	>1000.00	>111.67	>38.88	>12.36
NOEC	180.00	20.10	7.00	2.23

Reproduction

Endpoint	Value [mg test item/kg dry weight of the artificial soil]	Value [mg of dicamba/kg dry weight of the artificial soil]	Value [mg of nicosulfuron/kg dry weight of the artificial soil]	Value [mg of thifensulfuron-methyl/kg dry weight of the artificial soil]
EC_{10}	12.96 (8.53 – 18.10)	1.45 (0.95 – 2.02)	0.50 (0.33 – 0.70)	0.16 (0.11 – 0.22)
EC_{20}	42.59 (32.43 – 53.41)	4.76 (3.62 – 5.96)	1.66 (1.26 – 2.08)	0.53 (0.40 – 0.66)
EC_{50}	414.75 (343.98 – 513.90)	46.31 (38.41 – 57.38)	16.13 (13.38 – 19.98)	5.13 (4.25 – 6.35)
NOEC	10.00	1.12	0.39	0.12

The studies for formulation of **EVRITELL 162 OD** for *Folsomia candida* with risk assessment was accepted by zRMS only provisionally. The toxicity endpoints were based on nominal concentration. At the end on the study concentration of substances active was not reported. The analytical measurements should be performed and reported at least at the start, middle, and end of the study. The intermediate measurements should be to capture the degradation of the substance (i.e., designed substance property dependent). The TWA or geometric mean measured concentration should be calculated over the duration of the test and used if the concentration falls under 80% of nominal. Please complete the information regarding the analytical measurements of active substances during the study.

It should be considered at MSs level.

April 2025 updated
The information regarding the analytical measurements of active substances during the soil studies for formulation of EVRITELL 162 OD (DNT-162OD-R-CPd) with earthworms and *Folsomia candida* and *Hypoapsis aculeifer* was accepted by zRMS. No additional risk assessment for earthworms and other soil macroorganism is required.

It should be considered at MSs level.

Reference:	KCP 10.4.2.1-01
Report	DNT-162OD-R-CPd Collembolan (*Folsomia candida*) Reproduction Test according to OECD Guideline No. 232 (2016), STUDY CODE: G-31-23, Łukasiewicz Research Network – Institute of Industrial Organic Chemistry; Pieczka P., 2023
Guideline(s):	OECD Guideline for the Testing of Chemicals No. 232 (2016): "Collembolan reproduction test in soil" [1] and the Standard Operating Procedure SOP/G/87: "Collembolan *(Folsomia candida)* reproduction test".
Deviations:	No.
GLP:	Yes
Acceptability:	Yes

Material and Methods

Test item:	name: DNT-162OD-R-CPd; active ingredients content (analysed): Dicamba: 112 g/L Nicosulfuron: 39.0 g/L Thifensulfuron-methyl: 12.4 g/L, batch number: 1/23; manufacturing date: 09.02.2023; expiry date: 09.02.2025
Test organism:	The collembolan, *Folsomia candida* obtained from a standard laboratory culture at the Test Facility. The collembolans used in the study were 9 – 11 days old
Artificial soil	Components: - 5% sphagnum peat - 20% kaolin clay - 74.88% air-dried industrial sand with more than 50% of the particles between 50 and 200 µm - 0.12% calcium carbonate maximum water holding capacity: 35.27% pH: 6.23. soil dry weight content: 85.8%
Test conditions:	– temperature: 20.4-22.0°C - controlled light – dark cycles (16h : 8h)
Tested concentrations:	Eleven concentrations of the test item were used in the experiment (3.2, 5.6, 10.0, 18.0, 32.0, 56.0, 100.0, 180.0, 320.0, 560.0 and 1000 mg of the test item/kg of dry weight of the artificial soil). There were four replicates of each test concentration. At the same time, an untreated control group (eight replicates) was introduced to the soil without the test item. The test item in the form of a aqueous suspension was mixed with a suitable amounts of the artificial soil.
Study duration:	28 days
Observations:	After 28 days: mortality, number of juveniles
Endpoints:	NOEC, LOEC, EC_{10}, EC_{20}, EC_{50}, LC_{10} LC_{20}, LC_{50}

The aims of the study were to assess the impact of the test item on reproduction of the collembolan, *Folsomia candida* and to determine the EC_{10}, EC_{20}, EC_{50}, and NOEC.

Results
Mortality at the concentrations ranging from 3.2 to 1000 mg/kg dry weight of the artificial soil ranged from 5.0 to 37.5 %. As for the control group, it was equal to 6.3%.
After 28 days of the exposure of collembolans to the test item at the concentrations ranging from 3.2 to 1000 mg/kg dry weight of the artificial soil, the mean number of juveniles was between 911.3 – 373.8 per replicate. As for the control group, the number of juveniles was equal to 898.0 per replicate.

The endpoint values showing the impact of the test item on mortality and reproduction of *Folsomia candida* are presented in the table given below.

Endpoint	Value [mg test item/kg dry weight of the artificial soil]	Value [mg of dicamba/kg dry weight of the artificial soil]	Value [mg of nicosulfuron/kg dry weight of the artificial soil]	Value [mg of thifensulfuron-methyl/kg dry weight of the artificial soil]
LC_{10}	79.88 (39.98 – 159.61)	8.92 (4.46 – 17.82)	3.11 (1.55 – 6.21)	0.99 (0.49 – 1.97)
LC_{20}	392.18 (206.92 – 743.33)	43.79 (23.11 – 83.00)	15.25 (8.05 – 28.90)	4.85 (2.56 – 9.19)
LC_{50}	>1000.00	>111.67	>38.88	>12.36
NOEC	180.00	20.10	7.00	2.23

Endpoint	Value [mg test item/kg dry weight of the artificial soil]	Value [mg of dicamba/kg dry weight of the artificial soil]	Value [mg of nicosulfuron/kg dry weight of the artificial soil]	Value [mg of thifensulfuron-methyl/kg dry weight of the artificial soil]
EC_{10}	12.96 (8.53 – 18.10)	1.45 (0.95 – 2.02)	0.50 (0.33 – 0.70)	0.16 (0.11 – 0.22)
EC_{20}	42.59 (32.43 – 53.41)	4.76 (3.62 – 5.96)	1.66 (1.26 – 2.08)	0.53 (0.40 – 0.66)
EC_{50}	414.75 (343.98 – 513.90)	46.31 (38.41 – 57.38)	16.13 (13.38 – 19.98)	5.13 (4.25 – 6.35)
NOEC	10.00	1.12	0.39	0.12

Validity criteria:

The results are considered valid because the following criteria were satisfied in the controls:

- mean adult mortality: 6.3% (criterion: ≤ 20%),
- the mean number of juveniles per vessel at the end of the test: 898.0 (criterion: ≥ 100 juveniles at the end of the test),
- the coefficient of variation calculated for the number of juveniles: 7.1 (criterion: ≤ 30%).

