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| REGISTRATION REPORT  Part B  Section 9  Ecotoxicology  Detailed summary of the risk assessment |
| Product code: ADM.09250.H.1.A  Product name(s): **2,4-D 95 SP**  Chemical active substance:  2,4-dichlorophenoxy acetic acid**,** 80.4% or 804 g/kg |
| Central Zone  Zonal Rapporteur Member State: Poland |
| CORE ASSESSMENT  (authorisation) |
| Applicant: XXXX  Sponsor: XXXX  Submission date: March 2023  Evaluation date: December 2023  MS Finalisation date: March 2024 |

Version history

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| --- | --- |
| When | What |
| March 2023 | 1st applicant version |
| May 2023 | dRR submitted by applicant to the Polish Ministry of Agriculture and Rural Development |
| July 2023 | Submission to the evaluation unit |
| December 2023 | zRMS finalized dRR evaluation |

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Ecotoxicology (KCP 10)

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| **Review Comments:**  This document describes the acceptable use conditions required for registration of ADM.0950.H.1.A, a soluble powder containing 2,4-D 950 g/kg as sodium salt monohydrate (804 g/kg as acetic acid), for use as a herbicide in spring wheat.  This Part B document only reviews data and additional information that has not previously been considered within the EU review process.  Since this document is based on the information provided by the applicant, all review comments, additions and corrections have been made using commenting boxes or highlighted in grey. |

## 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 | Poland | Spring Wheat | F | Broadleaf Weeds  CENCY, VERPE, BRSNW, THLAR, CAPBP | Overall, Broadcast foliar spray | BBCH 15-25 | 1 | (-) | 0.9328 kg product (2,4-D acid)/ha | 0.75  (as acetic acid) | 200 -300 | - | - | A | A | A | A | A | A | 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 equipment (e.g. ULVA or LVA) it should be mentioned under “application: method/kind”.  (13) PHI - minimum pre-harvest interval  (14) Remarks may include: Extent of use/economic importance/restrictions |

### Overall conclusions

#### 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)

Based on the intended use of 2,4-D 95 SP on spring wheat, acceptable risk is demonstrated to birds and mammals at Tier 1. The risk to birds and mammals *via* drinking water is deemed low, and due to the low potential of 2,4-D to bioaccumulate the risk from secondary poisoning is considered acceptable.

The risk of secondary poisoning from the relevant metabolites 2,4-DCP and 2,4-DCA to earthworm-eating and fish-eating birds and mammals is also considered acceptable.

There is currently no guidance addressing terrestrial life stages of amphibians and reptiles in ecotoxicological risk assessments. Therefore, the risk assessment provided for birds and mammals is considered protective of terrestrial amphibian and reptile species.

#### Effects on aquatic organisms (KCP 10.2)

For the intended use of 2,4-D 95 SP on spring wheat, calculated PECSW/RAC ratios have indicated an acceptable risk to aquatic organisms using FOCUS Step 3 for the parent and Steps 1 to 2 for the metabolites 2,4-DCP, 2,4-DCA and 4-chlorophenol. No risk assessment has been conducted for metabolite 1,2,4-benzenetriol due to the transient nature of the metabolite.

#### Effects on bees (KCP 10.3.1)

Based on the intended use of 2,4-D 95 SP on spring wheat, the acute oral and acute contact risk to bees is deemed acceptable at the first tier.

#### Effects on arthropods other than bees (KCP 10.3.2)

Based on the intended use of 2,4-D 95 SP on spring wheat, acceptable in-field and off-field risk was demonstrated to non-target arthropods at the first tier.

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

Based on the intended use of 2,4-D 95 SP on spring wheat, the risk to earthworms and other non-target soil macrofauna is deemed acceptable at the first tier.

The risk to soil micro-organisms is also deemed acceptable with no significant effects (>25%) observed at concentrations greater than the predicted maximum soil concentrations.

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

Based on the intended use of 2,4-D 95 SP on spring wheat, acceptable risk is demonstrated to non-target terrestrial plants with mitigation of either 75% DRT or a 5 m buffer strip.

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

Not relevant.

### Grouping of intended uses for risk assessment

The following table documents the intended use of 2,4-D 95 SP according to the GAP. This is the relevant parameter for all areas of the risk assessment.

Table 9.1‑2: Critical use pattern of 2,4-D 95 SP

| Group | Intended use | Frequency / timing | Application rate |
| --- | --- | --- | --- |
| 1 | Spring wheat | 1 application at BBCH 15 - 25 | 0.75 kg a.s./ha (as acetic acid) |

### 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 2,4-D 95 SP is indicated in the table.

Table 9.1‑3 Metabolites of 2,4-D

| Metabolite | Molar mass | Chemical structure | Maximum occurrence in compartments | Risk assessment required? |
| --- | --- | --- | --- | --- |
| 2,4-DCP | 163 |  | Soil (aerobic): 8.7%  Water/Sediment: 32.1% | Soil: Yes  Surface water: Yes |
| 2,4-DCA | 177 |  | Soil (aerobic): 15%  Water/Sediment: 5.3% | Soil: Yes  Surface water: Yes |
| 4-CP | 128.6 |  | Soil (anaerobic): 33%  Water/Sediment: 6.9% | Soil: Yes  Surface water: Yes |
| 1,2,4-benzenetriol | 126.1 |  | Soil (aerobic): 0.1%  Water/Sediment: 31.7% | Soil: No  Surface water: Yes |

## 

## Effects on birds (KCP 10.1.1)

### Toxicity data

Avian toxicity studies have previously been carried out with 2,4-D its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

However, the provision of further data using the formulation 2,4-D 95 SP is not considered essential, because the high active substance concentration in the product allows for bridging from data with the active substance.

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process. A summary of these endpoints is presented below.

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

| Species | Substance | Exposure System | Results | Reference |
| --- | --- | --- | --- | --- |
| Canary (*Serinus canaria*) | 2,4-D acid | Oral 1 d Acute | LD50 = 633 mg/kg bw | EFSA Journal 2014;12(9):3812 |
| Japanese quail (*Coturnix coturnix japonica*) | 2,4-D acid | Oral 1 d Acute | LD50 = 617.3 mg/kg bw | EFSA Journal 2014;12(9):3812 |
| Bobwhite quail (*Colinus virginianus*) | 2,4-D acid | Oral 1 d Acute | LD50 = 500 mg/kg bw | EFSA Journal 2014;12(9):3812 |
| Geometric mean of three acute studies | | | **LD50 = 580.3 mg a.s./kg bw** | EFSA Journal 2014;12(9):3812 |
| Northern bobwhite  (*Colinus virginianus*) | 2,4-D | Dietary 8 d Short-term | LD50 >5620 mg/kg feed | EFSA Journal 2014;12(9):3812 |
| Mallard duck  (*Anas platyrhynchos*) | 2,4-D | Dietary 8 d Short-term | LD50 >5620 mg/kg feed | EFSA Journal 2014;12(9):3812 |
| Bobwhite quail (*Colinus virginianus*) | 2,4-D acid | Long-term | NOEL ~~NOEC~~ = 100 mg/kg bw/day | EFSA Journal 2014;12(9):3812 |
| Bobwhite quail (*Colinus virginianus*) | 2,4-D acid | Long-term | NOEL ~~NOEC~~ = 101 mg/kg bw/day | EFSA Journal 2014;12(9):3812 |
| Japanese quail (*Coturnix coturnix japonica*) | 2,4-D acid | Long-term | NOEL ~~NOEC~~ = 100 mg/kg bw/day | EFSA Journal 2014;12(9):3812 |
| Parent LD50/10 used as lowest endpoint in accordance with the current EFSA/2009/1438 | | | **NOEL ~~NOEC~~ = 58.03 mg/kg bw** | See section 9.2.1.1 |

Endpoints highlighted in **bold** have been used in the risk assessment.

#### Justification for new endpoints

The reproductive risk assessment has been conducted with the parent LD50/10 endpoint in accordance with the current EFSA/2009/1438 and as used in the EFSA Journal 2014;12(9):3812, where it is required that the lower of the acute LD50/10 and the long-term NOEL is used.

### Risk assessment for spray applications

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

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

Exposure of birds will be predominantly dietary through the consumption of residues on food items. Direct exposure of birds to 2,4-D 95 SP applications is considered unlikely, since at the time of application and for a short period thereafter, most birds will leave the immediate vicinity of spray operations in response to the human disturbance.

The acute ‘daily dietary dose’ (DDD) is calculated by multiplying the shortcut value (SV) based on the 90th percentile residues by the application rate in kg a.s./ha and the multiple application factor for 90th percentile residue data (MAF90).

DDD = application rate (kg a.s./ha) x MAF90 x SV

The long-term ‘daily dietary dose’ (DDD) is calculated by multiplying the shortcut value (SV) based on the mean residues by the application rate in kg a.s./ha, the appropriate multiple application factor (MAFM), and a time weighted average residue exposure (*f*TWA). The *f*TWA based upon a default DT50 of 10 days is 0.53, as given in EFSA/2009/1438.

DDD = application rate (kg a.s./ha) x SV x *f*TWA x MAFM

The TERA and TERLT values are calculated by dividing the acute and chronic toxicity endpoint by the respective daily dietary dose. The results of the acute and reproductive first-tier risk assessments are summarised in the following tables.

Table 9.2‑2: Screening step assessment of the acute and long-term/reproductive risk for birds due to the use of 2,4-D 95 SP in spring wheat

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Intended use | | Spring wheat | | | | |
| Active substance/product | | 2,4-D | | | | |
| Application rate (g a.s./ha) | | 1 × 750 | | | | |
| Acute toxicity (mg/kg bw) | | 580.3 | | | | |
| TER criterion | | 10 | | | | |
| Crop scenario | Generic focal species | | SV | MAF90 | DDD90  (mg/kg bw/d) | TERA |
| Cereals | Small omnivorous bird | | 158.8 | 1.00 | 119.1 | **4.9** |
| Reprod. toxicity (mg/kg bw/d) | | 58.03 | | | | |
| TER criterion | | 5 | | | | |
| Crop scenario | Generic focal species | | SV | MAFM × TWA | DDDM  (mg/kg bw/d) | TERLT |
| Cereals | Small omnivorous bird | | 64.8 | 1.00 x 0.53 | 25.8 | **2.25** |

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.

The TER values for the acute and long-term risk are below the respective trigger values, therefore a Tier I assessment is provided below.

Table 9.2‑3: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of 2,4-D 95 SP in spring wheat

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Intended use | | Spring wheat | | | | |
| Active substance/product | | 2,4-D | | | | |
| Application rate (g a.s./ha) | | 1 × 750 | | | | |
| Acute toxicity (mg/kg bw) | | 580.3 | | | | |
| TER criterion | | 10 | | | | |
| Crop scenario  Growth stage | Indicator species | | SV90 | MAF90 | DDD90  (mg/kg bw/d) | TERA |
| Cereals,  BBCH 10-29 | Small omnivorous bird "lark" | | 24.0 | 1.00 | 18.0 | 32 |
| Reprod. toxicity (mg/kg bw/d) | | 58.03 | | | | |
| TER criterion | | 5 | | | | |
| Crop scenario  Growth stage | Indicator species | | SVM | MAFM × TWA | DDDM  (mg/kg bw/d) | TERLT |
| Cereals,  BBCH 10-29 | Small omnivorous bird "lark" | | 10.9 | 1.00 x 0.53 | 4.3 | 13.5 |

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

|  |
| --- |
| **Review Comments:**  zRMS agrees with TERs calculations performed by the applicant. The risk assessment for large herbivorous birds “goose” is not required as product is proposed to be use only in spring cereals. |

#### Higher-tier risk assessment

Not required as the risk to birds is deemed acceptable at Tier 1.

#### 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 2,4-D 95 SP is not a 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 is not relevant.

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 K(f)oc of 58.6, 2,4-D belongs to the group of less sorptive substances.

|  |  |  |  |
| --- | --- | --- | --- |
| Effective application rate (g a.s./ha) = | 750 |  |  |
| Acute toxicity (mg/kg bw) = | 580.3 | quotient = | 1.3 |
| Reprod. toxicity (mg/kg bw/d) = | 58.03 | quotient = | 13 |

#### Effects of secondary poisoning

The log Pow of 2,4-D amounts to 1.54 at pH 4 (the highest value) and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

However, in line with the EU review, secondary poisoning assessments are required for two metabolites of 2,4-D, *i.e*. 2,4-DCP (log POW = 3.06) and 2,4-DCA (log POW = 3.36). Assessments are based on an assumed toxicity ten times higher than the reproductive endpoint used for the parent, *i.e*. based on an NOEL of 5.803 mg/kg bw/d.

Risk assessment for earthworm-eating birds via secondary poisoning

According to EFSA/2009/1438, the risk for vermivorous birds is assessed for a bird of 100 g body weight with a daily food consumption of 104.6 g. Bioaccumulation in earthworms is estimated based on predicted concentrations in soil.

Table 9.2.2.4‑2: Assessment of the risk for earthworm-eating birds due to exposure to 2,4-DCP via bioaccumulation in earthworms (secondary poisoning) for the intended use of 2,4-D 95 SP in spring wheat

| Parameter |  | Comments |
| --- | --- | --- |
| PECsoil (twa = 21 d) (mg/kg soil) | 0.0399 | Section 8 (Environmental Fate), Chapter 8.7, Table 8.7-5. |
| log Pow / Pow | 3.06/1148 | LoEP |
| Koc | 512 | LoEP |
| foc | 0.02 | Default |
| BCFworm | 1.43 | BCFworm/soil = (PECworm,ww/PECsoil,dw) = (0.84 + 0.012 × KOW) / fOC × KOC |
| PECworm | 0.057 | PECworm = PECsoil × BCFworm/soil |
| Daily dietary dose (mg/kg bw/d) | 0.060 | DDD = PECworm × 1.05 |
| NOEL (mg/kg bw/d) | 5.803 / 10 | 10 times toxicity of the parent (experimental acute LD50/10 for 2,4‑D acid or NOEL/10) |
| TERlt | 97 / 167 | Trigger ≥5 |

The TERLT is above the trigger value of 5 indicating an acceptable long-term risk to earthworm-eating birds from the metabolite 2,4-DCP.

Table 9.2.2.4‑2: Assessment of the risk for earthworm-eating birds due to exposure to 2,4-DCA via bioaccumulation in earthworms (secondary poisoning) for the intended use of 2,4-D 95 SP in spring wheat

| Parameter |  | Comments |
| --- | --- | --- |
| PECsoil (twa = 21 d) (mg/kg soil) | 0.078 | Section 8 (Environmental Fate), Chapter 8.7, Table 8.7-6. |
| log Pow / Pow | 3.36/2291 | LoEP |
| Koc | 1028 | LoEP |
| foc | 0.02 | Default |
| BCFworm | 1.43 | BCFWORM/SOIL = (PECWORM,WW/PECSOIL,DW) = (0.84 + 0.012 × POW) / foc × Koc |
| PECworm | ~~0.107~~ 0.11 | PECWORM = PECSOIL × BCFWORM/SOIL |
| Daily dietary dose (mg/kg bw/d) | ~~0.113~~ 0.12 | DDD = PECWORM × 1.05 |
| NOEL (mg/kg bw/d) | 5.803 / 10 | 10 times toxicity of the parent (experimental acute LD50/10 for 2,4‑D acid or NOEL/10) |
| TERlt | ~~51~~ 50 / 85 | Trigger ≥5 |

The TERLT is above the trigger value of 5 indicating an acceptable long-term risk to earthworm-eating birds from the metabolite 2,4-DCA.

Risk assessment for fish-eating birds via secondary poisoning

For fish, the BCF has been reported as 340 and 31 mL/g for 2-4-DCP and 2,4-DCA, respectively (EFSA Journal 2014;12(9):3812). Residues in fish were calculated in line with EFSA/2009/1438 according to the following equation:

PECfish = PECwater × TWA × BCFfish

and converted to a daily dietary dose by multiplying with a factor of 0.159, which is based on a 1000 g bird eating 159 g fish per day.

Table 9.2.2.4‑3: Assessment of the risk for fish-eating birds due to exposure to 2,4-DCP via bioaccumulation in fish (secondary poisoning) for the intended use of 2,4-D 95 SP in spring wheat

| Parameter |  | Comments |
| --- | --- | --- |
| PECsw (initial max.) (mg/L) | 0.046342 | Section 8 (Environmental Fate), Chapter 8.9, Table 8.9-8 ~~7~~. Step 1 |
| BCFfish | 340 | LoEP |
| BMF | - | biomagnification factor (relevant for BCF ≥ 2000) |
| PECfish | ~~16~~ 8.35 | PECFISH = PECWATER × BCFFISH × TWA |
| Daily dietary dose (mg/kg bw/d) | ~~2.5~~ 1.33 | DDD = PECFISH × 0.159 |
| NOEL (mg/kg bw/d) | 5.803 / 10 | 10 times toxicity of the parent (experimental acute LD50/10 for 2,4‑D acid or NOEL/10) |
| TERlt | **~~2.3~~ 4.4** / 7.5 | Trigger ≥5 |

The TERLT is below the trigger value of 5 indicating that further consideration is necessary to cover the long-term risk to fish-eating birds from the metabolite 2,4-DCP.

For the refined assessment of the risk to fish-eating birds from exposure to 2,4-DCP, PECsw values used are 21-day TWA values following FOCUS Step 2. As the submission is to be applied in Poland, the Northern Europe 21-day TWA PECsw of 0.005977 mg/L has been used.

Table 9.2.2.4‑5: Assessment of the risk for fish-eating birds due to exposure to 2,4-DCP via bioaccumulation in fish (secondary poisoning) for the intended use of 2,4-D 95 SP in spring wheat

| Parameter |  |  |
| --- | --- | --- |
| PECsw (initial max.) NEU (mg/L) | 0.005977 | Section 8 (Environmental Fate), Chapter 8.9, Table 8.9-9 ~~8~~.  FOCUS Step 2, NEU Mar - Sep |
| BCFfish | 340 | LoEP |
| BMF | - | biomagnification factor (relevant for BCF ≥ 2000) |
| PECfish | ~~2.0~~ 1.08 | PECFISH = PECWATER × BCFFISH × TWA |
| Daily dietary dose (mg/kg bw/d) | ~~0.32~~ 0.17 | DDD = PECFISH × 0.159 |
| NOEL (mg/kg bw/d) | ~~2.06~~ 5.803 | 10 times toxicity of the parent (experimental acute LD50/10 for 2,4‑D acid ~~NOEL for 2,4‑D acid~~) |
| TERlt | ~~18~~ 34 | Trigger ≥5 |

The TERLT is above the trigger value of 5 indicating an acceptable long-term risk to fish-eating mammals from the metabolite 2,4-DCP.

Table 9.2.2.4‑4: Assessment of the risk for fish-eating birds due to exposure to 2,4-DCA via bioaccumulation in fish (secondary poisoning) for the intended use of 2,4-D 95 SP in spring wheat

| Parameter |  | Comments |
| --- | --- | --- |
| PECsw (initial max.) (mg/L) | 0.0176208 | Section 8 (Environmental Fate), Chapter 8.9, Table 8.9-8 ~~7~~. Step 1 |
| BCFfish | 31 | LoEP |
| BMF | - | biomagnification factor (relevant for BCF ≥ 2000) |
| PECfish | ~~0.541~~ 0.30 | PECFISH = PECWATER × BCFFISH × TWA |
| Daily dietary dose (mg/kg bw/d) | ~~0.085~~ 0.046 | DDD = PECFISH × 0.159 |
| NOEL (mg/kg bw/d) | 5.803 / 10 | 10 times toxicity of the parent (experimental acute LD50/10 for 2,4‑D acid or NOEL/10) |
| TERlt | ~~68~~ 126 / 217 | Trigger ≥5 |

The TERLT is above the trigger value of 5 indicating an acceptable long-term risk to fish-eating birds from the metabolite 2,4-DCA.

#### Biomagnification in terrestrial food chains

Not relevant.

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

Not relevant.

### Overall conclusions

Based on the intended use of 2,4-D 95 SP on spring wheat, acceptable risk is demonstrated to birds at Tier 1. The risk to birds *via* drinking water is deemed low, and due to the low potential of 2,4-D to bioaccumulate the risk from secondary poisoning is considered acceptable. The risk of secondary poisoning from the relevant metabolites 2,4-DCP and 2,4-DCA to earthworm-eating and fish-eating birds is also considered acceptable.

|  |
| --- |
| **Review Comments:**  The acute and chronic risks of 2,4-D 95 SP to birds were assessed from toxicity exposure ratios between toxicity endpoints, estimated from study with active ingredient and maximum residues occurring on food items.  All TER values exceed the relevant triggers indicating that 2,4-D 95 SP does not pose an unacceptable risk to birds following applications according to recommended use pattern.  Evaluation of exposing to birds through the drinking water demonstrated the acceptable risk. The potential risk of secondary poisoning from the relevant metabolites 2,4-DCP and 2,4-DCA is low. |

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

### Toxicity data

The provision of further data using the formulation 2,4-D 95 SP is not considered essential because the high active substance concentration in the product allows for bridging from data with the active substance.

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process.

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

| Species | Substance | Exposure  System | Results | Reference |
| --- | --- | --- | --- | --- |
| Rat | 2,4-D acid | Oral 1 d Acute | LD50 = 699 mg/kg bw | EFSA Journal 2014;12(9):3812 |
| Rat | 2,4-D acid | Oral 1 d Acute | LD50 = 486 mg/kg bw | EFSA Journal 2014;12(9):3812 |
| Rat | 2,4-D acid | Oral 1 d Acute | LD50 >500 mg/kg bw | EFSA Journal 2014;12(9):3812 |
| Geometric mean of three acute studies | | | **LD50 >554 mg/kg bw** | EFSA Journal 2014;12(9):3812 |
| Rat | 2,4-D acid | Long-term | **NOAEL = 20.6 mg/kg bw/day** | EFSA Journal 2014;12(9):3812# |

Endpoints highlighted in **bold** have been used in the risk assessment.

# Endpoint agreed at Pesticides Peer Review Meeting 111, 4-7 February 2014, and is the food consumption endpoint for females

#### Justification for new endpoints

Not relevant, endpoints do not deviate from EU-agreed endpoints.

### Risk assessment for spray applications

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

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

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

Table 9.3‑2: Screening step assessment of the acute and long-term/reproductive risk for mammals due to the use of 2,4-D 95 SP in spring wheat

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Intended use | | Spring wheat | | | | |
| Active substance/product | | 2,4-D | | | | |
| Application rate (g a.s./ha) | | 1 × 750 | | | | |
| Acute toxicity (mg/kg bw) | | >554 | | | | |
| TER criterion | | 10 | | | | |
| Crop scenario | Generic focal species | | SV | MAF90 | DDD90  (mg/kg bw/d) | TERA |
| Cereals | Small herbivorous mammal | | 118.4 | 1.00 | 88.8 | **6.2** |
| Reprod. toxicity (mg/kg bw/d) | | 20.6 | | | | |
| TER criterion | | 5 | | | | |
| Crop scenario | Generic focal species | | SV | MAFM × TWA | DDDM  (mg/kg bw/d) | TERLT |
| Cereals | Small herbivorous mammal | | 48.3 | 1.00 x 0.53 | 19.2 | **1.1** |

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: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of 2,4-D 95 SP in spring wheat

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Intended use | | Spring wheat | | | | |
| Active substance/product | | 2,4-D | | | | |
| Application rate (g a.s./ha) | | 1 × 750 | | | | |
| Acute toxicity (mg/kg bw) | | >554 | | | | |
| TER criterion | | 10 | | | | |
| Crop scenario  Growth stage | Indicator/generic focal species | | SV90 | MAF90 | DDD90  (mg/kg bw/d) | TERA |
| Early (shoots) | Large herbivorous mammal “lagomorph” | | 42.1 | 1.00 | 31.6 | 18 |
| Cereals, BBCH 10-19 | Small insectivorous mammal "shrew" | | 7.6 | 1.00 | 5.7 | 97 |
| Cereals, BBCH 10-29 | Small omnivorous mammal "mouse" | | 17.2 | 1.00 | 12.9 | 43 |
| Cereals, BBCH > 20 | Small insectivorous mammal "shrew" | | 5.4 | 1.00 | 4.1 | 135 |
| Reprod. toxicity (mg/kg bw/d) | | 20.6 | | | | |
| TER criterion | | 5 | | | | |
| Crop scenario  Growth stage | Indicator/generic focal species | | SVM | MAFM × TWA | DDDM  (mg/kg bw/d) | TERLT |
| Early (shoots) | Large herbivorous mammal “lagomorph” | | 22.3 | 1.00 x 0.53 | 8.9 | **2.3** |
| Cereals, BBCH 10-19 | Small insectivorous mammal "shrew" | | 4.2 | 1.00 x 0.53 | 1.7 | 12 |
| Cereals, BBCH 10-29 | Small omnivorous mammal "mouse" | | 7.8 | 1.00 x 0.53 | 3.1 | 6.6 |
| Cereals, BBCH > 20 | Small insectivorous mammal "shrew" | | 1.9 | 1.00 x 0.53 | 0.8 | 27 |

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.

#### Higher-tier risk assessment

While acceptable risk has been shown at the first tier for the acute risk of 2,4-D to mammals, further consideration is necessary to cover the reproductive risk, for the generic focal species scenario large herbivorous “lagomorph”.

While the tier 1 assessment uses a default DT50 of 10 days, a refined DT50 of 2.39 days on spring cereals is proposed based on 22 residues trials available in the RAR Addendum, February 2014, B.9.3.3-12. This DT50 corresponds with a 21-day time-weighted-average factor (fTWA) of 0.163. This refinement was also applied in a precedent Central Zone dRR ‘Tricera’ with PL as the zRMS, finalised in May 2022.

|  |
| --- |
| **Review Comments:**  The refined DT50 of 2.39 days on spring cereals is accepted by zRMS. |

Table 9.3‑4: Higher-tier assessment of the long-term/reproductive risk for mammals due to the use of 2,4-D 95 SP in spring wheat

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Intended use | | | Spring wheat | | | | | |
| Active substance/product | | | 2,4-D | | | | | |
| Application rate (g a.s./ha) | | | 1 × 750 | | | | | |
| Reprod. toxicity (mg/kg bw) | | | 20.6 | | | | | |
| TER criterion | | | 5 | | | | | |
| Generic focal species | Food category,  % in diet | FIR/bw | | RUDm × DF  (mg/kg food) | MAFm × TWA | PT | DDDm  (mg/kg bw/d) | TERlt |
| Large herbivorous mammal “lagomorph” | 100% crop shoots | 0.41 | | 54.2 | 1 x 0.163 | 1.0 | 2.68 | 7.7 |

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.

Considering the above refinement, acceptable long-term risk towards mammals has been demonstrated.

#### 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 K(f)oc of 58.6, 2,4-D belongs to the group of less sorptive substances.

|  |  |  |  |
| --- | --- | --- | --- |
| Effective application rate (g a.s./ha) = | 750 |  |  |
| Acute toxicity (mg/kg bw) = | >~~544~~ 554 | quotient = | ~~1.38~~ 1.36 |
| Reprod. toxicity (mg/kg bw/d) = | 20.6 | quotient = | 36.4 |

#### Effects of secondary poisoning

The log Pow of 2,4-D amounts to 1.54 at pH 4 (the highest value) and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

However, in line with the most recent EU review, secondary poisoning assessments are required for the two metabolites of 2,4-D, i.e. 2,4-DCP (log POW = 3.06) and 2,4-DCA (log POW = 3.36). Assessments are based on an assumed toxicity ten times higher than the parent, i.e. based on an NOEL of 2.06 mg/kg bw/d.

Risk assessment for earthworm-eating mammals via secondary poisoning

According to EFSA/2009/1438, the risk for vermivorous mammals is assessed for a small mammal of 10 g body weight with a daily food consumption of 12.8 g. Bioaccumulation in earthworms is estimated based on measured/predicted concentrations in soil/porewater is based on experimental data.

Table 9.2.2.4‑2: Assessment of the risk for earthworm-eating mammals due to exposure to 2,4-DCP via bioaccumulation in earthworms (secondary poisoning) for the intended use of 2,4-D 95 SP in spring wheat

| Parameter |  | Comments |
| --- | --- | --- |
| PECsoil (twa = 21 d) (mg/kg soil) | 0.0399 | Section 8 (Environmental Fate), Chapter 8.7, Table 8.7-5. |
| log Pow / Pow | 3.06/1148 | LoEP |
| Koc | 512 | LoEP |
| foc | 0.02 | Default |
| BCFworm | 1.43 | BCFworm/soil = (PECworm,ww/PECsoil,dw) = (0.84 + 0.012 × KOW) / fOC × KOC |
| PECworm | 0.057 | PECworm = PECsoil × BCFworm/soil |
| Daily dietary dose (mg/kg bw/d) | 0.073 | DDD = PECworm × ~~1.05~~ 1.28 |
| NOEL (mg/kg bw/d) | 2.06 | 10 times toxicity of the parent (experimental NOEL for 2,4‑D acid) |
| TERlt | 28 | Trigger ≥5 |

The TERLT is above the trigger value of 5 indicating an acceptable long-term risk to earthworm-eating mammals from the metabolite 2,4-DCP.

Table 9.2.2.4‑2: Assessment of the risk for earthworm-eating mammals due to exposure to 2,4-DCA via bioaccumulation in earthworms (secondary poisoning) for the intended use of 2,4-D 95 SP in spring wheat

| Parameter |  | Comments |
| --- | --- | --- |
| PECsoil (twa = 21 d) (mg/kg soil) | 0.078 | Section 8 (Environmental Fate), Chapter 8.7, Table 8.7-6. |
| log Pow / Pow | 3.36/2291 | LoEP |
| Koc | 1028 | LoEP |
| foc | 0.02 | Default |
| BCFworm | 1.43 | BCFworm/soil = (PECworm,ww/PECsoil,dw) = (0.84 + 0.012 × KOW) / fOC × KOC |
| PECworm | 0.11 | PECworm = PECsoil × BCFworm/soil |
| Daily dietary dose (mg/kg bw/d) | 0.14 | DDD = PECworm × ~~1.05~~ 1.28 |
| NOEL (mg/kg bw/d) | 2.06 | 10 times toxicity of the parent (experimental NOEL for 2,4‑D acid) |
| TERlt | 15 | Trigger ≥5 |

The TERLT is above the trigger value of 5 indicating an acceptable long-term risk to earthworm-eating mammals from the metabolite 2,4-DCA.

Risk assessment for fish-eating mammals via secondary poisoning

For fish, the BCF has been reported as 340 and 31 mL/g for 2-4-dichlorophenol and 2,4-dichloroanisole, respectively (EFSA Journal 2014;12(9):3812). Residues in fish were calculated in line with EFSA/2009/1438 according to the following equation:

PECfish = PECwater × TWA × BCFfish

and converted to a daily dietary dose by multiplying with a factor of 0.142, which is based on a 3000 g mammal eating 425 g fish per day.

Table 9.2.2.4‑3: Assessment of the risk for fish-eating mammals due to exposure to 2,4-DCP via bioaccumulation in fish (secondary poisoning) for the intended use of 2,4-D 95 SP in spring wheat at FOCUS Step 1

| Parameter |  | Comments |
| --- | --- | --- |
| PECsw (initial max.) (mg/L) | 0.046342 | Section 8 (Environmental Fate), Chapter 8.9, Table 8.9-8 ~~7~~. Step 1 |
| BCFfish | 340 | LoEP |
| BMF | - | biomagnification factor (relevant for BCF ≥ 2000) |
| PECfish | ~~16~~ 8.35 | PECFISH = PECWATER × BCFFISH × TWA |
| Daily dietary dose (mg/kg bw/d) | ~~2.2~~ 1.19 | DDD = PECFISH × ~~0.159~~ 0.142 |
| NOEL (mg/kg bw/d) | 2.06a | 10 times toxicity of the parent (NOEL for 2,4‑D acid) |
| TERlt | **~~0.92~~ 1.73** | Trigger ≥5 |

The TERLT is below the trigger value of 5 indicating that further consideration is necessary to cover the long-term risk to fish-eating mammals from the metabolite 2,4-DCP.

For the refined assessment of the risk to fish-eating mammals from exposure to 2,4-DCP, PECsw values used are 21-day TWA values following FOCUS Step 2. As the submission is to be applied in Poland, the Northern Europe initial maximum PECsw of 0.005977 mg/L has been used.

Table 9.2.2.4‑4: Assessment of the risk for fish-eating mammals due to exposure to 2,4-DCP via bioaccumulation in fish (secondary poisoning) for the intended use of 2,4-D 95 SP in spring wheat

| Parameter |  |  |
| --- | --- | --- |
| PECsw (initial max.) NEU (mg/L) | 0.005977 | Section 8 (Environmental Fate), Chapter 8.9, Table 8.9-8.  FOCUS Step 2, NEU Mar - Sep |
| BCFfish | 340 | LoEP |
| BMF | - | biomagnification factor (relevant for BCF ≥ 2000) |
| PECfish | ~~2.0~~ 1.08 | PECFISH = PECWATER × BCFFISH × TWA |
| Daily dietary dose (mg/kg bw/d) | ~~0.29~~ 0.15 | DDD = PECFISH × ~~0.159~~ 0.142 |
| NOEL (mg/kg bw/d) | 2.06 | 10 times toxicity of the parent (NOEL for 2,4‑D acid) |
| TERlt | ~~7.1~~ 13.5 | Trigger ≥5 |

The TERLT is above the trigger value of 5 indicating an acceptable long-term risk to fish-eating mammals from the metabolite 2,4-DCP.