Conclusions

In the 28 - day collembolan reproduction study with DNT-162OD-R-CPd, the lowest endpoint NOEC of 10.0 mg/kg dry weight of the artificial soil was obtained and thus, it is proposed to be used in the risk assessment.

A 2.4.2.1.1 Study 2

Comments of zRMS:	The study has been provisionally accepted. The validity criteria of the test according to OECD 226 were met. **Validity criteria:** The results are considered valid because the following criteria were satisfied in the control: • mean adult mortality: 0.0% (criterion: ≤ 20%), • the mean number of juveniles per vessel at the end of the test: 114.4 (criterion: ≥ 50 juveniles at the end of the test), • the coefficient of variation for the number of juveniles: 12.2% (criterion: ≤ 30%). **Deviation of the study:** Deviations from the OECD Guideline No. 226 (2016): There are three deviations from the OECD Guideline No. 226 (2016), however they did not affect the results: 1. According to the OECD Guideline No. 226 (2016) the water content of the artificial soil should be maintained throughout the test by weighing and if needed re-watering the vessels periodically. In the study to maintain proper moisture content, a small sample of soil was dried at 105°C and re-weighed at the beginning, after 7 days of the test and at the end of the test. 2. Due to the use of the temperature extraction method, there was no need for euthanasia of the extracted organisms since the mites are fixed in a 70% ethanol solution. 3. Due to the use of the temperature extraction method, it was not possible to record the symptoms with behavioral and morphology changes of the extracted predatory mites. 4. Since the test guideline does not require the necessity of checking the concentration, homogeneity and stability of the test item, such analyses were not carried out. The waiver of these analyses constitutes a derogation from the principles of Good Laboratory Practice. **Toxicity endpoints:**

Concentration [mg/kg dry weight of the artificial soil]	Adult mites			Number of juveniles (mean)
	Number of tested mites	Dead mites after 14 days		
		No.	%	
0	80	0	0.0	114.4
18	40	0	0.0	125.3
32	40	0	0.0	114.8
56	40	0	0.0	117.5
100	40	2	5.0	123.0
180	40	1	2.5	116.3
320	40	0	0.0	122.5
560	40	2	5.0	120.3
1000	40	1	2.5	119.0

Endpoint values – the impact of the test item on reproduction and on mortality of the predatory mites *(Hypoaspis aculeifer)*.

Endpoint	Value [mg of the test item/kg dry weight of the artificial soil]	Value [mg of dicamba/kg dry weight of the artificial soil]	Value [mg of nicosulfuron/kg dry weight of the artificial soil]	Value [mg of thifensulfuron-methyl/kg dry weight of the artificial soil]
EC_{10}	> 1000	> 111.67	> 38.88	> 12.36
EC_{20}	> 1000	> 111.67	> 38.88	> 12.36
EC_{50}	> 1000	> 111.67	> 38.88	> 12.36
NOEC (reproduction)	≥ 1000	≥ 111.67	≥ 38.88	≥ 12.36
LC_{10}	> 1000	> 111.67	> 38.88	> 12.36
LC_{20}	> 1000	> 111.67	> 38.88	> 12.36
LC_{50}	> 1000	> 111.67	> 38.88	> 12.36
NOEC (survival)	≥ 1000	≥ 111.67	≥ 38.88	≥ 12.36

The studies for formulation of **EVRITELL 162 OD** for *Hypoaspis aculifera* with risk assessment was accepted by zRMS only provisionally. The toxicity endpoints were based on nominal concentration. At the end on the study concentration of substances active was not reported. The analytical measurements should be performed and reported at least at the start, middle, and end of the study. The intermediate measurements should be to capture the degradation of the substance (i.e., designed substance property dependent). The TWA or geometric mean measured concentration should be calculated over the duration of the test and used if the concentration falls under 80% of nominal. Please complete the information regarding the analytical measurements of active substances during the study.

	It should be considered at MSs level.
	April 2025 updated The information regarding the analytical measurements of active substances during the soil studies for formulation of EVRITELL 162 OD (DNT-162OD-R-CPd) with earthworms and *Folsomia candida* and *Hypoapsis aculeifer* was accepted by zRMS. No additional risk assessment for earthworms and other soil macroorganism is required. **It should be considered at MSs level.**

Reference:	KCP 10.4.2.1-02
Report	Predatory mite (*Hypoaspis* (*Geolaelaps*) *aculeifer*) Reproduction Test in soil according to the OECD Guideline No. 226 (2016), STUDY CODE: G-32-23, Łukasiewicz Research Network – Institute of Industrial Organic Chemistry; Pieczka P., 2023
Guideline(s):	OECD Guideline for the Testing of Chemicals No. 226 (2016): "Predatory mite *(Hypoaspis (Geolaelaps) aculeifer)* reproduction test in soil"
Deviations:	
GLP:	Yes
Acceptability:	Yes

Material and Methods

Test item:	name: DNT-162OD-R-CPd; active ingredients content (analysed): Dicamba: 112 g/L Nicosulfuron: 39.0 g/L Thifensulfuron-methyl: 12.4 g/L, batch number: 1/23; manufacturing date: 09.02.2023; expiry date: 09.02.2025
Test organism:	the predatory mites, Hypoaspis (Geolaelaps) aculeifer (adult female mites from a synchronized cohort) obtained from a standard laboratory culture at the Test Facility. The mites were introduced 7-14 days after becoming adult (i.e. 28 – 35 days after the start of the egg laying in the synchronisation).
Artificial soil	Components: - 5% sphagnum peat - 20% kaolin clay - 74.88% air-dried quartz sand - 0.12% calcium carbonate maximum water holding capacity: 32.78% pH: 6.00. soil dry weight content: 93.6%
Test conditions:	– temperature: 20.4 – 22.0ºC - controlled light – dark cycles (16h : 8h)

Tested concentrations:	Eight concentrations of the test item were used in the experiment (18, 32, 56, 100, 180, 320, 560 and 1000.00 mg of the test item/kg of dry weight of the artificial soil). There were four replicates of each test concentration. At the same time, an untreated control group (eight replicates) was introduced to the soil without the test item. The test item in the form of a aqueous suspension was mixed with a suitable amounts of the artificial soil.
Study duration:	14 days
Observations:	After 14 days: mortality, number of juveniles
Endpoints:	NOEC, LOEC, EC_{10}, EC_{20}, EC_{50}, LC_{10}, LC_{20}, LC_{50}

The aims of the study were to assess the impact of the test item on reproduction of predatory mite, *Hypoaspis aculeifer* and to determine the EC_{10}, EC_{20}, EC_{50}, and NOEC.