Table 9.2.2.4‑4: Assessment of the risk for fish-eating mammals due to exposure to 2,4-DCA via bioaccumulation in fish (secondary poisoning) for the intended use of 2,4-D 95 SP in spring wheat at FOCUS Step 1

| Parameter |  | Comments |
| --- | --- | --- |
| PECsw (initial max.) (mg/L) | ~~0.017438~~ 0.0176208 | Section 8 (Environmental Fate), Chapter 8.9, Table 8.9-8 Step 1 |
| BCFfish | 31 | LoEP |
| BMF | - | biomagnification factor (relevant for BCF ≥ 2000) |
| PECfish | ~~0.5~~ 2.30 | PECFISH = PECWATER × BCFFISH × TWA |
| Daily dietary dose (mg/kg bw/d) | ~~0.077~~ 0.041 | DDD = PECFISH × ~~0.159~~ 0.142 |
| NOEL (mg/kg bw/d) | 2.06 | 10 times toxicity of the parent (NOEL for 2,4‑D acid) |
| TERlt | ~~27~~ 50 | Trigger ≥5 |

The TERLT is above the trigger value of 5 indicating an acceptable long-term risk to fish-eating mammals from the metabolite 2,4-DCA.

#### Biomagnification in terrestrial food chains

Not relevant.

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

Not relevant.

### Overall conclusions

Based on the intended use of 2,4-D 95 SP to spring wheat, acceptable risk is demonstrated to mammals at Tier 1. The risk to mammals *via* drinking water is deemed low, and due to the low potential of 2,4-D to bioaccumulate the risk from secondary poisoning is considered acceptable. The risks of secondary poisoning from the metabolites 2,4-DCP and 2,4-DCA to earthworm-eating mammals and from the metabolite 2,4-DCP to fish-eating mammals are also considered acceptable.

|  |
| --- |
| **Review Comments:**  The acute and chronic risks of 2,4-D 95 SP to mammals were assessed from toxicity exposure ratios between toxicity endpoints, estimated from study with active ingredient and maximum residues occurring on food items.  All TER values exceed the relevant triggers indicating that 2,4-D 95 SP does not pose an unacceptable risk to mammals following applications according to recommended use pattern.  Evaluation of exposing to mammals through the drinking water demonstrated the acceptable risk. The potential risk of secondary poisoning from the relevant metabolites 2,4-DCP and 2,4-DCA is low. |

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

Not relevant.

## Effects on aquatic organisms (KCP 10.2)

### Toxicity data

Studies on the toxicity to aquatic organisms are available with 2,4-D and its relevant metabolites (2,4-DCP, 2,4-DCA, 4-chlorophenol and 1,2,4-benzenetriol).

The provision of further data using the formulation 2,4-D 95 SP is not considered essential, because the high active substance concentration in the product allows for bridging from data with the active substance.

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process. New data are available for metabolites and these are detailed in section 9.5.1.1.

Table 9.5‑1: Endpoints and effect values relevant for the risk assessment for aquatic organisms – 2,4-D and relevant metabolites

| Species | Substance | Exposure  System | Results | Reference |
| --- | --- | --- | --- | --- |
| Fathead minnow (*Pimephales promelas*) | 2,4-D | 96 hr (flow-through) | **LC50 = 100 mg/L NOM** | EFSA Journal 2014;12(9):3812 |
| Fathead minnow (*Pimephales promelas*) | 2,4-DCP | 96 hr (flow-through) | **LC50 = 10 mg/L NOM** | Refer to 9.5.1.1 |
| Rainbow trout (*Oncorhynchus mykiss*) | 2,4-DCA | 96 hr (static) | **LC50 >1.4 mg/L MM** | EFSA Journal 2014;12(9):3812 |
| Fathead minnow (*Pimephales promelas*) | 2,4-D | 32-d ELS (flow-through) | **NOEC (growth) = 63.4 mg/L MM** | EFSA Journal 2014;12(9):3812 |
| *Daphnia magna* | 2,4-D acid | 48 h (static) | **EC50 = 134.2 mg/L NOM** | EFSA Journal 2014;12(9):3812 |
| *Daphnia magna* | 2,4-DCP | 48 h (static) | **EC50 = 2.8 mg/L NOM** | EFSA Journal 2014;12(9):3812 |
| *Daphnia magna* | 2,4-DCA | 48 h | **EC50 = 6.4 mg/L MM** | EFSA Journal 2014;12(9):3812 |
| *Daphnia magna* | 2,4-D | 21 d (semi-static) | **NOEC = 38.4 mg/L NOM** | EFSA Journal 2014;12(9):3812 |
| *Daphnia magna* | 2,4-D | 21 d (flow-through) | NOEC (reproduction) = 79 mg/L MM | EFSA Journal 2014;12(9):3812 |
| *Pseudokirchneriella subcapitata* | 2,4-D | 72 h (static) | EyC50 >78 mg/L MM  ErC50 >78 mg/L MM | EFSA Journal 2014;12(9):3812 |
| *Navicula pelliculosa* | 2,4-D acid | 72 h | EyC50 >100 mg/L NOM  ErC50 >100 mg/L NOM | EFSA Journal 2014;12(9):3812 |
| *Desmodesmus subspicatus* | 2,4-D acid | 72 h | EyC50 >582.2 mg/L MM  ErC50 >582.2 mg/L MM | EFSA Journal 2014;12(9):3812 |
| *Skeletonema costatum* | 2,4-D acid | 120 h (static) | EyC50 = 0.68 mg/L NOM  **ErC50 = 4.58 mg/L NOM** | EFSA Journal 20H14;12(9):3812 |
| *Pseudokirchneriella subcapitata* | 2,4-DCP | 72 h | EyC50 = 1.13 mg/L MM  **ErC50 = 3.44 mg/L MM** | EFSA Journal 2014;12(9):3812 |
| *Pseudokirchneriella subcapitata* | 2,4-DCA | 72 h | EyC50 = 2.2 mg/L MM  **ErC50 = 4.3 mg/L MM** | EFSA Journal 2014;12(9):3812 |
| *Lemna minor* | 2,4-D acid | 7 d (static) | Fronds, EyC50 = 10.66 mg/L NOM  Fronds, ErC50 = 17.51 mg/L NOM  Dry weight, EyC50 = 18.50 mg/L NOM  Dry weight, ErC50 = >100 mg/L | EFSA Journal 2014;12(9):3812 |
| *Myriophyllum spicatum* | 2,4-D acid | 14 d | **Shoot length:**  EyC50 = 0.169 mg/L NOM  **ErC50 = 0.346 mg/L NOM** | Test provided with EU Bridging report 2018  Gonsior, G. 2014, S14-03290  Refer to 9.5.1.1 |
| *Myriophyllum spicatum* | 2,4-D | 14 d | Total root length  EC50 = 0.011 mg/L NOM | EFSA Journal 2014;12(9):3812# |
| *Lemna gibba* | 2,4-DCP | 10 d | **Fronds:**  **EC50 = 1.5 mg/L MM** | EFSA Journal 2014;12(9):3812 |
| *Myriophyllum aquaticum* | 2,4-DCP | 10 d (static) | Fresh weight:  EyC50 = 12.4 mg/L MM  ErC50 = 25.8 mg/L MM | EFSA Journal 2014;12(9):3812 |
| *Myriophyllum aquaticum* | 2,4-DCA | 10 d (static) | **Shoot length:**  EyC50 = 1.16 mg/L MM  **ErC50 = 1.42 mg/L MM** | EFSA Journal 2014;12(9):3812 |
| *Lemna gibba* | 2,4-DCA | 7 d | Fronds:  EyC50 = 2.1 mg/L MM  ErC50 = 4.5 mg/L MM | EFSA Journal 2014;12(9):3812 |
| *Myriophyllum spicatum* | 4-chlorophenol | 14 d | **Shoot length:**  EyC50 = 10.4 mg/L GEO  **ErC50 = 13.1 mg/L GEO** | Gonsior, 2015  S15-00666 |

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; GEO: based on geometric mean measured concentration

Endpoints highlighted in **bold** have been used in the risk assessment.

# Endpoint agreed at the Pesticides Peer Review Meeting 111 (04 – 07 February 2013) and it is the geometric mean value for root length from the available 6 ring test studies with *Myriophyllum*.

#### Justification for new endpoints

As the formulation is nominally 95.0% active substance, it is considered acceptable to read-across data using the active substance in place of formulation data.

New data are now available with the rooted aquatic macrophyte *Myriophyllum spicatum* exposed to the active substance and the metabolites 1,2,4-benzenetriol and 4-chlorophenol. In addition, new acute fish and *Daphnia* studies are now available conducted with 1,2,4-benzenetriol; while an assessment of the toxicity of 4-chlorophenol to fish and *Daphnia* are taken from peer reviewed literature.

At the time of Annex I renewal, the selection of the aquatic plant endpoint, upon which the EFSA risk assessment was based, was decided during the EFSA Peer Review Meeting 111 (04 – 07 February 2014). In this Peer Review Meeting, in the absence of a GLP, guideline study, the aquatic plant endpoint was selected based on reported effects on root length determined in a non-GLP ring-test using a water-only screening level test method (Maletzki, 2011)[[1]](#footnote-1). This is despite significant reservations about the suitability of this test method, and the use of a root length endpoint, which are summarized below:

* *Myriophyllum* sp. cuttings, without roots, were used resulting in an unrealistic exposure scenario for assessing exposure and risk to rooted aquatic plants since subsequent root development and growth is unnatural due to the lack of a sediment substrate.
* The exposure of roots to light results in atypical root development, and the addition of sucrose to the test media will interfere with photosynthesis and hence the normal physiology and metabolism of the plants.
* The use of sterile test conditions in this test system means that important biotic degradation processes for 2,4-D were omitted. Thus, the ecological relevance of any effects on root endpoints will be highly questionable.

In addition to these reservations, the ring test (from which the endpoint was selected) was not conducted to GLP, certified test material was not used, and exposure concentrations were not verified during the test. So, as well as the conditions not being ecologically relevant (no roots at test start, exposure of roots to light, addition of sucrose, and no sediment), the reliability, reproducibility and repeatability of this test system is in question.

Since Annex I renewal, new GLP compliant studies with 2,4-D were conducted for the aquatic plant *Myriophyllum spicatum* in accordance with OECD Test Guideline 239 (water-sediment exposure) and the latest EFSA Aquatic Guidance, using certified test material, and with analytical verification of exposure. The biomass (fresh and dry weight) measurements were performed with whole plants so that any significant effects on root development were implicitly measured with the biomass endpoints. These new studies are more reliable, and environmentally relevant, than the screening level value evaluated for Annex I Renewal. In addition, it should be noted that the OECD Test Guideline 238 (water-only exposure) for testing aquatic plants states that ‘the inclusion of root endpoints is questionable’ for molecules with an auxin-type mode of action.  Thus, the critical endpoint for aquatic plants provided in the EFSA Conclusion Report has been revised with the relevant growth rate endpoint taken from the new water-sediment studies with 2,4-D.

Therefore, a more appropriate endpoint of 346 µg/L for *Myriophyllum spicatum* has been used in the risk assessment based on the recent findings in these GLP compliant studies with 2,4-D acid conducted in laboratory sediment-water test systems.

Where data is not available for the metabolites, a surrogate endpoint of 10x the toxicity of the parent has been used.

Studies with the metabolite 1,2,4-benzenetriol were submitted as part of a precedent Central Zone dRR ‘Tricera’ with PL as the zRMS finalised in May 2022 (formulation code ADM.3304.H.1.A). The conclusion of the zRMS was that studies with the metabolite 1,2,4-benzenetriol are considered not valid since no reliable endpoints could be derived due to the test item measured concentrations dropping <LOD already after 1 hours after the test initiation. Since the studies were performed under the static exposure regime, it was not possible to calculate the mean measured concentrations, relevant for not stable substance for endpoints derivation. Nevertheless, the studies provided clear information that 1,2,4-benzenetriol is a transient metabolite of 2,4-D and for this reason no significant exposure from this compound is expected. Taking this into account, the risk assessment performed for the parent compound is deemed sufficient to cover the risk from this transient metabolite.

### Risk assessment

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) in the context of Regulation (EC) No 1107/2009”, as provided by the Commission Services (SANTE-2015-00080, 15 January 2015).

The relevant PECSW for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.9, Tables 8.9-7 to 8.9-9.

The relevant global maximum FOCUS Step 1, 2 and 3 PECSW for risk assessments covering the proposed use pattern and the resulting PEC/RAC ratios are presented in the table below.

A risk assessment for metabolite 1,2,4-benzenetriol has not been conducted due to the transient nature of the metabolite, meaning the risk assessment for the parent is considered to cover the risk from this metabolite.

Since available data demonstrate that *Myriophyllum spicatum* is the aquatic species most sensitive to 2,4‑D, inclusion of other species in the risk assessment for the metabolite 4-chlorophenol is not deemed necessary.

In the following table, the ratios between predicted environmental concentrations in surface water bodies (PECSW, PECSED) and regulatory acceptable concentrations (RAC) for aquatic organisms are given per intended use for each FOCUS scenario and each organism group.

**Active substance 2,4-D**

Table 9.5‑3: Aquatic organisms: acceptability of risk (PEC/RAC <1) for 2,4-D for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of 2,4-D 95 SP in spring wheat

| Group |  | Fish acute | Fish prolonged | Inverteb. acute | Inverteb. prolonged | Algae | Aquatic macrophyte |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Test species |  | *Pimephales promelas* | *Oncorhynchus mykiss* | *Daphnia magna* | *Daphnia magna* | *Skeletonema costatum* | *Myriophyllum spicatum* |
| Endpoint |  | LC50 | NOEC | EC50 | NOEC | ErC50 | EC50 |
| (µg/L) |  | 100000 | 63400 | 134200 | 38400 | 4580 | 346 |
| AF |  | 100 | 10 | 100 | 10 | 10 | 10 |
| RAC (µg/L) |  | 1000 | 6340 | 1342 | 3840 | 458 | 34.6 |
| FOCUS Scenario | PEC GL-MAX (µg/L) |  |  |  |  |  |  |
| Step 1 |  |  |  |  |  |  |  |
|  | 238.78 | 0.24 | 0.038 | 0.18 | 0.062 | 0.52 | **6.9** |
| Step 2 |  |  |  |  |  |  |  |
| N-Europe | 29.366 | 0.029 | 0.0046 | 0.022 | 0.0076 | 0.064 | 0.85 |
| S-Europe | 53.104 | 0.053 | 0.0084 | 0.040 | 0.014 | 0.12 | **1.5** |
| Step 3 |  |  |  |  |  |  |  |
| D1/ditch 1 | 4.867 | - | - | - | - | - | 0.14 |

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

1 Worst-case FOCUS Step 3 scenario presented

For the intended use on spring wheat, calculated PEC/RAC ratios indicate an acceptable risk for the most sensitive group of aquatic organisms to 2,4-D (risk for aquatic macrophytes as characterised by an ErC50 for *Myriophyllum spicatum* of 346 µg/L in connection with an assessment factor of 10) at FOCUS Step 3. No further consideration is necessary.

**Metabolite 2,4-DCP**

Table 9.5‑3: Aquatic organisms: acceptability of risk (PEC/RAC <1) for 2,4-DCP for each organism group based on FOCUS Steps 1 and 2 calculations for the use of 2,4-D 95 SP in spring wheat

| Group |  | Fish acute1 | Inverteb. acute | Algae | Aquatic macrophyte |
| --- | --- | --- | --- | --- | --- |
| Test species |  | *Pimephales promelas* | *Daphnia magna* | *Pseudokirchneriella subcapitata* | *Lemna gibba* |
| Endpoint |  | LC50 | EC50 | ErC50 | EC50 |
| (µg/L) |  | 10000 | 2800 | 3440 | 1500 |
| AF |  | 100 | 100 | 10 | 10 |
| RAC (µg/L) |  | 100 | 28 | 344 | 150 |
| FOCUS Scenario | PEC GL-MAX (µg/L) |  |  |  |  |
| Step 1 |  |  |  |  |  |
|  | 46.342 | 0.46 | **1.7** | 0.13 | 0.31 |
| Step 2 |  |  |  |  |  |
| N-Europe | 5.977 | 0.060 | 0.21 | 0.017 | 0.040 |
| S-Europe | 10.861 | 0.11 | 0.39 | 0.032 | 0.072 |

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

1 10x toxicity of the parent used

For the intended use on spring wheat, calculated PEC/RAC ratios indicated an acceptable risk for the most sensitive group of aquatic organisms to 2,4-DCP (risk for invertebrates as characterised by an EC50 for *Daphnia magna* of 2800 µg/L in connection with an assessment factor of 100) at FOCUS Step 2. No further assessment is necessary.

**Metabolite 2,4-DCA**

Table 9.5‑3: Aquatic organisms: acceptability of risk (PEC/RAC <1) for 2,4-DCA for each organism group based on FOCUS Steps 1 and 2 calculations for the use of 2,4-D 95 SP in spring wheat

| Group |  | Fish acute | Inverteb. acute | Algae | Aquatic macrophyte |
| --- | --- | --- | --- | --- | --- |
| Test species |  | *Oncorhynchus mykiss* | *Daphnia magna* | *Pseudokirchneriella subcapitata* | *Myriophyllum spicatum* |
| Endpoint |  | LC50 | EC50 | ErC50 | EC50 |
| (µg/L) |  | >1400 | 6400 | 4300 | 1420 |
| AF |  | 100 | 100 | 10 | 10 |
| RAC (µg/L) |  | 14 | 64 | 430 | 142 |
| FOCUS Scenario | PEC GL-MAX (µg/L) |  |  |  |  |
| Step 1 |  |  |  |  |  |
|  | 17.438 | **1.2** | 0.27 | 0.041 | 0.12 |
| Step 2 |  |  |  |  |  |
| N-Europe | 2.552 | 0.18 | 0.040 | 0.0059 | 0.018 |
| S-Europe | 4.951 | 0.35 | 0.077 | 0.012 | 0.035 |

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 use on spring wheat, calculated PEC/RAC ratios indicated an acceptable risk for the most sensitive group of aquatic organisms to 2,4-DCA (risk for fish as characterised by an LC50 for *Pimephales promelas* of 1400 µg/L in connection with an assessment factor of 100) at FOCUS Step 2. No further assessment is necessary.

**Metabolite 4-Chlorophenol**

Table 9.5‑3: Aquatic organisms: acceptability of risk (PEC/RAC <1) for 4-CP for each organism group based on FOCUS Steps 1 and 2 calculations for the use of 2,4-D 95 SP in spring wheat

| Group |  | Fish acute | Inverteb. acute | Algae | Aquatic macrophyte |
| --- | --- | --- | --- | --- | --- |
| Test species |  | - | - | - | *Myriophyllum spicatum* |
| Endpoint |  | - | - | - | EC50 |
| (µg/L) |  | - | - | - | 13100 |
| AF |  | - | - | - | 10 |
| RAC (µg/L) |  | - | - | - | 1310 |
| FOCUS Scenario | PEC GL-MAX (µg/L) | - | - | - |  |
| Step 1 |  |  |  |  |  |
|  | 46.987 | **-** | **-** | **-** | 0.036 |

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 use on spring wheat, calculated PEC/RAC ratios indicated an acceptable risk for the most sensitive group of aquatic organisms to 4-CP (risk for fish as characterised by an LC50 for *Oncorhynchus mykiss* of 1900 µg/L in connection with an assessment factor of 100) at FOCUS Step 2. No further assessment is necessary.

### Overall conclusions

For the intended use of 2,4-D 95 SP on spring wheat, calculated PECSW/RAC ratios have indicated an acceptable risk to aquatic organisms using FOCUS Step 3 for the parent and Steps 1 to 2 for the metabolites 2,4-DCP, 2,4-DCA and 4-chlorophenol. No risk assessment has been conducted for metabolite 1,2,4-benzenetriol due to the transient nature of the metabolite.

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| **Review Comments:**  The relevant predicted environmental concentrations in water (PECsw) for risk assessments covering the proposed use pattern are taken from Part B Section 8 (Environmental Fate). The initial risk assessment was based on the worst case PECsw values and the results of laboratory toxicity testing.  For 2,4-D and relevant metabolites PECSW calculations were performed with FOCUS STEPS 1-2 and FOCUS STEP 3 (active substance; D1/ditch – worst case value).  The calculated PEC/RAC ratios indicate an acceptable risk for all groups of aquatic organisms without the need for any mitigation measures. |

## Effects on bees (KCP 10.3.1)

### Toxicity data

Studies on the toxicity to bees are available with 2,4-D. 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 2,4-D 95 SP were not evaluated as part of the EU assessment of 2,4-D. New data submitted with this application are listed in Appendix 1.

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

| Species | Substance | Exposure  System | Results | Reference |
| --- | --- | --- | --- | --- |
| Honey bees (*Apis mellifera*) | 2,4-D | Acute oral | **LD50 = 94 µg/bee** | EFSA Journal 2014;12(9):3812 |
| Honey bees (*Apis mellifera*) | 2,4-D | Acute contact | **LD50 >100 µg/bee** | EFSA Journal 2014;12(9):3812 |
| Honey bee (*Apis mellifera*) | 2,4-D 95 SP | Chronic oral  (10-day) | LDD50 = 30.44 µg a.s./bee/day1 | New study |
| Honey bee (*Apis mellifera*) | 2,4-D 95 SP | Chronic larval  (22-day) | NOED = 7.937 µg a.s./larva1 | New study |

Endpoints highlighted in **bold** have been used in the risk assessment.

1 Endpoint derived from draft report

#### Justification for new endpoints

No deviation from EU-agreed endpoints for the acute oral and contact tests. As the formulation is nominally 95.0% active substance, it is considered acceptable to read-across data using the active substance in place of formulation data.

New studies are available to cover the chronic risk and risk to honeybee development as required under Commission Regulation 283/2013, summaries are included in Appendix 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).

#### Hazard quotients for bees

Table 9.6‑2: First-tier assessment of the risk for bees due to the use of 2,4-D 95 SP in spring wheat

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Intended use | | Spring wheat | | |
| Active substance | | 2,4-D | | |
| Application rate (g a.s./ha) | | 1 × 750 | | |
| Test design | LD50 (lab.)  (µg/bee) | | Single application rate  (g/ha) | QHO, QHC  criterion: QH ≤ 50 |
| Oral toxicity | 94 | | 750 | 8.0 |
| Contact toxicity | >100 | | 7.5 |

QHO, QHC: Hazard quotients for oral and contact exposure. QH values shown in bold breach the relevant trigger.

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

Not relevant.

### Effects on bumble bees

As the “EFSA Guidance Document on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees)“ (EFSA Journal 2013;11(7):3295 – updated 2014) has not been noted, and as studies on bumble bees are not required under Regulation (EC) No 1107/2009, no further consideration is necessary.

### Effects on solitary bees

As the “EFSA Guidance Document on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees)“ (EFSA Journal 2013;11(7):3295 – updated 2014) has not been noted, and as studies on solitary bees are not required under Regulation (EC) No 1107/2009, no further consideration is necessary.

### Overall conclusions

Based on the intended use of 2,4-D 95 SP on spring wheat, the acute oral and acute contact risk to bees is deemed acceptable at the first tier.

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| **Review Comments:**  The evaluation of the acute 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). The submitted risk assessment, based on laboratory studies, has been accepted. It can therefore be concluded that there will be negligible acute risk associated with the exposure of *Apis mellifera* to 2,4-D 95 SP.  The data requirements in accordance with Commission Regulation (EU) No 284/2013 for the chronic toxicity to adult honeybees and honeybee larvae are partly met. The study by Wilkins, S. (2023b), Report no.: FR/002602-11, need to be supplemented by an assessment of storage stability of the analytical samples.  There is not harmonized approach for the chronic risk assessment for bees, therefore, Concerned Member States should perform the evaluation, if necessary at national level. |

## Effects on arthropods other than bees (KCP 10.3.2)

### Toxicity data

Studies on the toxicity to non-target arthropods are available with 2,4-DMA 600 SL.

Effects on non-target arthropods of 2,4-D 95 SP were not evaluated as part of the EU assessment of 2,4‑D. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

| Species | Substance | Exposure  System | Results | Reference |
| --- | --- | --- | --- | --- |
| *Aphidius rhopalosiphi* | 2,4-D DMA 600 SL | Laboratory test glass plates (2D) | LR50 = >3000 g a.s./ha | EFSA Journal 2014;12(9):3812 |
| *Aphidius rhopalosiphi* | 2,4-D 95 SP | Laboratory test glass plates (2D) | **LR50 = >1000 g a.s./ha** | New study |
| *Typhlodromus pyri* | 2,4-D DMA 600 SL | Laboratory test glass plates (2D) | LR50 = >3000 g a.s./ha | EFSA Journal 2014;12(9):3812 |
| *Typhlodromus pyri* | 2,4-D 95 SP | Laboratory test glass plates (2D) | **LR50 = >1000 g a.s./ha** | New study |
| *Aleochara bilineata* | Herbizid Marks | Arenas containing sand (glass beakers)  4 weeks + 5 weeks | Mortality: 0% effect  Beneficial capacity: 1.3% effect | EFSA Journal 2014;12(9):3812 |
| *Poecilus cupreus* | Herbizid Marks | Arenas containing sand (plastic trays)  14 days | Mortality: 0% effect  Feeding reduction: 29.6% effect | EFSA Journal 2014;12(9):3812 |
| *Pardosa ssp.* | Herbizid Marks | Arenas containing sand (plastic containers)  14 days | Mortality: 5%  Food consumption: 0% effect | EFSA Journal 2014;12(9):3812 |

Endpoints highlighted in **bold** have been used in the risk assessment.

#### Justification for new endpoints

New data have been provided to cover the risk to non-target arthropods from the new formulation 2,4-D 95 SP.

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.

The risk assessment is based on data using the formulated product, therefore data using the active substance are not provided.

### 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.

#### 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 2,4-D 95 SP in spring wheat

|  |  |  |  |
| --- | --- | --- | --- |
| Intended use | Spring wheat | | |
| Active substance/product | 2,4-D | | |
| Application rate (g a.s./ha) | 1 × 750 | | |
| MAF | 1 | | |
| Test species  Tier I | LR50 (lab.)  (g/ha) | PERIN‑FIELD  (g/ha) | HQIN-FIELD  criterion: HQ ≤ 2 |
| *Typhlodromus pyri* | >1000 | 750 | <0.75 |
| *Aphidius rhopalosiphi* | >1000 | <0.75 |

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient;

\* If an LR50 or ER50 from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤50 % effect.

#### Risk assessment for off-field exposure

Table 9.7‑3: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of 2,4-D 95 SP in spring wheat

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Intended use | | Spring wheat | | | | |
| Active substance/product | | 2,4-D | | | | |
| Application rate (g a.s./ha) | | 1 × 750 | | | | |
| MAF | | 1 | | | | |
| vdf | | 10 (Tier 1) | | | | |
| Test species  Tier I | LR50 (lab.)  (g/ha) | | Drift rate | PEROFF‑FIELD  (g/ha) | CF | HQOFF-FIELD  criterion: HQ ≤2 |
| *Typhlodromus pyri* | >1000 | | 0.0277 | 2.0775 | 10 | <0.021 |
| *Aphidius rhopalosiphi* | >1000 | | <0.021 |

MAF: Multiple application factor; vdf: Vegetation distribution factor; (corr.) PER: (corrected) Predicted environmental rate; CF: Correction factor; HQ: Hazard quotient.

\* If an LR50 or ER50 from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤50 % effect.

#### Additional higher-tier risk assessment

Not relevant.

#### Risk mitigation measures

No risk mitigation needed.

### Overall conclusions

Based on the intended use of 2,4-D 95 SP on spring wheat, acceptable in-field and off-field risk was demonstrated to non-target arthropods at the first tier.

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| **Review Comments:**  Based on the results of the conducted risk assessment, it can be concluded that low risk for non-target arthropods is expected from the use of 2,4-D 95 SP according to the proposed use pattern. No unacceptable effects on non-target arthropods are expected in in-field and off-field habitats. |

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

### Toxicity data

Studies on the toxicity to earthworms and other non-target soil organisms (meso- and macrofauna) are available with 2,4-D and its relevant metabolites.

The provision of further data using the formulation 2,4-D 95 SP is not considered essential, because the high active substance concentration in the product allows for bridging from data with the active substance.

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process. New data are available for metabolites and are detailed in section 9.5.1.1. Summaries are available 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 |
| --- | --- | --- | --- | --- |
| *Eisenia fetida* | 2,4-D | 8 weeks (chronic)  10% peat | **NOEC = 62.5 mg a.s./kg dw soil** | EFSA Journal 2014;12(9):3812 |
| *Eisenia fetida* | 2,4-DCA | 8 weeks (chronic)  5% peat | NOEC >10 mg/kg dw soil  **NOECcorr >5 mg/kg dw soil** | EFSA Journal 2014;12(9):3812 |
| *Eisenia fetida* | 2,4-DCP | 8 weeks (chronic)  5% peat | NOEC = 10 mg/kg dw soil  **NOECcorr = 5 mg/kg dw soil** | EFSA Journal 2014;12(9):3812 |
| *Eisenia fetida* | 4-chlorophenol | 8 weeks (chronic)  10% peat | NOEC = 10 mg/kg dw soil  **NOECcorr = 5 mg/kg dw soil** | Wagenhoff, 2015  S15-00154  Study evaluated in ‘Tricera’ |
| *Folsomia candida* | 2,4-D | - | n.r. | EFSA Journal 2014;12(9):3812 |
| *Folsomia candida* | 2,4-DCP | 28 d (chronic)  5% peat | NOEC = 1.25 mg a.s./kg dw soil  **NOECcorr = 0.625 mg/kg dw soil** | EFSA Journal 2014;12(9):3812 |
| *Folsomia candida* | 2,4-DCA | 28 d (chronic)  5% peat | NOEC = 10 mg a.s./kg dw soil  **NOECcorr = 5 mg/kg dw soil** | EFSA Journal 2014;12(9):3812 |
| *Hypoaspis aculeifer* | 2,4-D | - | n.r. | EFSA Journal 2014;12(9):3812 |
| *Hypoaspis aculeifer* | 2,4-DCP | 14 d (chronic)  5% peat | NOEC = 5 mg a.s./kg dw soil  **NOECcorr = 2.5 mg/kg dw soil** | EFSA Journal 2014;12(9):3812 |
| *Hypoaspis aculeifer* | 2,4-DCA | 14 d (chronic)  5% peat | NOEC = 10 mg a.s./kg dw soil  **NOECcorr = 5 mg/kg dw soil** | EFSA Journal 2014;12(9):3812 |

\* Corrected value derived by dividing the endpoint by a factor of 2 in accordance with the EPPO earthworm scheme 2002 due to metabolites having a Log Pow >2.

n.r.: not required

#### Justification for new endpoints

The provision of further data on the formulation 2,4-D 95 SP is not considered essential due to the high concentration of active substance present allowing for read-across from studies using the active substance.

New data since the previous review ~~are~~ is available for the risk to earthworms of the relevant metabolite~~s~~ ~~2,4-DCP, 2,4-DCA and~~ 4-chlorophenol.

### 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).

#### First-tier risk assessment

The relevant PECSOIL for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.2, Tables 8.7-3 to 8.7-7. According to the assessment of environmental-fate data, multi-annual accumulation in soil does not need to be considered for 2,4-D and the relevant metabolites.

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 2,4-D 95 SP in spring wheat

|  |  |  |  |
| --- | --- | --- | --- |
| Intended use | Spring wheat | | |
| Chronic effects on earthworms | | | |
| Substance | NOEC  (mg/kg dw) | PECSOIL  (mg/kg dw) | TERLT  (criterion TER ≥5) |
| 2,4-D | 62.5 | 1.000 | 63 |
| 2,4-DCP | 5 | ~~0.067~~ 0.0642 | ~~75~~ 78 |
| 2,4-DCA | 5 | ~~0.163~~ 0.120 | ~~31~~ 42 |
| 4-Chlorophenol | 5 | ~~0.201~~ 0.192 | ~~25~~ 26 |
| Chronic effects on other soil macro- and mesofauna | | | |
| Substance | NOEC  (mg/kg dw) | PECSOIL  (mg/kg dw) | TERLT  (criterion TER ≥5) |
| *Folsomia candida* | | | |
| 2,4-DCP | 0.625 | ~~0.067~~ 0.0642 | ~~9.3~~ 9.7 |
| 2,4-DCA | 5 | ~~0.163~~ 0.120 | ~~31~~ 42 |
| *Hyposaspis aculeifer* | | | |
| 2,4-DCP | 2.5 | ~~0.067~~ 0.0642 | ~~37~~ 39 |
| 2,4-DCA | 5 | ~~0.163~~ 0.120 | ~~31~~ 42 |

TER values shown in bold fall below the relevant trigger.

#### Higher-tier risk assessment

Not relevant.