Results

After the application of the test item at the concentrations ranging from 18 to 1000.00 mg/kg dry weight of the artificial soil, mortality was between 0.0 to 5.0%. As for the control group, it was equal to 0.0%. The concentration of the test item causing a 50% mortality of adults within the exposure period (LC_{50}) was not determined due to mathematical reasons.

After the application of the test item at the concentrations ranging from 18 to 1000.00 mg/kg dry weight of the artificial soil, the mean number of juveniles was between 114.8 – 125.3 per replicate. As for the control group, the mean number of juveniles was equal to 114.4 per replicate.

The endpoint values showing the impact of the test item on mortality of *Predatory mite* are presented in the table given below.

Endpoint	Value [mg of the test item/kg dry weight of the artificial soil]	Value [mg of dicamba/kg dry weight of the artificial soil]	Value [mg of nicosulfuron/kg dry weight of the artificial soil]	Value [mg of thifensulfuron-methyl/kg dry weight of the artificial soil]
LC_{10}	> 1000	> 111.67	> 38.88	> 12.36
LC_{20}	> 1000	> 111.67	> 38.88	> 12.36
LC_{50}	> 1000	> 111.67	> 38.88	> 12.36
NOEC (survival)	≥ 1000	≥ 111.67	≥ 38.88	≥ 12.36

The endpoint values showing the impact of the test item on reproduction of *Predatory mite* are presented in the table given below.

Endpoint	Value [mg of the test item/kg dry weight of the artificial soil]	Value [mg of dicamba/kg dry weight of the artificial soil]	Value [mg of nicosulfuron/kg dry weight of the artificial soil]	Value [mg of thifensulfuron-methyl/kg dry weight of the artificial soil]
EC_{10}	> 1000	> 111.67	> 38.88	> 12.36
EC_{20}	> 1000	> 111.67	> 38.88	> 12.36
EC_{50}	> 1000	> 111.67	> 38.88	> 12.36
NOEC (reproduction)	≥ 1000	≥ 111.67	≥ 38.88	≥ 12.36

Validity criteria:

The results are considered valid because the following criteria were satisfied in the control:
• mean adult mortality: 0.0% (criterion: ≤ 20%),
• the mean number of juveniles per vessel at the end of the test: 114.4 (criterion: ≥ 50 juveniles at the end of the test,
• the coefficient of variation for the number of juveniles: 12.2 (criterion: ≤ 30%).

Conclusions

In the 14 - day *Hypoaspis* reproduction study with DNT-162OD-R-CPd, NOEC of ≥ 1000 mg of test item/ kg dry weight of the artificial soil for reproduction was obtained and thus, it is proposed to be used in the risk assessment.

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 is acceptable. The validity criteria of the test according to OECD 216 were met. Validity criteria: The coefficients of variation (CV) in the control group were: 4.3; 11.3, 10.5, 6.9 and 5.1%, after 0, 7, 14, 28 and 42 days of incubation respectively (data obtained from statistical analysis performed in ToxRat – Appendix No. 5). The validity criterion was met, because the variation between replicate control samples was less than ± 15%. Deviation of the study: none Agreed toxicity endpoints:

Nitrate formation rate* [mg nitrate /kg dry weight of soil/day] for selected time intervals

Interval of sampling days (X-Y)	Control				PEC 1.35 mg of the test item/kg dry weight of soil (0.15 mg of dicamba/kg dry weight of soil. 0.05 mg of nicosulfuron/kg dry weight of soil and 0.02 mg thifensulfuron-methyl/kg dry weight of soil)				Upper PEC 6.74 mg of the test item/kg dry weight of soil (0.77 mg of dicamba/kg dry weight of soil. 0.26 mg of nicosulfuron/kg dry weight of soil and 0.08 mg thifensulfuron-methyl/kg dry weight of soil)			
	Replicate			Mean ± SD	Replicate			Mean ± SD	Replicate			Mean ± SD
	1	2	3		1	2	3		1	2	3	
0-7^	1.781	0.697	3.567	2.015 ± 1.449	3.476	4.261	1.966	3.234 ± 1.167	3.554	2.404	2.250	2.736 ± 0.713
7-14^	11.843	17.200	12.986	14.010 ± 2.822	22.741	17.813	26.527	22.360 ± 4.370	17.026	11.097	16.454	14.859 ± 3.270
14-28^	7.631	4.917	6.131	6.226 ± 1.360	2.476	2.190	4.690	3.119 ± 1.368	4.869	0.798	6.155	3.940 ± 2.797
28-42^	4.083	1.798	2.833	2.905 ± 1.145	2.964	2.000	2.214	2.393 ± 0.506	5.929	1.500	2.750	3.393 ± 2.283
Additional calculation at the request of the Sponsor:												
0-7*	1.781	0.697	3.567	2.015 ± 1.449	3.476	4.261	1.966	3.234 ± 1.167	3.554	2.404	2.250	2.736 ± 0.713
0-14*	6.929	9.607	7.500	8.012 ± 1.411	12.988	10.524	14.881	12.797 ± 2.185	9.881	6.916	9.595	8.797 ± 1.635
0-28*	7.821	6.464	7.071	7.119 ± 0.680	7.637	7.494	8.744	7.958 ± 0.684	6.833	4.798	7.476	6.369 ± 1.398
0-42*	6.107	5.345	5.690	5.714 ± 0.382	6.294	5.972	6.044	6.103 ± 0.169	6.222	4.746	5.163	5.377 ± 0.761

*Nitrate formation rates were calculated as follows: Nitrate formation rate = $\frac{\text{Concentration of nitrate on day Y} - \text{Concentration of nitrate on day X}}{\text{Y days}}$ [mg/kg dry weight of soil/day]

^ Mean, SD based on the data obtained from ToxRat statistical analysis (Appendix No. 6).
* Mean, SD based on the data obtained from ToxRat statistical analysis (Appendix No. 7).

The difference in the nitrate formation rate between control soil and treated soils exceed 25% on 28 day of analysis and measurements were continued until the difference between PEC, 5xPEC and the control soil was lower than 25% which occurred on day 42.

Reference:	KCP 10.5
Report	Soil Microorganisms: Nitrogen Transformation Test according to the OECD Guideline No. 216 (2000), STUDY CODE: EMI/4/67/2022, Ecomelius Institute Sp. z o. o., Sajdok-Czernecka S. 2023

Guideline(s):	Yes. According to the OECD Guideline for the Testing of Chemicals No. 216 (2000)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Reference:	KCP 10.5
Report	Soil Microorganisms: Nitrogen Transformation Test according to the OECD Guideline No. 216 (2000), STUDY CODE: EMI/4/67/2022, Ecomelius Institute Sp. z o. o., Sajdok-Czernecka S. 2023
Guideline(s):	Yes. According to the OECD Guideline for the Testing of Chemicals No. 216 (2000)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

1. Test material: DNT-162OD-R-CPd

2. Batch number: 13/22
   Concentration of Dicamba: 115 g/L, Nicosulfuron: 39.40 g/L, Thifensulfuron-methyl: 12.03 g/L

3. Soil: Agricultural soil (Type 5M) purchased from LUFA Speyer Obere Langgasse 40, 67346 Speyer.

4. Test design: Three portions of soil with lucerne: one control group and two groups containing the test item. Every portion was divided into three replicates. Test duration: 42 days.

5. Concentrations of the test item:

   PEC: 1.35 mg of the test item/kg dry weight of soil (0.15 mg of dicamba/kg dry weight of soil, 0.05 mg of nicosulfuron/kg dry weight of soil, 0.02 mg of thifensulfuron - methyl/kg dry weight of soil), 5xPEC: 6.74 mg of the test item/kg dry weight of soil (0.77 mg of dicamba/kg dry weight of soil, 0.26 mg of nicosulfuron/kg dry weight of soil, 0.08 mg of thifensulfuron - methyl/kg dry weight of soil).

The aim of this study was to detect long-term adverse effects of the test item (DNT-162OD-R-CPd) on the process of nitrogen transformation in aerobic surface soils.
The soil was divided into three portions – two treated portions and control soil; each of them was divided into three replicates.
On certain days of experiment soil samples from each test vessel were collected and soil extracts with 0.1M KCl were prepared. Particle-free soil extracts were frozen from -20.0 to -22.3°C and after defrosting quantities of nitrate were determined in it. The method was based on spectrophotometrical measurement. The nitrate formation rate in each treated group was compared to that in the control and the percent deviation of the treated from the control was calculated.

Results:
The difference in the nitrate formation rate between control soil and treated soils exceed 25% on 28 day of analysis and measurements were continued until the difference between PEC, 5xPEC and the control soil was lower than 25% which occurred on day 42.

The nitrate formation rate [mg/kg dry weight of soil/day] for selected time intervals of soil incubation, i.e. 0 - 7, 7 – 14, 14 – 28, 28 – 42 days and at 0-7, 0-14, 0-28 and 0- 42 days.

Interval of sampling days (X-Y)	Control				PEC 1.35 mg of the test item/kg dry weight of soil (0.15 mg of dicamba/kg dry weight of soil. 0.05 mg of nicosulfuron/kg dry weight of soil and 0.02 mg thifensulfuron-methyl/kg dry weight of soil)				Upper PEC 6.74 mg of the test item/kg dry weight of soil (0.77 mg of dicamba/kg dry weight of soil. 0.26 mg of nicosulfuron/kg dry weight of soil and 0.08 mg thifensulfuron-methyl/kg dry weight of soil)			
	Replicate 1	2	3	Mean ± SD	Replicate 1	2	3	Mean ± SD	Replicate 1	2	3	Mean ± SD
0-7^	1.781	0.697	3.567	2.015 ± 1.449	3.476	4.261	1.966	3.234 ± 1.167	3.554	2.404	2.250	2.736 ± 0.713
7-14^	11.843	17.200	12.986	14.010 ± 2.822	22.741	17.813	26.527	22.360 ± 4.370	17.026	11.097	16.454	14.859 ± 3.270
14-28^	7.631	4.917	6.131	6.226 ± 1.360	2.476	2.190	4.690	3.119 ± 1.368	4.869	0.798	6.155	3.940 ± 2.797
28-42^	4.083	1.798	2.833	2.905 ± 1.145	2.964	2.000	2.214	2.393 ± 0.506	5.929	1.500	2.750	3.393 ± 2.283
Additional calculation at the request of the Sponsor:												
0-7*	1.781	0.697	3.567	2.015 ± 1.449	3.476	4.261	1.966	3.234 ± 1.167	3.554	2.404	2.250	2.736 ± 0.713
0-14*	6.929	9.607	7.500	8.012 ± 1.411	12.988	10.524	14.881	12.797 ± 2.185	9.881	6.916	9.595	8.797 ± 1.635
0-28*	7.821	6.464	7.071	7.119 ± 0.680	7.637	7.494	8.744	7.958 ± 0.684	6.833	4.798	7.476	6.369 ± 1.398
0-42*	6.107	5.345	5.690	5.714 ± 0.382	6.294	5.972	6.044	6.103 ± 0.169	6.222	4.746	5.163	5.377 ± 0.761

Percent deviation from the control in nitrate formation rate calculated for selected time intervals i.e. 0 - 7, 7 – 14, 14 – 28, 28 – 42 days and additionally: 0-7, 0-14, 0-28 and 0-42 days.

Day of incubation	PEC 1.35 mg of the test item/kg dry weight of soil (0.15 mg of dicamba/kg dry weight of soil, 0.05 mg of nicosulfuron/kg dry weight of soil and 0.02 mg thifensulfuron-methyl/kg dry weight of soil)	Upper PEC 6.74 mg of the test item/kg dry weight of soil (0.77 mg of dicamba/kg dry weight of soil, 0.26 mg of nicosulfuron/kg dry weight of soil and 0.08 mg thifensulfuron-methyl/kg dry weight of soil)
0-7^	-60.5	-35.8
7-14^	-59.6	-6.1
14-28^	49.9	36.7
28-42^	17.6	-16.8
Additional calculation at the request of the Sponsor:		
0-7*	-60.5	-35.8
0-14*	-59.7	-9.8
0-28*	-11.8	10.5
0-42*	-6.8	5.9

Validity criteria:
The coefficients of variation (CV) in the control group were 4.3, 11.3, 10.6, 6.9 and 5.1 %, after 0, 7, 14, 28 and 42 days of incubation. The validity criterion was met, because the variation between replicate control samples is less than ± 15%.

Conclusions:
The difference in the nitrate formation rate between control soil and treated soils exceed 25% on 28 day of analysis and measurements were continued until the difference between PEC, 5xPEC and the control soil was lower than 25% which occurred on day 42.