### Overall conclusions

Based on the intended use of 2,4-D 95 SP on spring wheat, the risk to earthworms and other non-target soil macrofauna is deemed acceptable with ~~TER~~~~A~~ ~~and~~ TERLT values exceeding the respective trigger ~~values~~ of ~~10 and~~ 5 at the first tier.

|  |
| --- |
| **Review Comments:**  The long-term risks of 2,4-D 95 SP to soil meso- and macro-organisms were assessed from toxicity exposure ratios between toxicity endpoints and maximum PECsoil. The relevant predicted environmental concentration in soil (PECsoil) for risk assessment covering the proposed use pattern was taken from Part B Section 8 (Environmental Fate).  Safe use of 2,4-D 95 SP was confirmed based on TERLT calculations for 2,4-D and its relevant metabolites. |

## Effects on soil microbial activity (KCP 10.5)

### Toxicity data

Studies on effects soil microorganisms are available with 2,4-D and its relevant metabolites. The provision of further data on the formulation 2,4-D 95 SP is not considered essential due to the high concentration of active substance present allowing for read-across from studies using the active substance.

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process.

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

| Endpoint | Substance | Exposure  System | Results | Reference |
| --- | --- | --- | --- | --- |
| N-mineralisation | 2,4-D | 28 days | **No effect at 3 mg a.s./kg soil** | EFSA Journal 2014;12(9):3812 |
| N-mineralisation | LAF-74 | 56 days | No effect at 29.9 mg a.s./kg soil | EFSA Journal 2014;12(9):3812 |
| N-mineralisation | 2,4-DCP | 42 days | **No effect at 5 mg a.s./kg soil** | EFSA Journal 2014;12(9):3812 |
| N-mineralisation | 2,4-DCA | 28 days | **No effect at 5 mg a.s./kg soil** | EFSA Journal 2014;12(9):3812 |

#### Justification for new endpoints

Not relevant, endpoints do not deviate from EU-agreed endpoints.

### 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 PECSOIL for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.2, Tables 8.7-3 to 8.7-6 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 2,4-D 95 SP in spring wheat

|  |  |  |  |
| --- | --- | --- | --- |
| Intended use | Spring wheat | | |
| N-mineralisation | | | |
| Product/active substance | Max. conc. with effects ≤25% (mg/kg dw) | PECSOIL  (mg/kg dw) | Risk acceptable? |
| 2,4-D | 3 (at 28 d) | 1.000 | Yes |
| 2,4-DCP | 5 (at 42 d) | ~~0.067~~ 0.0642 | Yes |
| 2,4-DCA | 5 (at 28 d) | ~~0.163~~ 0.120 | Yes |

### Overall conclusions

Based on the intended use of 2,4-D 95 SP on spring wheat, the risk to soil micro-organisms is deemed acceptable with no significant effects (>25%) observed at concentrations greater than the predicted maximum soil concentrations.

|  |
| --- |
| **Review Comments:**  The use of 2,4-D 95 SP at the proposed rates poses no unacceptable risk to soil micro-organisms. |

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

### Toxicity data

Studies on the toxicity to non-target terrestrial plants have been carried out with 2,4-D 95 SP. New data submitted with this application are listed in Appendix 1 summarised in Appendix 2.

The selection of studies and endpoints for the risk assessment deviates from the results of the EU review process. Justifications are provided below.

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

| Species | Substance | Exposure  System | Results | Reference |
| --- | --- | --- | --- | --- |
| Lettuce (*Lactuca sativa*) | LAF-74 | 21d Seedling emergence | 27 g a.s./ha | EFSA Journal 2014;12(9):3812 |
| Turnip (*Brassica rapa*) | 2,4-D 95 SP | 21d Seedling emergence | **ER50 = 28.69 g a.s./ha**  **Dry weight** | New Study  Jarratt, 2023a FR/002603-09, 000109114 |
| Lettuce (*Lactuca sativa*) | LAF-74 | 21 d Vegetative vigour | 19 g a.s./ha | EFSA Journal 2014;12(9):3812 |
| Turnip (*Brassica rapa*) | 2,4-D 95 SP | 21d Vegetative vigour | **ER50 = 29.4 g a.s./ha**  **Fresh weight** | New Study  Jarratt, 2023b FR/002603-08, 000109115 |

m: monocotyledonous; d: dicotyledonous

#### Justification for new endpoints

New data have been provided to cover the risk to non-target terrestrial plants from the new formulation 2,4-D 95 SP.

The risk assessment is based on data using the formulated product, therefore data using the active substance are not provided.

### Risk assessment

#### Tier-1 risk assessment (based screening data)

Not relevant.

#### 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 2,4-D 95 SP in spring wheat

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Intended use | | Spring wheat | | | |
| Active substance/product | | 2,4-D | | | |
| Application rate (g a.s./ha) | | 1 × 750 | | | |
| MAF | | 1 | | | |
| Test species | ER50  (g a.s./ha) | | Drift rate | PEROFF‑FIELD  (g/ha) | TER  criterion: TER ≥5 |
| Seedling emergence  *Brassica rapa* | 28.69 | | 2.77 | 20.78 | **1.4** |
| Vegetative vigour  *Brassica rapa* | 29.4 | | 2.77 | 20.78 | **1.4** |

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

TERs for both seedling emergence and vegetative vigour are below the trigger, indicating an unacceptable risk. Therefore, further consideration is required.

#### Higher-tier risk assessment

Not relevant.

#### 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 1 or 5 m; drift-reducing nozzles with reduction by 50% or 75%) are summarised in the following table. The worst-case ER50 value of 28.63 g a.s./ha is taken from the seedling emergence study of Jarratt, 2022a.

Table 9.10‑3: Risk assessment for non-target terrestrial plants due to the use of 2,4-D 95 SP in spring wheat considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Intended use | | Spring wheat | | | |
| Active substance/product | | 2,4-D | | | |
| Application rate (g a.s./ha) | | 1 × 750 | | | |
| MAF | | 1 | | | |
| ER50 (g a.s/ha) | | 28.63 | | | |
| Mitigation | | | |  | |
| Buffer strip (m) | Drift rate (%) | | DRT (%) | PEROFF-FIELD  (g a.s./ha) | TER |
| 1 | 2.77 | | 0 | 21 | **1.4** |
| 1 | 2.77 | | 50 | 10 | **2.8** |
| 1 | 2.77 | | 75 | 5.2 | 5.5 |
| 5 | 0.57 | | 0 | 4.3 | 6.7 |

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

### Overall conclusions

Based on the intended use of 2,4-D 95 SP on spring wheat, acceptable risk is demonstrated to non-target terrestrial plants with mitigation of either 75% Drift Reducing Nozzles (DRT) or a 5 m buffer strip.

|  |
| --- |
| **Review Comments:**  The risk assessment is based on the “Guidance Document on Terrestrial Ecotoxicology”, (SANCO/10329/2002 rev.2 final, 2002).  Based on the risk assessment it can be concluded that the proposed use of 2,4-D 95 SP poses no unacceptable risk to non-target plants, if applied according to the recommended use pattern. Particular precautions to reduce the environmental concentrations resulting from 2,4-D 95 SP applications are not required. |

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

Not relevant.

## Monitoring data (KCP 10.8)

Not relevant.

## Classification and Labelling

Classification and labelling of ADM.09250.H.1.A is proposed in accordance with Regulation 1272/2008/EC.

Taking into account the *Myriophyllum spicatum* ErC50 of 0.054 mg/L, the formulation is classified for acute aquatic hazard in category 1 (H400).

Taking into account the *Myriophyllum spicatum* NOErC of 0.004 mg/L, the formulation is classified for chronic aquatic hazard in category 1 (H410).

The following labelling is considered relevant:

|  |  |
| --- | --- |
| **Hazard pictograms:** | GHS09  **Aquatic-pollut-red** |
| **Signal word:** | Warning |
| **Hazard statement(s):** | H410 - Very toxic to aquatic life with long lasting effects |
| **Precautionary statement(s):** | P391: Collect spillage  P501: Dispose of contents/container to hazardous or special waste collection point, in accordance with local, regional, national and/or international regulation |

1. Lists of data considered in support of the evaluation

List of data submitted by the applicant and relied on

| Data point | Author(s) | Year | Title Company Report No. Source (where different from company) GLP or GEP status Published or not | Vertebrate study Y/N | Owner |
| --- | --- | --- | --- | --- | --- |
| CP 10.2.1/01 | Gonsior, G. | 2015 | 4-Chlorophenol: Growth Inhibition of *Myriophyllum spicatum* in a Water/Sediment System S15-00666; DAS study No: 141226 GLP, unpublished | N | EU 2,4-D Annex III Taskforce |
| CP 10.3.2.1/01 | Milner, S. | 2022a | Acute Dose-Response Toxicity of 2,4-D 95 SP to the Parasitic Wasp *Aphidius rhopalosiphi* (De Stefani-Perez) (Hymenoptera, Braconidae, Aphidiinae). FR/002603-07, 000109117 GLP, unpublished | N | Adama |
| CP 10.3.2.1/02 | Milner, S. | 2022b | Acute Dose-Response Toxicity of 2,4-D 95 SP to the Predacious Mite *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae). FR/002603-06, 000109116 GLP, unpublished | N | Adama |
| CP 10.3.1.2/01 | Wilkins, S. | 2023a | 2,4-D 95 SP: 10-Day chronic oral toxicity test for adult honeybees (*Apis mellifera* L.) FR/002602-10, 000109119 GLP, unpublished | N | Adama |
| CP 10.3.1.3/02 | Wilkins, S. | 2023b | 2,4-D 95 SP: In vitro 22-day toxicity test - repeated exposure to larval stage honeybees (*Apis mellifera* L.) **Interim report**  FR/002602-11, 000109120 GLP, unpublished | N | Adama |
| CP 10.4.1.1/01 | Wagenhoff, E. | 2015 | 4-Chlorophenol: Sublethal Toxicity to the Earthworm, *Eisenia fetida* (Annelida, Lumbricidae) in Artificial Soil with 10 % Peat S15-00154; DAS Study No. 141229 GLP, unpublished | N | EU 2,4-D Annex III Taskforce |
| CP 10.6.2/01 | Jarratt, N. | 2023a | 2,4-D 95 SP: Terrestrial Plant Seedling Emergence Test FR/002603-08, 000109115 GLP, unpublished | N | Adama |
| CP 10.6.2/02 | Jarratt, N. | 2023b | 2,4-D 95 SP: Terrestrial Plant Test: Vegetative Vigour Test FR/002603-09, 000109114 GLP, unpublished | N | Adama |

List of data submitted or referred to by the applicant and relied on, but already evaluated at EU peer review

| Data point | Author(s) | Year | Title Company Report No. Source (where different from company) GLP or GEP status Published or not | Vertebrate study Y/N | Owner |
| --- | --- | --- | --- | --- | --- |
| As the majority of the endpoints for 2,4-D and the relevant metabolites were taken from the EU review (EFSA Journal 2014;12(9):3812), for the list of respective studies please refer to Volume 2 of the RAR (2013). | | | | | |

The following tables are to be completed by MS

List of data submitted by the applicant and not relied on

| Data point | Author(s) | Year | Title Company Report No. Source (where different from company) GLP or GEP status Published or not | Vertebrate study Y/N | Owner |
| --- | --- | --- | --- | --- | --- |
| CP XX | Author | YYYY | Title  Company Report No  Source  GLP/Not GLP/GEP/Not GEP, Published/Unpublished | Y/N | Owner |
|  |  |  |  |  |  |

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

| Data point | Author(s) | Year | Title Company Report No. Source (where different from company) GLP or GEP status Published or not | Vertebrate study Y/N | Owner |
| --- | --- | --- | --- | --- | --- |
| CP XX | Author | YYYY | Title  Company Report No  Source  GLP/Not GLP/GEP/Not GEP, Published/Unpublished | Y/N | Owner |
|  |  |  |  |  |  |

1. Detailed evaluation of the new studies
   1. KCP 10.1 Effects on birds and other terrestrial vertebrates
      1. KCP 10.1.1 Effects on birds
         1. KCP 10.1.1.1 Acute oral toxicity
         2. KCP 10.1.1.2 Higher tier data on birds
      2. KCP 10.1.2 Effects on terrestrial vertebrates other than birds
         1. KCP 10.1.2.1 Acute oral toxicity to mammals
         2. KCP 10.1.2.2 Higher tier data on mammals
      3. KCP 10.1.3 Effects on other terrestrial vertebrate wildlife (reptiles and amphibians)
   2. KCP 10.2 Effects on aquatic organisms
      1. KCP 10.2.1 Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes

|  |  |
| --- | --- |
| Comments of zRMS: | Study with the metabolite 4-Chlorophenol was submitted as part of a precedent Central Zone dRR ‘Tricera’ with PL as the zRMS finalised in May 2022 (formulation code ADM.3304.H.1.A).  The study is considered acceptable with following endpoints relevant for the risk assessment (all based on geometric mean measured concentrations):  Total shoot length  ErC50 = 13.1 mg pm/L  EyC50 = 10.4 mg pm/L  Fresh weight  ErC50 = 48.0 mg pm/L  EyC50 = 18.2 mg pm/L  Dry weight  ErC50 = 56.7 mg pm/L  EyC50 = 15.4 mg pm/L |
| Reference: | KCP 10.2.1/01 |
| Report: | 4-Chlorophenol: Growth Inhibition of *Myriophyllum spicatum* in a Water/Sediment System. Gonsior, G., (2015). S15-00666 |
| Guideline(s): | OECD Guideline 239 |
| Deviations: | No major deviations |
| GLP: | Yes |
| Acceptability: | - |

**Executive Summary**

Following exposure of the aquatic macrophyte *Myriophyllum spicatum* to 4-Chlorophenol for 14 days, the ErC50 and EyC50 values based on nominal concentrations and total shoot length were 12.9 mg/L and 10.6 mg/L respectively. The NOEC for growth rate and yield based on total shoot length was 4.88 mg/L. Based on geometric mean measured concentrations, the ErC50, EyC50 and NOEC values were 13.1, 10.4 and 4.39 mg test item/L respectively.

The ErC50 and EyC50 values based on nominal concentrations and biomass (fresh weight) were 38.7 mg/L and 17.2 mg/L respectively. The NOEC for growth rate and yield based on biomass (fresh weight) was 4.88 mg/L. Based on geometric mean measured concentrations, the ErC50, EyC50 and NOEC values were 48.0, 18.2 and 4.39 mg test item/L respectively.

The ErC50 and EyC50 values based on nominal concentrations and biomass (dry weight) were 43.8 mg/L and 15.1 mg/L respectively. The NOEC for growth rate and yield based on biomass (dry weight) was 1.53 mg/L. Based on geometric mean measured concentrations, the ErC50, EyC50 and NOEC values were 56.7, 15.4 and 0.979 mg test item/L respectively.

**Materials and Methods**

**Materials**

|  |  |  |
| --- | --- | --- |
| **Test Material:** | 4-Chlorophenol | |
| **Description:** | Solid white | |
| **Lot/batch, density:** | MKBJ7452V, TSN304318 | |
| **Concentration/Purity:** | 100 % w/w | |
| **Stability of test compound:** | Re-certification date: 26 January 2015 | |
|  |  | |
| **Vehicle and/or control:** | Untreated sterilised sediment overlaid with SMART ANBARKO medium | |
|  |  | |
| **Test species:** | Rooted aquatic macrophyte, *Myriophyllum spicatum* | |
| **Source:** | *Myriophyllum spicatum* plants have been maintained under laboratory conditions at Eurofins Agroscience Services EcoChem GmbH since November 2010. The cultures obtained from Umweltbundesamt Berlin, Germany were based on a culture of the Landesanstalt für Gewässerkunde Koblenz, Germany.  *M. spicatum* is cultivated under sterile conditions submersed in a modified, aqueous ANDREWS medium containing sucrose. This laboratory stock culture is used to provide uniform plants throughout the year, eliminating seasonal variation in plant quality and contamination by other species (e.g. algae). The stock culture plants were held under the same environmental conditions as used in the test. | |
| **Acclimation period:** | Nine days prior to test initiation, submerged apical shoots of the same size were planted in an aquarium in an artificial sterilised sediment overlaid with SMART AND BARKO medium under the same temperature, light, and water quality conditions as used during the exposure of the plants in the test. Shoot were anchored in an upright position using glass rings and were maintained under controlled environment conditions. | |
| **Culture medium:** | ANDREWS Medium | |
| **Test vessel:** | Plants were grown in a static water-sediment system using artificial sterilized sediment overlaid with SMART AND BARKO medium under the same conditions as used in the pre-culture. The study was conducted in 2 L glass-beakers measuring approx. 12 cm in diameter and 24 cm height. Only one shoot per test vessel was planted. The volume of added water was recorded, and the level marked on the outside of the test vessels.  Sediment used in the test (percentages based on dry weight):   * 4 % sphagnum peat (approximately pH 5.5 – 6.0; no visible plant remains, finely ground, air dried); * 20 % kaolin clay (kaolinite content above 30 %); * 75 – 76 % quartz sand (fine sand with more than 50 % of the particles between 50 and 200 microns); * approximately 0.2 % calcium carbonate, precipitated extra pure, to adjust the sediment pH to 7.0 ± 0.5 at the start of the test before adding the test item; * organic carbon content of the final mixture should be 2 % (± 0.5 %) and was adjusted by the use of appropriate amounts of peat and sand; * 100 mg ammonium chloride and sodium phosphate per kg sediment (dry weight).   The dry constituents were blended in the correct proportions and mixed thoroughly in an electric mixer. The dry sediment was sterilised in a heating chamber at 110 °C for at least 2 hours prior to use to minimise algal contamination of the test systems.  SMART AND BARKO medium:   * CaCl2 × 2 H2O: 91.7 mg/L * MgSO4 × 7 H2O: 69.0 mg/L * NaHCO3: 58.4 mg/L * KHCO3:15.4 mg/L * pH (air equilibrium) approximately 7.9 | |
| **Number of replicates:** | Five replicates per test item concentration and ten replicates for the control were used | |
| **Untreated variant:** | Test vessel/medium without test substance | |
| **Reference substance:** | None | |
|  |  | |
| **Environmental conditions during testing** | | |
| **Temperature** | Test solution temperature (range): 19.9 ± 0.3  C | |
| **pH** | Test solution pH (range): 7.93 ± 0.23 | |
| **Oxygen-concentration [mg/L]** | The oxygen saturation was determined to be 92 ± 10 % | |
| **Photoperiod** | Photoperiod: 16 h day length | |
| **Light intensity** | 120 – 160 µEm-2s-1 | |
|  | |

**Study Design**

Plants were grown in a static water-sediment system using artificial sterilized sediment overlaid with Smart and Barko medium under the same conditions as used in the pre-culture. The study was conducted in 2 L glass-beakers measuring approx. 12 cm in diameter and 24 cm height. Only one shoot per test vessel was planted. The volume of added water was recorded, and the level marked on the outside of the test vessels. Each vessel contained approx. 350 g of moist sediment containing growth nutrients (ammonium chloride and sodium phosphate), with the sediment surface overlaid with moist sediment without nutrients, and a thin layer of washed quartz sand, to minimize displacement of the sediment when the growth medium was added. Afterwards the test vessels were filled carefully with growth medium (1.5 L). Two days after preparation of the test vessels and before application of the test item, one rooted apical shoot per vessel was planted carefully, ensuring the plant was rooted into the sediment. Shortly afterwards, application of the test item was performed and mixed in with gentle stirring. The test item was spiked to the water at nominal concentrations of 0.477, 1.53, 4.88, 15.6 and 50.0 mg 4-Chlorophenol /L. Ten replicates were used for the control and five for each test item group. On day 0 fifteen additional plants, representative of those used in the test, were selected from the available plant material. The plants were blotted dry prior to assessment of plant fresh weight and shoot length. The plants were placed separately in labelled glass beakers and dried at 60 °C for > 48 hours.

Test concentrations were nominally 0.477, 1.53, 4.88, 15.6 and 50.0 mg test item /L

Chemical analysis and validation was conducted using HPLC MS/MS. The limit of detection (LOD) was defined as 30 % of the limit of quantification (= 0.0300 mg/L of 4-Chlorophenol). Limit of Quantitation: The limit of quantification was 0.0700 mg/L of 4-Chlorophenol in water and 0.500 mg/kg 4-Chlorophenol in sediment. Recoveries from QC fortifications: (70 ± 110 % mean recovery, ≤ 20 % RSD)

Samples taken: 0 hours, 1 day, 3 day, 7 days and 14 days

Test item concentrations in the definitive test were verified by analyses of 4-Chlorophenol at all concentration levels by analyzing the overlaying water at test start, 1, 3 and 7 days after test start and at test end and wet sediment at termination on day 14.

The weight of the dry plant samples was recorded. On day 14 plants were harvested from each treatment group for assessment of total plant fresh weight, total plant dry weight, shoot length and number and length of side shoots. In addition, observations on shoot and root development (e.g. necrosis, deformation) were documented

Temperature (°C), pH and oxygen saturation (%) of the test solutions was measured after 0, 7 and 14 days.

All data were subjected to ANOVA. A test for normality of the data was carried out by calculating the Shapiro-Wilk’s statistic. For homogeneity of variances across treatment groups a Bartlett’s or Levene’s test was performed. If data were normally distributed and variance was homogeneous a Dunnett’s t-test was performed. If Shapiro Wilkʼs test indicated a non-normal distribution of residuals a Bonferroni-U Exact Test was performed to determine significant differences from controls (SAS® Proprietary Software 9.3).

The ECx (yield and growth rate) was calculated where possible using Probit analysis. The EC10 and EC20 (yield and growth rate) were calculated when the C.V. in the control cultures was low enough to allow for reliable estimates to be determined.

For example, estimates of EC10 and EC20 values are only reliable if the C.V. in control plants is below the effect level being estimated, i.e. C.V. should be < 20 % for robust estimation of an EC20 and C.V. should be < 10 % for robust estimation of an EC10.

Only concentrations within a clear dose response were used for calculations.

**Results and Discussion**

Validity criteria according to OECD 239 were met:

* The control plants showed uniform growth over the test period and the mean total shoot length increased 3-fold, fresh weight biomass increased more than 4-fold, and mean dry weight biomass more than 4.5-fold.
* The coefficient of variation (C.V.) for control growth based on shoot length, fresh weight and dry weight was 15.1 %, 14.8 % and 9.1 % respectively. The mean C.V. for control yield based on shoot length, fresh weight and for dry weight was 25.5 %, 28.2 % and 17.9 % respectively.

Since shoot biomass and length more than doubled within the test duration, and the C.V. for fresh weight and shoot length yield was below 35 %, the performance of the plants used for this test was considered acceptable.

The measured concentration of the test item based on the 4-Chlorophenol content in the overlaying water in the test vessels at test start ranged between 117 and 132 % of nominal. At test end, concentrations of 4-Chlorophenol ranged between < LOD and 90 % of nominal. At test end, concentrations of 4-Chlorophenol in the sediment were detectable at between 10 – 15 % of the amount applied in the three highest test concentrations 4.88, 15.6 and 50.0 mg/L. `Since the initial mean measured concentrations of 4-Chlorophenol were > 80 % of nominal, and the mean measured concentrations at test termination were below 80 % of nominal, all toxicological endpoints were evaluated using nominal and the geometric mean measured concentrations based on 4-Chlorophenol concentration. Results of analytical measurements are presented in tables below.

**Table 10.2.1/01-1: Measured concentrations in overlying water**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Time | Nominal concentration | | Overlaying water (measured concentrations) | |
|  | Test item | 4-Chlorophenol | 4-Chlorophenol | |
| [d] | [mg/L] | [mg/L] | [mg/L] | [% of nominal] |
| 0 | Control | 0 | nd | - |
| 1 | nd | - |
| 3 | nd | - |
| 7 | nd | - |
| 14 | nd | - |
| 0 | 0.477 | 0.477 | 0.558 | 117 |
| 1 | 0.48 | 101 |
| 3 | 0.472 | 99 |
| 7 | 0.279 | 58 |
| 14 | nd | - |
| 0 | 1.53 | 1.53 | 1.81 | 118 |
| 1 | 1.54 | 101 |
| 3 | 1.45 | 95 |
| 7 | 1.04 | 68 |
| 14 | 0.397 | 26 |
| 0 | 4.88 | 4.88 | 6.34 | 130 |
| 1 | 5.4 | 111 |
| 3 | 5.23 | 107 |
| 7 | 4.08 | 84 |
| 14 | 2.54 | 52 |
| 0 | 15.6 | 15.6 | 19.5 | 125 |
| 1 | 18.8 | 121 |
| 3 | 17.9 | 115 |
| 7 | 16.1 | 103 |
| 14 | 12.4 | 79 |
| 0 | 50 | 50 | 66.1 | 132 |
| 1 | 65.9 | 132 |
| 3 | 60.8 | 122 |
| 7 | 49.5 | 99 |
| 14 | 44.9 | 90 |

LOQ = 0.0700 mg/L

n.d. = not detectable

**Table 10.2.1/01-2: Measured concentrations in sediment**

|  |  |  |  |
| --- | --- | --- | --- |
| Time [d] | Sample | | Sediment |
| Test item | 4-Chlorophenol | 4-Chlorophenol |
| [mg/L] | [mg/L] | [mg/kg] |
| 14 | Control | 0 | n.d. |
| 0.477 | 0.477 | n.d. |
| 1.53 | 1.53 | <LOQ |
| 4.88 | 4.88 | 1.24 |
| 15.6 | 15.6 | 4.49 |
| 50 | 50 | 18.7 |

LOQ = 0.500 mg/L

n.d. = not detectable

1) based on wet weight

**A s**ummary of the biological results are shown in the following tables.

**Table 10.2.1/01-3: Mean total shoot length including side shoots (cm)**

| Nominal concentration (µg/L) | Days after application | | Yield (cm) | Reduction in yield (%) | Growth rate (1/day) | Reduction in growth rate (%) |
| --- | --- | --- | --- | --- | --- | --- |
| 0 1) | 14 |
| Control | 8.2 | 24.4 | 16.2 | - | 0.0769 | - |
| 0.477 | 8.2 | 24.4 | 16.2 | 0.0 | 0.0778 | -1.2 |
| 1.53 | 8.2 | 23.4 | 15.2 | 6.2 | 0.0716 | 6.9 |
| 4.88 | 8.2 | 21.9 | 13.7 | 15.4 | 0.0694 | 9.8 |
| 15.6 | 8.2 | 14.9 | 6.7\* | 58.6\* | 0.0420\* | 45.4\* |
| 50.0 | 8.2 | 7.6 | -0.6\* | 103.7\* | -0.0058\* | 107.5\* |

\* Significantly different reduction compared to the control.

1) Based on 15 additional plants, representative of those used in the test.

**Table 10.2.1/01-4: Mean total plant fresh weight (g)**

| Nominal concentration (µg/L) | Days after application | | Yield (cm) | Reduction in yield (%) | Growth rate (1/day) | Reduction in growth rate (%) |
| --- | --- | --- | --- | --- | --- | --- |
| 0 1) | 14 |
| Control | 0.2271 | 0.9237 | 0.6966 | - | 0.0988 | - |
| 0.477 | 0.2271 | 0.8922 | 0.6651 | 4.5 | 0.0975 | 1.3 |
| 1.53 | 0.2271 | 0.8166 | 0.5895 | 15.4 | 0.0880 | 10.9 |
| 4.88 | 0.2271 | 0.7649 | 0.5378 | 22.8 | 0.0847 | 14.3 |
| 15.6 | 0.2271 | 0.6149 | 0.3878\* | 44.3\* | 0.0699\* | 29.3\* |
| 50.0 | 0.2271 | 0.4111 | 0.1840\* | 73.6\* | 0.0412\* | 58.3\* |

\* Significantly different reduction compared to the control.

1) Based on 15 additional plants, representative of those used in the test.

**Table 10.2.1/01-5: Mean total plant dry weight (g)**

| Nominal concentration (µg/L) | Days after application | | Yield (cm) | Reduction in yield (%) | Growth rate (1/day) | Reduction in growth rate (%) |
| --- | --- | --- | --- | --- | --- | --- |
| 0 1) | 14 |
| Control | 0.0265 | 0.1306 | 0.1041 | - | 0.1132 | - |
| 0.477 | 0.0265 | 0.1250 | 0.0985 | 5.4 | 0.1104 | 2.5 |
| 1.53 | 0.0265 | 0.1078 | 0.0813 | 21.9 | 0.0983 | 13.2 |
| 4.88 | 0.0265 | 0.0996 | 0.0731\* | 29.8\* | 0.0931\* | 17.8\* |
| 15.6 | 0.0265 | 0.0868 | 0.0603\* | 42.1\* | 0.0839\* | 25.9\* |
| 50.0 | 0.0265 | 0.0524 | 0.0259\* | 75.1\* | 0.0481\* | 57.5\* |

\* Significantly different reduction compared to the control.

1) Based on 15 additional plants, representative of those used in the test.

The calculated EC50 values, NOEC and LOEC based on growth rate and yield for each of the measured parameters (total shoot length, fresh weight and dry weight) are presented below. Where the C.V. in the control cultures allowed for reliable estimates of EC10 and/or EC20 to be determined, these were also reported below.

**Table 10.2.1/01-6: Summary of biological results based on nominal concentrations of 4-Chlorophenol**

| Parameter (mg/L) | Total shoot length | | Fresh weight | | Dry weight | |
| --- | --- | --- | --- | --- | --- | --- |
| Growth rate | Yield | Growth rate | Yield | Growth rate | Yield |
| 14-day EC50 | 12.9 | 10.6 | 38.7 | 17.2 | 43.8 | 15.1 |
| 95% Conf. Limits | 11.0 – 15.3 | 9.07 – 12.4 | 27.1 – 62.7 | 13.0 – 24.1 | 28.8 – 78.6 | 11.2 – 21.6 |
| 14-day EC20 | 5.69 | - | 6.23 | - | 5.40 | 2.14 |
| 95% Conf. Limits | 4.62 – 6.78 | - | 4.42 – 8.39 | - | 3.67 – 7.52 | 1.40 – 2.97 |
| 14-day EC10 | - | - | - | - | 1.81 | - |
| 95% Conf. Limits | - | - | - | - | 0.999 – 2.76 | - |
| 14-day NOEC | 4.88 | 4.88 | 4.88 | 4.88 | 1.53 | 1.53 |
| 14-day LOEC | 15.6 | 15.6 | 15.6 | 15.6 | 4.88 | 4.88 |

(-) Values not reliable, control CV exceeded the effect level.

**Table 10.2.1/01-7: Summary of biological results based on geometric mean measured concentrations of 4-Chlorophenol**

| Parameter (mg/L) | Total shoot length | | Fresh weight | | Dry weight | |
| --- | --- | --- | --- | --- | --- | --- |
| Growth rate | Yield | Growth rate | Yield | Growth rate | Yield |
| 14-day EC50 | 13.1 | 10.4 | 48.0 | 18.2 | 56.7 | 15.4 |
| 95% Conf. Limits | 10.9 – 16.0 | 8.68 – 12.6 | 31.0 – 87.2 | 12.8 – 27.7 | 33.8 – 118 | 10.6 – 24.1 |
| 14-day EC20 | 4.95 | - | 5.15 | - | 4.26 | 1.33 |
| 95% Conf. Limits | 3.85 – 6.11 | - | 3.36 – 7.43 | - | 2.62 – 6.43 | 0.776 – 2.02 |
| 14-day EC10 | - | - | - | - | 1.10 | - |
| 95% Conf. Limits | - | - | - | - | 0.516 – 1.88 | - |
| 14-day NOEC | 4.39 | 4.39 | 4.39 | 4.39 | 0.979 | 0.979 |
| 14-day LOEC | 16.7 | 16.7 | 16.7 | 16.7 | 4.39 | 4.39 |

(-) Values not reliable, control CV exceeded the effect level.

**Conclusion**

Following exposure of the aquatic macrophyte *Myriophyllum spicatum* to 4-Chlorophenol for 14 days, the ErC50 and EyC50 values based on nominal concentrations and total shoot length were 12.9 mg/L and 10.6 mg/L respectively. The NOEC for growth rate and yield based on total shoot length was 4.88 mg/L. Based on geometric mean measured concentrations, the ErC50, EyC50 and NOEC values were 13.1, 10.4 and 4.39 mg test item/L respectively.

The ErC50 and EyC50 values based on nominal concentrations and biomass (fresh weight) were 38.7 mg/L and 17.2 mg/L respectively. The NOEC for growth rate and yield based on biomass (fresh weight) was 4.88 mg/L. Based on geometric mean measured concentrations, the ErC50, EyC50 and NOEC values were 48.0, 18.2 and 4.39 mg test item/L respectively.