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 is acceptable. The validity criteria of the test according to OECD 208 were met. **Validity criteria:** On the basis of the obtained results, it was stated that the following validity criteria of the study aimed at evaluating the impact of **EVRITELL 162 OD** on seedling emergence and seedling growth of terrestrial plants were met: - the seedling emergence in the control (validity criterion: at least 70%) was as follows: 100.0% – tomato, 100.0% – cabbage, 95.2% – lettuce, 100.0% – oilseed rape, 100.0% – pea, 90.0% – sugar beet, 90.0% – carrot, 95.0% – onion, 100.0% – perennial ryegrass, 100.0% – oats, - the mean survival of the emerged control seedlings was 100% for each tested plant species (validity criterion: 90%); - the control seedlings did not exhibit any visible phytotoxic effects; - environmental conditions for all plants of the same species were identical. **Deviation from OECD Guideline No. 208:** According to OECD Guideline No. 208 (2006), the light intensity should be 350 ± 50µE/m2/s. However, these values are recommended for tests conducted in greenhouses. The experiment was conducted in a test room, where only artificial lighting was used. The light intensity was between 205.7 and 237.2 µE/m2/s. Good control plant vigour was observed. Therefore, it was concluded that the light intensity was suitable for plant growing. **Agreed toxicity endpoints:**

The ER_{50} and NOER values determined on the basis of emergence of plants, survival of plants, shoot length and shoot dry weight measurements and ER_{50} values for plant damages at the end of the exposure period expressed as mL of the test item/ha for all test species are given below.

	Tomato *Solanum lycopersicon*	**Cabbage** *Brassica oleracea var. capitata*	**Lettuce** *Lactuca sativa*	**Oilseed rape** *Brassica napus*	**Pea** *Pisum sativum*
Seedling emergence					
ER_{50}	> 1000.00	> 1000.00	> 1000.00	> 1000.00	> 1000.00
NOER	400.00	≥ 1000.00	400.00	400.00	≥ 1000.00
Post-emergence survival					
ER_{50}	> 1000.00	> 1000.00	> 1000.00	> 1000.00	> 1000.00
NOER	≥ 1000.00	≥ 1000.00	≥ 1000.00	≥ 1000.00	≥ 1000.00
Shoot length					
ER_{50}	283.58 (245.37 – 327.73)	339.32 (262.20 – 439.13)	432.98 (339.85 – 573.46)	364.51 (277.19 – 479.35)	758.20 (528.78 – >1000.00)
NOER	25.60	25.60	64.00	64.00	25.60
Shoot dry weight					
ER_{50}	170.87 (136.99 – 213.13)	206.34 (159.08 – 267.65)	225.34 (140.38 – 361.73)	179.75 (140.75 – 229.57)	202.91 (163.59 – 251.68)
NOER	25.60	25.60	0.70	10.20	10.20
Plant damages					
ER_{50}	106.62 (99.53 – 114.22)	188.40 (170.08 – 208.69)	119.64 (108.64 – 130.68)	131.40 (119.71 – 144.24)	59.09 (52.55 – 66.45)
NOER	10.20	25.60	25.60	n.d.*	0.30

*n.d. – not determined

	Sugar beet *Beta vulgaris*	**Carrot** *Daucus carota*	**Onion** *Allium cepa*	**Perennial reygrass** *Lolium perenne*	**Oats** *Avena sativa*
Seedling emergence					
ER_{50}	> 1000.00	> 1000.00	307.31 (132.82 – 711.05)	> 1000.00	> 1000.00
NOER	≥ 1000.00	400.00	25.60	400.00	≥ 1000.00
Post-emergence survival					
ER_{50}	> 1000.00	> 1000.00	> 1000.00	> 1000.00	> 1000.00
NOER	≥ 1000.00	≥ 1000.00	≥ 1000.00	≥ 1000.00	≥ 1000.00
Shoot length					
ER_{50}	477.94 (390.41 – 585.11)	279.93 (215.44 – 363.73)	80.80 (64.79 – 100.77)	160.80 (131.05 – 197.29)	> 1000.00
NOER	25.60	25.60	10.20	64.00	160.00
Shoot dry weight					
ER_{50}	146.27 (116.14 – 184.21)	730.09 (452.89 ->1000.00)	> 1000.00	183.91 (147.61 – 229.13)	> 1000.00
NOER	25.60	160.00	25.60	64.00	400.00
Plant damages					
ER_{50}	90.09 (83.13 – 97.65)	122.27 (103.32 -144.76)	75.48 (68.09 – 83.68)	124.83 (115.49 – 134.93)	> 1000.00
NOER	10.20	10.20	10.20	25.60	160.00

Reference: KCP 10.6.2/01

Report: DNT-162OD-R-CPd Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test according to OECD Guideline No. 208 (2006), Pieczka P., 2023

	STUDY CODE: G-34-23 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry
Guideline(s):	Yes. According to the OECD Guideline for the Testing of Chemicals No. 208 "Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test"
Deviations:	Yes.
GLP:	Yes
Acceptability:	Yes

Materials and methods

1. Test material: DNT-162OD-R-CPd

2. Batch number: 1/23
   Concentration of dicamba – 112.0 g/L nicoslufuron – 39.0 g/L thifensulfuron-methyl – 12.4 g/L

3. Test organism: Test organism: Ten plant species were used. These were: tomato (*Solanum lycopersicon*), cabbage (*Brassica oleracea var. capitata*), lettuce (*Lactuca sativa*), oilseed rape (*Brassica napus*), pea (*Pisum sativum*), sugar beet (Beta vulgaris), carrot (Daucus carota), onion (*Allium cepa*), perennial ryegrass (*Lolium perenne*), oats (*Avena sativa*).

4. Test design:

The study, aimed at evaluating the effect of DNT-162OD-R-CPd on seedling emergence and seedling growth of 10 terrestrial plants, was conducted on 6 dicotyledonous and 4 monocotyledonous species.

The test item was sprayed onto the soil surface. Ten application rates (0.3, 0.7, 1.6, 4.1, 10.2, 25.6, 64.0, 160.0, 400.0, 1000.0 mL test item/ha) were used for pea, cabbage and lettuce; nine application rates (0.7, 1.6, 4.1, 10.2, 25.6, 64.0, 160.0, 400.0, 1000.0 mL test item/ha) for onion and perennial ryegrass; eleven application rates (0.1, 0.3, 0.7, 1.6, 4.1, 10.2, 25.6, 64.0, 160.0, 400.0, 1000.0 mL test item/ha) for sugar beet; Five application rates (25.6, 64.0, 160.0, 400.0, 1000.0 mL test item/ha) for oats; seven application rates (4.1, 10.2, 25.6, 64.0, 160.0, 400.0, 1000.0 mL test item/ha) for carrot and oilseed rape; six application rates (10.2, 25.6, 64.0, 160.0, 400.0, 1000.0 mL test item/ha) for tomato. There was also a concurrent control group. Selected number of plants per pot provide the adequate growth conditions and avoid overcrowding during the experiment.