The ErC50 and EyC50 values based on nominal concentrations and biomass (dry weight) were 43.8 mg/L and 15.1 mg/L respectively. The NOEC for growth rate and yield based on biomass (dry weight) was 1.53 mg/L. Based on geometric mean measured concentrations, the ErC50, EyC50 and NOEC values were 56.7, 15.4 and 0.979 mg test item/L respectively.

|  |
| --- |
| **Assessment and conclusion by applicant:**  This is a new study that has not been previously evaluated.  Validity criteria according to OECD 239 were met:   * The control plants showed uniform growth over the test period and the mean total shoot length increased 3-fold, fresh weight biomass increased more than 4-fold, and mean dry weight biomass more than 4.5-fold. * The coefficient of variation (C.V.) for control growth based on shoot length, fresh weight and dry weight was 15.1 %, 14.8 % and 9.1 % respectively. The mean C.V. for control yield based on shoot length, fresh weight and for dry weight was 25.5 %, 28.2 % and 17.9 % respectively.   For total shoot length, based on geometric mean measured concentrations, the ErC50, EyC50 and NOEC values were 13.1, 10.4 and 4.39 mg test item/L respectively.  For biomass (fresh weight), based on geometric mean measured concentrations, the ErC50, EyC50 and NOEC values were 48.0, 18.2 and 4.39 mg test item/L respectively.  For biomass (dry weight), based on geometric mean measured concentrations, the ErC50, EyC50 and NOEC values were 56.7, 15.4 and 0.979 mg test item/L respectively. |

* + 1. KCP 10.2.2 Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms
    2. KCP 10.2.3 Further testing on aquatic organisms
  1. KCP 10.3 Effects on arthropods
     1. KCP 10.3.1 Effects on bees
        1. KCP 10.3.1.1 Acute toxicity to bees
           1. KCP 10.3.1.1.1 Acute oral toxicity to bees
           2. KCP 10.3.1.1.2 Acute contact toxicity to bees
        2. KCP 10.3.1.2. Chronic toxicity to bees

|  |  |
| --- | --- |
| Comments of zRMS: | The study was conducted to OECD guidance TG 245 and according to the principles of GLP. All validity criteria were met.  The study is considered to be reliable and suitable for the risk assessment. |
| Reference: | KCP 10.3.1.2/01 |
| Report: | 2,4-D 95 SP: 10-Day chronic oral toxicity test for adult honeybees (*Apis mellifera* L.)  Wilkins, S. (2023a), Report no.: FR/002602-10, Document no.: 000109119 |
| Guideline(s): | OECD 245 (2017) |
| Deviations: | * Single bee escaped from one replicate on day 6. As feed intake is calculated based on number of live bees per unit, and as no mortalities were observed within said replicate, this did not have any impact on the integrity or outcome of the study. * A dilution series was prepared in water rather than sucrose solution in error, however nominal doses were within 5% of the target therefore this deviation had no impact on the study. |
| GLP: | Yes |
| Acceptability: | Yes |

**Executive Summary**

The honeybee *Apis mellifera* was exposed to 2,4-D 95 SP over 10 days in a chronic feeding test.

Test organisms were exposed to nominal concentrations of 162.3, 324.5, 649.0, 1298 and 2595 mg a.s./kg, equivalent to a mean dose of 4.526, 9.768, 18.33, 28.16 and 34.48 µg a.s./bee/day, respectively, along with a control and dimethoate toxic reference.

Mean mortalities after 10 days exposure of 10.3, 0.0, 3.3, 10.0 and 96.7% were observed at test doses of 4.526, 9.768, 18.33, 28.16 and 34.48 µg a.s./bee/day, respectively. Percent mortality in the control was 3.3%, and in the toxic reference was 100%.

The 10-day NOEDD and LDD50 were determined to be 28.16 and 30.44 µg a.s./bee/day, respectively.

**Materials and Methods**

**Materials**

|  |  |
| --- | --- |
| **Test Material** | 2,4-D 95 SP |
| **Lot/Batch #:** | 2111140274 |
| **Purity:** | 95.1% |
| **Description:** | White/beige powder |
| **Stability of test compound:** | Not reported |
| **Reanalysis/Expiry date:** | 1st November 2023 |
| **Density:** | Not reported |
| **Treatments** |  |
| **Test rates:** | Nominal concentrations: 162.3, 324.5, 649.0, 1298 and 2595 mg a.s./kg, equivalent to: Mean dose: 4.526, 9.768, 18.33, 28.16 and 34.48 µg a.s./bee/day |
| **Solvent/vehicle:** | None |
| **Analysis of test concentrations:** | None |
| **Test organisms** |  |
| **Species:** | *Apis mellifera* adults |
| **Source:** | Fera National Bee Unit |
| **Feeding:** | 50% aqueous sucrose solution *ad libitum* |
| **Treatment for disease:** | None reported |
| **Test design** |  |
| **Test vessel:** | Plastic pots of 4.5 x 11 cm |
| **Replication:** | Three replicates per test concentration and control |
| **No. animals/vessel:** | 10 bees |
| **Duration of test:** | 10 days |
| **Environmental test conditions** |  |
| **Temperature:** | 32.2 – 33.1°C (mean 33.0°C) |
| **Relative humidity** | 45.7 – 68.8% (mean 66.9%) |
| **Photoperiod:** | Constant darkness |

**Study Design**

The honeybee *Apis mellifera* was exposed to 2,4-D 95 SP over 10 days in a chronic feeding test.

Newly emerged bees aged between 0 and 48 hours at test start were used. Three replicates of 10 bees were exposed in each treatment group. Bees were exposed to nominal concentrations of 162.3, 324.5, 649.0, 1298 and 2595 mg a.s./kg, equivalent to a mean dose of 4.526, 9.768, 18.33, 28.16 and 34.48 µg a.s./bee/day, respectively, along with a control and dimethoate toxic reference. Test concentrations were adjusted for purity of the test item.

An initial test item stock solution was prepared in water at 51.9 mg a.s./mL. This was then used to prepare a range of dosing solutions in water with nominal concentrations of 3.245, 6.485, 12.98, and 25.95 mg a.s./mL. These dosing solutions were used to spike a 50% w/v aqueous sucrose solution at a rate of 50 µL/g to make the ‘dosed feed’ solutions.

Following analysis of the dosed feed samples it was seen that the difference between expected concentration and analysed content was less than 20%. Therefore, the results have been expressed as nominal values.

Each cage of bees was offered approximately 1.5 mL of treated or control diet each day.

Observations of mortality and behaviour were recorded at 24-hour intervals. Organisms were defined as alive, affected, moribund or dead.

Statistical evaluations were performed using validated software R V 3.4.4 on mean mortality after 10 days.

**Results and Discussion**

Validity criteria according to the OECD 245 (2017) were met. In the control, the following criteria were met:

* Mean control mortality ≤15% at the end of the test (actual: 3.3%);
* Mean mortality in the reference item to be ≤50% (actual: 100 %).

Mean mortalities after 10 days exposure of 10.3, 0.0, 3.3, 10.0 and 96.7% were observed at test doses of 4.526, 9.768, 18.33, 28.16 and 34.48 µg a.s./bee/day, respectively. Percent mortality in the control was 3.3%, and in the toxic reference was 100%.

The only abnormal behaviour noted was pre-mortality, e.g. bees stumbling or knocked down prior to death.

**Table 10.3.1.2/01-1: Mean percentage mortality of honeybees over 10 days**

| Treat. | Nominal conc.  (mg a.s./ha) | Mean dose (µg a.s./ bee/day) | Mean mortality1 (%) by day | | | | | | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| Control | 0 | - | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 | 3.3 | 3.3 | 3.3 | 3.3 |
| 2,4-D | 162.3 | 4.526 | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 | 3.3 | 6.9 | 10.3 | 10.3 | 10.3 |
| 324.5 | 9.768 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 649.0 | 18.33 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 | 3.3 | 3.3 | 3.3 |
| 1298 | 28.16 | 0.0 | 0.0 | 3.3 | 3.3 | 3.3 | 6.7 | 10.0 | 10.0 | 10.0 | 10.0 |
| 2595 | 34.48 | 0.0 | 10.0 | 36.7 | 56.7 | 63.3 | 70.0 | 80.0 | 86.7 | 93.3 | 96.7\* |
| Dimethoate | 1.0 | 0.028 | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 | 3.3 | 3.3 | 100 | 100 | 100 |
| 1 mean of four replicates  \* Significantly different from the control (p<0.05) | | | | | | | | | | | | |

**Conclusion**

The honeybee *Apis mellifera* was exposed to 2,4-D 95 SP over 10 days in a chronic feeding test.

Test organisms were exposed to nominal concentrations of 162.3, 324.5, 649.0, 1298 and 2595 mg a.s./kg, equivalent to a mean dose of 4.526, 9.768, 18.33, 28.16 and 34.48 µg a.s./bee/day, respectively, along with a control and dimethoate toxic reference.

Mean mortalities after 10 days exposure of 10.3, 0.0, 3.3, 10.0 and 96.7% were observed at test doses of 4.526, 9.768, 18.33, 28.16 and 34.48 µg a.s./bee/day, respectively. Percent mortality in the control was 3.3%, and in the toxic reference was 100%.

The 10-day NOEDD and LDD50 were determined to be 28.16 and 30.44 µg a.s./bee/day, respectively.

|  |
| --- |
| **Assessment and conclusion by applicant:**  This is a new study that has not been previously evaluated.  Validity criteria according to the OECD 245 (2017) were met. In the control, the following criteria were met:   * Mean control mortality ≤15% at the end of the test (actual: 3.3%); * Mean mortality in the reference item to be ≤50% (actual: 100 %).   The study is therefore considered acceptable.  Mean mortalities after 10 days exposure of 10.3, 0.0, 3.3, 10.0 and 96.7% were observed at test doses of 4.526, 9.768, 18.33, 28.16 and 34.48 µg a.s./bee/day, respectively. Percent mortality in the control was 3.3%, and in the toxic reference was 100%.  The 10-day NOEDD and LDD50 were determined to be 28.16 and 30.44 µg a.s./bee/day, respectively. |

* + - 1. KCP 10.3.1.3 Effects on honey bee development and other honey bee life stages

|  |  |
| --- | --- |
| Comments of zRMS: | The study was conducted to OECD guidance TG 239 and according to the principles of GLP. All validity criteria were met.  The study is considered to be reliable.  Nevertheless, it should be highlighted that at the time of production of this interim report an assessment of storage stability of the analytical samples has not been completed. |
| Reference: | KCP 10.3.1.3/01 |
| Report: | 2,4-D 95 SP: In vitro 22-day toxicity test - repeated exposure to larval stage honeybees (*Apis mellifera* L.)  Wilkins, S. (2023b), Report no.: FR/002602-11, Document no.: 000109120 |
| Guideline(s): | OECD 239 (2016) |
| Deviations: | * On Day 8, a dish of saturated NaCl salt solution is placed in the desiccator with the larval plates to produce a humidity range of 80 ±5%. However, in this test the humidity was higher than this with a maximum of 90.4% RH recorded. There was no mould recorded in the test (which high humidity at this stage can cause) and the control emergence was unaffected (93.75%) and so there is no impact of this deviation on the test. |
| GLP: | Yes |
| Acceptability: | Yes |

**Executive Summary**

Larvae of the honeybee *Apis mellifera* was exposed to 2,4-D 95 SP over 22 days in a repeated exposure toxicity test, with the test item incorporated into larval diet for four days.

Test organisms were exposed to nominal doses of 3.942, 9.856, 24.64, 61.60 and 154.0 µg formulation/larva, equivalent to 3.175, 7.937, 19.84, 49.61 and 124.0 µg a.s./larva, respectively, along with a control and dimethoate toxic reference.

After 22-days, the adult emergence rate was 93.75, 87.50, 66.67, 14.58 and 0.00% at test doses 3.175, 7.937, 19.84, 49.61 and 124.0 µg a.s./larva, respectively. Emergence in the control and toxic reference were 93.75 and 0.00%, respectively.

The 22-day NOED and LD50 were determined to be 7.937 and 27.14 µg a.s./larva, respectively.

**Materials and Methods**

**Materials**

|  |  |
| --- | --- |
| **Test Material** | 2,4-D 95 SP |
| **Lot/Batch #:** | 2111140274 |
| **Purity:** | 95.1% |
| **Description:** | Fine crystalline solid substance, white to beige in colour |
| **Stability of test compound:** | Deemed stable in dosing solutions after validation test |
| **Reanalysis/Expiry date:** | 1st November 2023 |
| **Density:** | Not reported |
| **Treatments** |  |
| **Test rates:** | Nominal 3.942, 9.856, 24.64, 61.60 and 154.0 µg formulation/larva, equivalent to 3.175, 7.937, 19.84, 49.61 and 124.0 µg a.s./larva, |
| **Solvent/vehicle:** | Demineralised water |
| **Analysis of test concentrations:** | No |
| **Test organisms** |  |
| **Species:** | *Apis mellifera* adults |
| **Source:** | Fera National Bee Unit |
| **Feeding:** | Diet Solution A: aqueous solution containing 2% yeast extract, 12% glucose and 12% fructose  Diet Solution B: aqueous solution containing 3% yeast extract, 15% glucose and 15% fructose  Diet Solution C: aqueous solution containing 4% yeast extract, 18% glucose and 18% fructose. |
| **Treatment for disease:** | None reported |
| **Test design** |  |
| **Test vessel:** | Crystal polystyrene grafting cells of 9 m diameter and 8 mm depth |
| **Replication:** | Three replicates per test concentration and control |
| **No. animals/vessel:** | 16 |
| **Duration of test:** | 22 days |
| **Environmental test conditions** |  |
| **Temperature:** | 34 - 35°C |
| **Relative humidity** | Days 1-8: 95% ± 5%  Days 8-15: 80% ± 5%  Days 15-22: 65% ± 15% |
| **Photoperiod:** | Constant darkness |

**Study Design**

Larvae of the honeybee *Apis mellifera* was exposed to 2,4-D 95 SP over 22 days in a repeated exposure toxicity test, with the test item incorporated into larval diet for four days.

Larval honey bees less than 24 hours old were collected from colonies and grafted (D1) into artificial queen cell cups and fed an artificial diet. On Days 3-6 (dosing days) diet dosed with appropriate levels of test/reference item as required was fed to each larva. Equivalent undosed (water) controls were set up as well as a toxic reference treated group.

Three replicates of 16 bees from three different colonies were exposed in each treatment group. Bees were exposed to nominal concentration of 3.942, 9.856, 24.64, 61.60 and 154.0 µg formulation/larva, equivalent to 3.175, 7.937, 19.84, 49.61 and 124.0 µg a.s./larva, respectively, along with a control and dimethoate toxic reference.

The analysed content of 2,4-D in the dosed diet samples collected on each dosing day (D3-6) all showed an average deviation of less than 20% from expected. Therefore, the results are reported based on the nominal concentrations.

On D1 the larvae were fed 20 μL of untreated diet A. On D3 they were fed 20 μL of diet B treated with the test solutions. On D4, D5, and D6 the larvae were fed 30, 40 or 50 μL diet C treated with the test solutions respectively. The diet was well mixed using a vortex mixer following dosing, warmed in the incubator prior to feeding to the larvae (to prevent temperature shock to the larvae), and mixed again before feeding. The treatments were visually assessed for homogeneity.

Treatment-related mortality checks were made on D3 to D8, before feeding on feeding days (D3-6). An immobile larva, one which did not respond when the cell cup was tapped, was recorded as dead and removed. At D8 a qualitative assessment was made as to whether the larvae were smaller than would be expected or had remaining food.

Between D8 and D15 the plates were examined daily and any larvae/pupae that appeared to be dead (black in colour or decomposing) or that showed signs of fungal growth were removed.

On the D15 mortality check any developing bees that were still larvae were removed along with any dead pupae that had not previously been removed. The number alive was recorded along with any observations of discolouration, abnormal positioning (developing pupae should be face up in the cups) or incorrect growth stage (e.g. white eyed pupae).

On the D22 emergence assessments each plate was checked for the number of emerged adult bees. A bee was recorded as emerged if it had developed wings even if the bee had subsequently died. It was also recorded if the bees (whether alive or dead) had any morphological problems such as wings that had not fully expanded. Of those bees that did not successfully emerge it was recorded whether the pupae had reached the final development stage (almost a fully developed bee but with wings not expanded at all).

Statistical evaluations were performed using validated software R V 3.4.4.

**Results and Discussion**

Validity criteria according to the OECD 239 (2016) were met. In the control, the following criteria were met:

* ≤15% pre-pupal mortality (D3 – D8) in the control plate (actual: 0.0%)
* An adult emergence rate ≥70% on D22 in the control plate (across all replicates) (actual: 93.75%)
* A dimethoate pre-pupal mortality rate of ≥ 50% (on D8 across all replicates) (actual: 97.9%)

After 22-days, the adult emergence rate was 93.75, 87.50, 66.67, 14.58 and 0.00% at test doses 3.175, 7.937, 19.84, 49.61 and 124.0 µg a.s./larva, respectively. Emergence in the control and toxic reference were 93.75 and 0.00%, respectively.

Morphological observations included under-developed wings, pupae in the wrong position in cells and colour differences. Many of these observations may be a result of the artificial test system and did not follow a dose-response, therefore are not thought to be test-item related.