The number of seeds per pot as well as the total number of seeds per concentration for each of the tested species is presented below:
- pea: 3 plants/pot – 21 plants/rate (7 pots/rate)
- cabbage: 2 plants/pot – 20 plants/rate (10 pots/rate)
- tomato: 2 plants/pot – 20 plants/rate (10 pots/rate)
- carrot: 5 plants/pot – 20 plants/rate (4 pots/rate)
- lettuce - 3 plants/pot – 21 seeds/rate (7 pots/rate)
- oilseed rape: 3 plants/pot – 21 plants/rate (7 pots/rate)
- onion: 5 plants/pot – 20 plants/rate (4 pots/rate)
- oats - 5 plants/pot – 20 plants/rate (4 pots/rate)
- perennial ryegrass: 5 plants/pot – 20 plants/rate (4 pots/rate)
- sugar beet - 2 plants/pot – 20 plants/rate (10 pots/rate).

The experiment was conducted in a special plant growth chamber where suitable environmental conditions were provided. During the study, the plants were observed for emergence (every day before the emergence of 50% of the control seedlings and then every 2 – 3 days) and visual phytotoxicity (7 and 14 day after the emergence of 50% of the control seedlings). Phytotoxic effects and plant damage were recorded. The experiment finished 14 days after the germination of 50% of the control seedlings. At the end of the experiment, the number of surviving plants was counted. Next, the plants were cut down, and the lengths of their shoots were determined. Finally, they were dried at 60ºC to a constant weight and weighed.

Results and discussions:

The results concerning the emergence, the shoot length, and the dry weight were statistically analyzed to determine the ER_{10}, ER_{25}, ER_{50}, NOER, and LOER.

The values determined on the basis of seedling emergence, plants number at the end of the experiment, shoot length and shoot dry weight measurements, phytotoxic symptoms expressed as mL of the test item/ ha for all test species are given below.

	Tomato *Solanum lycopersicon*	**Cabbage** *Brassica oleracea var. capitata*	**Lettuce** *Lactuca sativa*	**Oilseed rape** *Brassica napus*	**Pea** *Pisum sativum*
Seedling emergence					
ER_{50}	> 1000.00	> 1000.00	> 1000.00	> 1000.00	> 1000.00
NOER	400.00	≥ 1000.00	400.00	400.00	≥ 1000.00
Post-emergence survival					
ER_{50}	> 1000.00	> 1000.00	> 1000.00	> 1000.00	> 1000.00
NOER	≥ 1000.00	≥ 1000.00	≥ 1000.00	≥ 1000.00	≥ 1000.00
Shoot length					
ER_{50}	283.58 (245.37 – 327.73)	339.32 (262.20 – 439.13)	432.98 (339.85 – 573.46)	364.51 (277.19 – 479.35)	758.20 (528.78 – >1000.00)
NOER	25.60	25.60	64.00	64.00	25.60
Shoot dry weight					
ER_{50}	170.87 (136.99 – 213.13)	206.34 (159.08 – 267.65)	225.34 (140.38 – 361.73)	179.75 (140.75 – 229.57)	202.91 (163.59 – 251.68)
NOER	25.60	25.60	0.70	10.20	10.20
Plant damages					
ER_{50}	106.62 (99.53 – 114.22)	188.40 (170.08 – 208.69)	119.64 (108.64 – 130.68)	131.40 (119.71 – 144.24)	59.09 (52.55 – 66.45)
NOER	10.20	25.60	25.60	n.d.*	0.30

	Sugar beet *Beta vulgaris*	**Carrot** *Daucus carota*	**Onion** *Allium cepa*	**Perennial reygrass** *Lolium perenne*	**Oats** *Avena sativa*
Seedling emergence					
ER_{50}	> 1000.00	> 1000.00	307.31 (132.82 – 711.05)	> 1000.00	> 1000.00
NOER	≥ 1000.00	400.00	25.60	400.00	≥ 1000.00
Post-emergence survival					
ER_{50}	> 1000.00	> 1000.00	> 1000.00	> 1000.00	> 1000.00
NOER	≥ 1000.00	≥ 1000.00	≥ 1000.00	≥ 1000.00	≥ 1000.00
Shoot length					
ER_{50}	477.94 (390.41 – 585.11)	279.93 (215.44 – 363.73)	80.80 (64.79 – 100.77)	160.80 (131.05 – 197.29)	> 1000.00
NOER	25.60	25.60	10.20	64.00	160.00
Shoot dry weight					
ER_{50}	146.27 (116.14 – 184.21)	730.09 (452.89 ->1000.00)	> 1000.00	183.91 (147.61 – 229.13)	> 1000.00
NOER	25.60	160.00	25.60	64.00	400.00
Plant damages					
ER_{50}	90.09 (83.13 – 97.65)	122.27 (103.32 -144.76)	75.48 (68.09 – 83.68)	124.83 (115.49 – 134.93)	> 1000.00
NOER	10.20	10.20	10.20	25.60	160.00

1. On the basis of the obtained results it was proved that the test item i.e. DNT-162OD-R CPd had diversified impact on seedling emergence and growth of tested plant species.
2. Delayed emergence of plants in comparison to the control group was not noticed in cultivation of lettuce, pea, sugar beet, carrot, onion, perennial ryegrass. Mortality of plants was noticed in cultivation of sugar beet and carrot.
3. On the basis of NOER, ER_{10}, ER_{25} and ER_{50} values determined from the plant emergence during exposure period it was proved that the test item inhibited the process of seedling emergence of lettuce, oilseed rape, carrot and onion. Slight effect was observed in cultivation of perennial ryegrass and tomato. The test item did not inhibited process of seedling emergence of cabbage, pea, oats, sugar beet.
4. On the basis of NOER, ER_{10}, ER_{25} and ER_{50} values determined from the survival of plants it was proved that the test item had slight impact on carrot. No effect was noticed in cultivation of tomato, cabbage, lettuce, oilseed rape, pea, onion, perennial ryegrass, oats and sugar beet.
5. On the basis of NOER, ER_{10}, ER_{25} and ER_{50} values determined from the shoot length it was proved that the test item inhibited the process of growth of all tested plant species.
6. On the basis of NOER, ER_{10}, ER_{25} and ER_{50} values determined from the dry shoot weight it was proved that the test item inhibited process of the growth of all tested plant species.
7. During the exposure period, the phytotoxic symptoms of the test item were observed in cultivation of all tested plant species.

Validity of the test:

- Seedling emergence in the control was at least 90% (sugar beet and carrot).
- the control seedlings did not exhibit any visible phytotoxic symptoms,
- Mean survival of plants in control was 100% for every species (required at least 90%)
- Environmental conditions and soil were identical for all used in the experiment plants species

Conclusion:
The lowest ER_{50} was determined for onion (plant damages) and is equal to 59.09 mL of the test item/ ha.

Comments of zRMS:	The study is acceptable. The validity criteria of the test according to OECD 227 were met. **Validity criteria:** **Deviation from OECD Guideline No. 227:** On the basis of the obtained results, it was stated that the following validity criteria of the study aimed at evaluating the impact of DNT-162OD-R-CPd, on vegetative vigour of terrestrial plants were met: - the seedling emergence of plants (validity criterion: at least 70%) was as follows: 92.9 – 100.0 – pea, 92.9 – 100.0 – cabbage, 83.3 – 92.9 – lettuce, 92.9 – 100.0 – oilseed rape, 95.0 – 100.0 – tomato, 90.0 – 100.0 –sugar beet, 90.0 – 100.0 – onion, 92.5 – 100.0 – perennial ryegrass, 90.0 – 97.5 – carrot, 95.0 – 100.0 – oats; - the mean plant survival of the control was 100% for all tested species (validity criterion: at least 90%), - the control plants did not exhibit any visible phytotoxic symptoms, - environmental conditions for all plants belonging to the same species were identical. **Deviation from OECD Guideline No. 227:** According to OECD Guideline No. 227 (2006), the light intensity should be 350 ± 50µE/m2/s. However, these values are recommended for tests conducted in greenhouses. The experiment was conducted in a test room, where only artificial lighting was used. **Agreed toxicity endpoints:** The values determined on the basis of plants number at the end of the experiment, shoot length and shoot dry weight measurements, phytotoxic symptoms expressed as mL of the test item/ ha for all test species are given below.

	Tomato *Solanum lycopersicon*	**Cabbage** *Brassica oleracea var. capitata*	**Lettuce** *Lactuca sativa*	**Oilseed rape** *Brassica napus*	**Pea** *Pisum sativum*
Plant number at the end of the experiment					
ER_{50}	799.90 (527.31 – >1000.0)	>1000.00	>1000.00	>1000.00	>1000.00
NOER	160.0	≥1000.00	400.00	≥1000.00	≥1000.00
Shoot length					
ER_{50}	105.30 (91.22 – 122.25)	>1000.00	320.39 (243.94 – 443.54)	903.19 (697.88 – >1000.0)	249.62 (218.58 – 285.15)
NOER	4.10	64.0	25.60	10.20	64.00
Shoot dry weight					
ER_{50}	138.20 (119.99 – 159.68)	519.57 (442.67 – 623.39)	337.34 (285.52 – 405.06)	250.93 (188.01 – 341.11)	353.14 (313.73 – 397.99)
NOER	10.20	25.60	25.60	1.60	64.00
Plant damages					
ER_{50}	42.01 (39.61 – 44.55)	153.19 (138.59 – 169.32)	80.01 (71.87 – 89.07)	27.85 (23.09 – 33.61)	128.20 (115.14 – 142.74)
NOER	4.10	n.d.	10.20	n.d.	n.d.

	Sugar beet *Beta vulgaris*	**Carrot** *Daucus carota*	**Onion** *Allium cepa*	**Perennial ryegrass** *Lolium perenne*	**Oats** *Avena sativa*
Plant number at the end of the experiment					
ER_{50}	699.73 (442.35 – >1000.00)	>1000.00	>1000.00	540.19 (382.12 – 763.65)	>1000.00
NOER	64.00	160.00	160.00	160.00	≥1000.00
Shoot length					
ER_{50}	312.12 (247.56 – 409.59)	438.74 (340.28 – 565.68)	213.22 (188.03 – 242.21)	335.96 (272.88 – 413.62)	687.61 (628.19 – 757.69)
NOER	10.20	25.60	10.20	64.00	64.00
Shoot dry weight					
ER_{50}	79.31 (70.14 – 89.72)	179.18 (149.11 – 215.32)	449.95 (376.84 – 537.23)	422.55 (384.29 – 464.62)	620.24 (549.02 – 709.44)
NOER	10.20	10.20	64.00	64.00	64.00
Plant damages					
ER_{50}	112.32 (101.14 – 124.75)	75.96 (53.83 – 107.63)	75.70 (61.84 –92.67)	103.04 (89.82 – 118.20)	410.92 (322.33 – 523.87)
NOER	4.10	10.20	n.d.	25.60	64.00

Reference: KCP 10.6.2/02

Report: DNT-162OD-R-CPd Terrestrial Plant Test: Vegetative Vigour Test according to OECD Guideline No. 227 (2006), Pieczka P., 2023 STUDY CODE: G-33-23 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry

Guideline(s): Yes. According to the OECD Guideline for the Testing of Chemicals No. 227 “Terrestrial Plant Test: Vegetative Vigour Test”.

Deviations: Yes.

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GLP: Yes

Acceptability: Yes

Materials and methods

1. Test material: DNT-162OD-R-CPd

2. Batch number: 1/23
   Concentration of dicamba – 112.0 g/L nicosulfuron – 39.0 g/L thifensulfuron-methyl – 12.4 g/L

3. Test organism: Test organism: Ten plant species were used. These were: Cabbage (*Brassica oleracea var. capitata*), Tomato (*Solanum lycopersicon*), oilseed rape (*Brassica napus*), Pea (*Pisum sativum*), Sugar beet (*Beta vulgaris*), Lettuce (*Lactuca sativa*), Carrot (*Daucus carota*), Onion (*Allium cepa*), Oats (*Avena sativa*), Perennial ryegrass (*Lolium perenne*).

1. Test design:

The study, aimed at evaluating the effect of DNT-162OD-R-CPd on vegetative vigour of 10 terrestrial plants, was conducted on 7 dicotyledonous and 3 monocotyledonous species.

Seeds of the test plant species were sown in plastic pots (6 seeds/pot for pea, cabbage, oilseed rape and lettuce; 10 seeds/pot for tomato and sugar beet, 4 seeds/pot for carrot, onion, perennial ryegrass and oats). The plants were grown to the 2- to 4- true leaf stage. Then, some of them were removed.

As a result, the number of plants per pot as well as the total number of plants per concentration were:
- pea: 3 plants/pot – 21 plants/rate (7 pots/rate)
- cabbage: 3 plants/pot – 21 plants/rate (7 pots/rate)
- tomato: 2 plants/pot – 20 plants/rate (10 pots/rate)
- carrot: 5 plants/pot – 20 plants/rate (4 pots/rate)
- lettuce - 3 plants/pot – 21 seeds/rate (7 pots/rate)
- oilseed rape: 3 plants/pot – 21 plants/rate (7 pots/rate)
- sugar beet - 2 plants/pot – 20 plants/rate (10 pots/rate).
- onion: 5 plants/pot – 20 plants/rate (4 pots/rate)
- oats - 5 plants/pot – 20 plants/rate (4 pots/rate)
- perennial ryegrass: 5 plants/pot – 20 plants/rate (4 pots/rate)

The pot is defined as a replicate. The test item was sprayed onto the plants. Seven application rates (4.1, 10.2, 25.6, 64.0, 160.0, 400.0, 1000.0 mL test item/ha) were used for cabbage, tomato, perennial ryegrass, sugar beet and onion; six application rates (10.2, 25.6, 64.0, 160.0, 400.0, 1000.0 mL test item/ha) for lettuce, cabbage, pea and oats; nine application rates 0.7, 1.6, 4.1, 10.2, 25.6, 64.0, 160.0, 400.0, 1000.0 mL test item/ha) for oilseed rape. Untreated control group was conducted simultaneously.

The experiment was conducted in a plant growth room where suitable environmental conditions for each test species were provided. During the experiment, the plants were observed for visual phytotoxicity (7, 14 and 21 days after the test item application). The experiment finished 21 days after the spraying. At the end of the experiment, the number of surviving plants was counted. Next, the plants were cut down, and the lengths of their shoots were determined. Finally, they were dried at 60ºC to a constant weight and weighed.

The results concerning the phytotoxic effects, the shoot length, the dry weight, and the number of plants at the end of the experiment were statistically analyzed to determine the ER_{10}, ER_{25}, ER_{50}, NOER, and LOER.

Results and discussions:

The results concerning the shoot length, the dry weight, and the number of plants at the end of the experiment were statistically analyzed to determine the ER_{10}, ER_{25}, ER_{50}, NOER, and LOER.

The values determined on the basis of plants number at the end of the experiment, shoot length and shoot dry weight measurements, phytotoxic symptoms expressed as mL of the test item/ ha for all test species are given below.

	Tomato *Solanum lycopersicon*	**Cabbage** *Brassica oleracea var. capitata*	**Lettuce** *Lactuca sativa*	**Oilseed rape** *Brassica napus*	**Pea** *Pisum sativum*
Plant number at the end of the experiment					
ER_{50}	799.90 (527.31 – >1000.0)	>1000.00	>1000.00	>1000.00	>1000.00
NOER	160.0	≥1000.00	400.00	≥1000.00	≥1000.00
Shoot length					
ER_{50}	105.30 (91.22 – 122.25)	>1000.00	320.39 (243.94 – 443.54)	903.19 (697.88 – >1000.0)	249.62 (218.58 – 285.15)
NOER	4.10	64.0	25.60	10.20	64.00
Shoot dry weight					
ER_{50}	138.20 (119.99 – 159.68)	519.57 (442.67 – 623.39)	337.34 (285.52 – 405.06)	250.93 (188.01 – 341.11)	353.14 (313.73 – 397.99)
NOER	10.20	25.60	25.60	1.60	64.00
Plant damages					
ER_{50}	42.01 (39.61 – 44.55)	153.19 (138.59 – 169.32)	80.01 (71.87 – 89.07)	27.85 (23.09 – 33.61)	128.20 (115.14 – 142.74)
NOER	4.10	n.d.	10.20	n.d.	n.d.

	Sugar beet *Beta vulgaris*	**Carrot** *Daucus carota*	**Onion** *Allium cepa*	**Perennial ryegrass** *Lolium perenne*	**Oats** *Avena sativa*
Plant number at the end of the experiment					
ER_{50}	699.73 (442.35 – >1000.00)	>1000.00	>1000.00	540.19 (382.12 – 763.65)	>1000.00
NOER	64.00	160.00	160.00	160.00	≥1000.00
Shoot length					
ER_{50}	312.12 (247.56 – 409.59)	438.74 (340.28 – 565.68)	213.22 (188.03 – 242.21)	335.96 (272.88 – 413.62)	687.61 (628.19 – 757.69)
NOER	10.20	25.60	10.20	64.00	64.00
Shoot dry weight					
ER_{50}	79.31 (70.14 – 89.72)	179.18 (149.11 – 215.32)	449.95 (376.84 – 537.23)	422.55 (384.29 – 464.62)	620.24 (549.02 – 709.44)
NOER	10.20	10.20	64.00	64.00	64.00
Plant damages					
ER_{50}	112.32 (101.14 – 124.75)	75.96 (53.83 – 107.63)	75.70 (61.84 –92.67)	103.04 (89.82 – 118.20)	410.92 (322.33 – 523.87)
NOER	4.10	10.20	n.d.	25.60	64.00

1. The test item, i.e. DNT-162OD-R-CPd, had a varied impact on vegetative vigour of all tested plant species.
2. On the basis of NOER, ER_{10}, ER_{25} and ER_{50} values determined from the plant number at the end of the experiment it was proved that the test item inhibited the process of growth of tomato, sugar beet, carrot, onion, perennial ryegrass, lettuce. No influence was noticed in cultivation of cabbage, oilseed rape, pea, oats.
3. On the basis of NOER, ER_{10}, ER_{25} and ER_{50} values determined from the shoot length it was proved that the test item inhibited the process of growth of all tested plant species.
4. On the basis of NOER, ER_{10}, ER_{25} and ER_{50} values determined from the dry shoot weight it was proved that the test item inhibited the process of growth of all tested plant species.
5. During the experiment the phytotoxic symptoms of the test item were noticed in cultivation of all tested plant species.

Validity of the test:

- Seedling emergence in the control was at least 83.3% (lettuce)
- In none of the control replications of any plants species there were any signs of intoxications visible
- Mean survival of plants in control was 100% for every species (required at least 90%)
- Environmental conditions and soil were identical for all used in the experiment plants species

Conclusion:
The lowest ER_{50} was determined for oilseed rape (plant damages) and is equal to 27.85 mL of the test item/ha.

A 2.6.3 KCP 10.6.3 Extended laboratory studies on non-target plants

A 2.7 KCP 10.7 Effects on other terrestrial organisms (flora and fauna)

A 2.8 KCP 10.8 Monitoring data