**Table 10.3.1.3/01-1: Percent corrected cumulative mortality of honeybees over 22 days**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Nominal concentration  (mg formulation /kg diet) | Nominal Dose  (µg formulation /larva/test period) | Day 4 | Day 5 | Day 6 | Day 7 | Day 8 | Day 15 | Day 22 |
| Water Control | 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 6.25 | 6.25 |
| 25.60 | 3.942 | 2.08 | 2.08 | 2.08 | 2.08 | 2.08 | -4.44 | 0.00 |
| 64.00 | 9.856 | 0.00 | 2.08 | 2.08 | 2.08 | 2.08 | 2.22 | 6.67 |
| 160.0 | 24.64 | 0.00 | 4.17 | 8.33 | 10.42 | 16.67 | 22.22 | 28.89 |
| 400.0 | 61.60 | 0.00 | 0.00 | 35.42 | 52.08 | 56.25 | 73.33 | 84.44 |
| 1000 | 154.0 | 4.17 | 52.08 | 100 | 100 | 100 | 100 | 100 |
| Toxic ref  10 mg a.s./kg | 7.39 µg a.s./larva | 68.75 | 79.17 | 91.67 | 97.92 | 97.92 | 100.0 | 100.0 |

Values corrected to the control using Abbott 1925.

**Table 10.3.1.3/01-2 Toxicity effects after 22 days exposure**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Nominal concentration  (mg formulation /kg diet) | Nominal Dose1  (µg formulation /larva/test period) | Larval mortality rate (D8)  (%) | Pupal mortality rate (D15)  (%) | Adult emergence rate (D22)  (%) |
| Water Control | 0 | 0.000 | 6.250 | 93.75 |
| 25.60 | 3.942 | 2.083 | 4.255 | 93.75 |
| 64.00 | 9.856 | 2.083 | 10.64 | 87.50 |
| 160.0 | 24.64 | 16.67 | 20.00 | 66.67\* |
| 400.0 | 61.60 | 56.25 | 66.67 | 14.58\* |
| 1000 | 154.0 | 100 | N/A\*\* | 0.00\* |
| Toxic ref  10 mg a.s./kg | 7.39 µg a.s./larva | 97.92 | 100 | 0.00 |

\* Statistically different to controls (p=<0.05%) (Only D22 data assessed)

\*\*All larvae were dead by D8 so there was no pupal development

**Conclusion**

Larvae of the honeybee *Apis mellifera* was exposed to 2,4-D 95 SP over 22 days in a repeated exposure toxicity test, with the test item incorporated into larval diet for four days.

Test organisms were exposed to nominal doses of 3.942, 9.856, 24.64, 61.60 and 154.0 µg formulation/larva, equivalent to 3.175, 7.937, 19.84, 49.61 and 124.0 µg a.s./larva, respectively, along with a control and dimethoate toxic reference.

After 22-days, the adult emergence rate was 93.75, 87.50, 66.67, 14.58 and 0.00% at test doses 3.175, 7.937, 19.84, 49.61 and 124.0 µg a.s./larva, respectively. Emergence in the control and toxic reference were 93.75 and 0.00%, respectively.

The 22-day NOED and LD50 were determined to be 7.937 and 27.14 µg a.s./larva, respectively.

|  |
| --- |
| **Assessment and conclusion by applicant:**  This is a new study that has not been previously evaluated.  Validity criteria according to the OECD 239 (2016) were met. In the control, the following criteria were met:   * ≤15% pre-pupal mortality (D3 – D8) in the control plate (actual: 0.0%) * An adult emergence rate ≥70% on D22 in the control plate (across all replicates) (actual: 93.75%) * A dimethoate pre-pupal mortality rate of ≥ 50% (on D8 across all replicates) (actual: 97.9%)   The study is therefore considered acceptable.  After 22-days, the adult emergence rate was 93.75, 87.50, 66.67, 14.58 and 0.00% at test doses 3.175, 7.937, 19.84, 49.61 and 124.0 µg a.s./larva, respectively. Emergence in the control and toxic reference were 93.75 and 0.00%, respectively.  The 22-day NOED and LD50 were determined to be 7.937 and 27.14 µg a.s./larva, respectively. |

* + - 1. KCP 10.3.1.4 Sub-lethal effects
      2. KCP 10.3.1.5 Cage and tunnel tests
      3. KCP 10.3.1.6 Field tests with honeybees
      4. KCP 10.3.2 Effects on non-target arthropods other than bees

|  |  |
| --- | --- |
| Comments of zRMS: | The study follows the guideline specified by Mead Briggs *et al.* and according to the principles of GLP. The study is considered to be valid and suitable for the risk assessment. |
| Reference: | KCP 10.3.2.1/01 |
| Report: | Acute Dose-Response Toxicity of 2,4-D 95 SP to the Parasitic Wasp *Aphidius rhopalosiphi* (De Stefani-Perez) (Hymenoptera, Braconidae, Aphidiinae).  Milner, S. (2022a), Report no.: FR/002603-07, Document no.: 000109117 |
| Guideline(s): | Mead-Briggs *et al*. (2000) |
| Deviations: | Yes:   1. Feeding solution prepared as 1:3 w/w in error, not as 1:3 v/v as per the study plan. 2. Humidity fell to 59.8% for 30 minutes, below the target 60 – 90%. 3. Potential photoperiod deviation occurred.   These deviations are not expected to have affected the integrity of the study. |
| GLP: | Yes |
| Acceptability: | Yes |

**Executive Summary**

The parasitoid wasp , *Aphidius rhopalosiphi*, was exposed to 2,4-D 95 SP for 48 hours in a glass-plate exposure test.

Test organisms were exposed to rates of 3.906, 15.625, 62.5, 250.0 and 1000.0 g a.s./ha, in an application volume of 200 L/ha, equivalent to: 4.61, 18.45, 73.79, 295.16 and 1180.64 g/ha 2,4-D sodium salt, a control group was also tested.

Mean mortalities of 2.5, 0.0, 2.5, 5.0 and 0.0% were observed at test concentrations 3.906, 15.625, 62.5, 250.0 and 1000 g a.s./ha. Percent mortality in the control was 8.3%. Mean mortalities of 92.5% were observed in the reference item group.

The NOER was determined to be 1000.0 g a.s./ha, equivalent to 1180.6 g 2,4-D sodium salt/ha (equivalent to 1180.6 g/ha 2,4-D sodium salt in an application volume 200 L/ha). The LR50 could not be calculated, as none of the test item treatments had a significant effect on mortality.

**Materials and Methods**

**Materials**

|  |  |
| --- | --- |
| **Test Material** | 2,4-D 95 SP |
| **Lot/Batch #:** | 2111140274 |
| **Purity:** | 95.0 ± 1.5% |
| **Description:** | White/beige powder |
| **Stability of test compound:** | Not reported |
| **Reanalysis/Expiry date:** | November 2023 |
| **Density:** | Not reported |
| **Treatments** |  |
| **Test rates:** | Nominal: 3.906, 15.625, 62.5, 250.0 and 1000.0 g a.s./ha, in an application volume of 200 L/ha.  Equivalent to: 4.61, 18.45, 73.79, 295.16 and 1180.64 g/ha 2,4-D sodium salt |
| **Solvent/vehicle:** | None |
| **Analysis of test concentrations:** | None |
| **Test organisms** |  |
| **Species:** | *Aphidius rhopalosiphi* adults between 0 and 48 hours old |
| **Source:** | Obtained as mummies from Bias Labs Ltd, Kirkcaldy, Scotland |
| **Feeding:** | Cotton wool pad soaked in a 1:3 w/w honey water solution available |
| **Treatment for disease:** | None reported |
| **Test design** |  |
| **Test vessel:** | Glass plates (approx. 9 x 9 cm) fitted to stainless steel frame. |
| **Replication:** | Four replicates per test concentration and control |
| **No. animals/vessel:** | 10 wasps |
| **Duration of test:** | 48 hours |
| **Environmental test conditions** |  |
| **Temperature:** | 19.7 – 21.2°C |
| **Relative humidity** | 59.8 – 71.3% |
| **Photoperiod:** | 16 h light :8 h dark at 1491 - 2280 lux |

**Study Design**

This study was conducted in order to assess the influence of 2,4-D 95 SP on mortality of the parasitoid wasp *Aphidius rhopalosiphi* in an acute glass-plate test over 48 hours.

Test organisms were aged between 0 and 48 hours at test start. Ten wasps were exposed in each of four replicates to rates of 3.906, 15.625, 62.5, 250.0 and 1000.0 g a.s./ha, in an application volume of 200 L/ha, equivalent to: 4.61, 18.45, 73.79, 295.16 and 1180.64 g/ha 2,4-D sodium salt and a control. A toxic reference (dimethoate) was applied at a nominal application rate of 0.025 g/ha, in an application volume of 200 L/ha was included to indicate the relative susceptibility of the test organisms and the test system.

All treatments were applied to test units using a calibrated track sprayer on day 0. The sprayer was calibrated to deliver 2 ± 0.2 mg spray solution per cm2, corresponding to an application volume of 200 L/ha by weighing the mass of water deposited onto the glass plates. All dilutions were prepared in deionised water on the day of application and stored at room temperature until required. After application, the glass plates were left to dry at room temperature (approximately 2 hours ± 1 hour) before the test units were assembled. No analytical determinations were carried out for this test.

The mortality of organisms was assessed at 2, 24 and 48 hours of the test. Observations of the wasps was defined as unaffected, affected, moribund, dead or not seen.

Wasps were fed using a cotton wool pad soaked in a 1:3 w/w honey water solution.

Statistical evaluations were performed using validated software R V 3.4.4.

**Results and Discussion**

Validity criteria according to the Mead-Briggs *et al*. (2000) were met. In the control, the following criteria were met:

* Mean adult mortality <13% at the end of the test (actual: 8.3%);
* Mean mortality in the reference item to be >50% after 48 hours exposure (actual: 92.5%).

Mean mortalities of 2.5, 0.0, 2.5, 5.0 and 0.0% were observed at test concentrations 3.906, 15.625, 62.5, 250.0 and 1000 g a.s./ha. Percent mortality in the control was 8.3%.

Mean mortalities of 92.5% were observed in the reference item group.

**Table 10.3.2.1/01-1: Mortality of adult wasps 48 hours post-exposure**

| Treatment | g a.s./ha | g 2,4-D sodium salt/ha | Mean mortality1 (%) |
| --- | --- | --- | --- |
| Control | 0 | 0 | 8.3 |
| 2,4-D 95 SP | 3.906 | 4.61 | 2.5 |
| 15.625 | 18.45 | 0.0 |
| 62.5 | 73.79 | 2.5 |
| 250.0 | 295.2 | 5.0 |
| 1000.0 | 1180.6 | 0.0 |
| Dimethoate | 0.025 | - | 92.5 |
| 1 mean of four replicates | | | |

**Conclusion**

This study was conducted in order to assess the influence of 2,4-D 95 SP on mortality of the parasitoid wasp *Aphidius rhopalosiphi* in an acute glass-plate test over 48 hours.

Mean mortalities of 2.5, 0.0, 2.5, 5.0 and 0.0% were observed at test concentrations 3.906, 15.625, 62.5, 250.0 and 1000 g a.s./ha. Percent mortality in the control was 8.3%.

The NOER was determined to be 1000.0 g a.s./ha, equivalent to 1180.6 g 2,4-D sodium salt/ha.

|  |
| --- |
| **Assessment and conclusion by applicant:**  This is a new study that has not been previously evaluated.  Validity criteria according to the Mead-Briggs *et al*. (2000) were met. In the control, the following criteria were met:   * Mean adult mortality <13% at the end of the test (actual: 8.3%); * Mean mortality in the reference item to be >50% after 48 hours exposure (actual: 92.5%).   The study is therefore considered acceptable.  At the highest application rate, 1000 g a.s./ha, 0.0% mortality was observed. Percent mortality in the control was 8.3%.  The NOER was determined to be 1000.0 g a.s./ha, equivalent to 1180.6 g 2,4-D sodium salt/ha. |

|  |  |
| --- | --- |
| Comments of zRMS: | The study follows the guideline specified by Blümel *et al.* (2000) and according to the principles of GLP.  The study is considered to be valid and suitable for the risk assessment. |
| Reference: | KCP 10.3.2.1/02 |
| Report: | Acute Dose-Response Toxicity of 2,4-D 95 SP to the Predacious Mite *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae).  Milner, S. (2022B), Report no.: FR/002603-06, Document no.: 000109116 |
| Guideline(s): | Blümel *et al*. (2000) |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |

**Executive Summary**

The predacious mite, *Typhlodromus pyri*, was exposed to 2,4-D 95 SP for 48 hours in a glass-plate exposure test.

Test organisms were exposed to rates of 62.5, 125, 250, 500 and 1000 g a.s./ha, in an application volume of 200 L/ha, equivalent to: 73.8, 147.6, 295.2, 590.3 and 1180.6 g/ha 2,4-D sodium salt, a control group was also tested.

Mean mortalities of 10.0, 11.7, 6.7, 13.3 and 10.0% were observed at test concentrations 32.5, 125, 250, 500 and 1000 g a.s./ha. Percent mortality in the control was 6.7%. Mean mortalities of 58.3% were observed in the reference item group.

The NOER was determined to be 1000.0 g a.s./ha, equivalent to 1180.6 g 2,4-D sodium salt/ha (equivalent to 1180.6 g/ha 2,4-D sodium in an application volume 200 L). The LR50 could not be calculated, as none of the test item treatments had a significant effect on mortality.

**Materials and Methods**

**Materials**

|  |  |
| --- | --- |
| **Test Material** | 2,4-D 95 SP |
| **Lot/Batch #:** | 2111140274 |
| **Purity:** | 95. 0± 1.5% |
| **Description:** | White/beige powder |
| **Stability of test compound:** | Not reported |
| **Reanalysis/Expiry date:** | November 2023 |
| **Density:** | Not reported |
| **Treatments** |  |
| **Test rates:** | Nominal: 62.5, 125, 250, 500 and 1000 g a.s./ha, in an application volume of 200 L/ha.  Equivalent to: 73.8, 147.6, 295.2, 590.3 and 1180.6 g/ha 2,4-D sodium salt |
| **Solvent/vehicle:** | None |
| **Analysis of test concentrations:** | None |
| **Test organisms** |  |
| **Species:** | *Typhlodromus pyri* protonymphs between 0 and 24 hours old |
| **Source:** | Obtained as eggs from Bias Labs Ltd, Kirkcaldy, Scotland |
| **Feeding:** | Fresh broad bean pollen at test start and then every 2 – 3 days |
| **Treatment for disease:** | None reported |
| **Test design** |  |
| **Test vessel:** | Two cover slides approx. 2 x 6 cm with an area of approx. 10 cm2 |
| **Replication:** | Three replicates per test concentration and control |
| **No. animals/vessel:** | 20 protonymphs |
| **Duration of test:** | 7 days |
| **Environmental test conditions** |  |
| **Temperature:** | 24.5 – 25.3°C |
| **Relative humidity** | 68.1 – 85.5% |
| **Photoperiod:** | 16 h light :8 h dark at 1870 - 1940 lux |

**Study Design**

This study was conducted in order to assess the influence of 2,4-D 95 SP on mortality of the predacious mite *Typhlodromus pyri* in an acute glass-plate test over 7 days.

Test organisms were aged between 0 and 24 hours at test start. Twenty mites were exposed in each of three replicates to rates of 62.5, 125, 250, 500 and 1000 g a.s./ha, in an application volume of 200 L/ha, equivalent to: 73.8, 147.6, 295.2, 590.3 and 1180.6 g/ha 2,4-D sodium salt and a control. A toxic reference (dimethoate) was applied at a nominal application rate of 5.0 g/ha, in an application volume of 200 L/ha was included to indicate the relative susceptibility of the test organisms and the test system.

All treatments were applied to test units using a calibrated track sprayer on day 0. The sprayer was calibrated to deliver 2 ± 0.2 mg spray solution per cm2, corresponding to an application volume of 200 L/ha by weighing the mass of water deposited onto the glass plates. All dilutions were prepared in deionised water on the day of application and stored at room temperature until required. After application, the glass plates were left to dry at room temperature (approximately 2 hours ± 1 hour) before the test units were assembled. No analytical determinations were carried out for this test.

The mortality of organisms was assessed at day 3 and day 7. The number of dead and missing mites was recorded (same time of day as setup ± 2 hour).

2, 24 and 48 hours of the test. Observations of the wasps was defined as unaffected, affected, moribund, dead or not seen.

Mites were fed with broad bean pollen at test start, and again every two to three days.

Statistical evaluations were performed using validated software R V 3.4.4.

**Results and Discussion**

Validity criteria according to the Blümel *et al*. (2000) were met. In the control, the following criteria were met:

* Mean adult mortality <20% at the end of the test (actual: 6.7%);
* Mean mortality in the reference item to be >50% after 7 days exposure (actual: 58.3%).

Mean mortalities of 10.0, 11.7, 6.7, 13.3 and 10.0% were observed at test concentrations 32.5, 125, 250, 500 and 1000 g a.s./ha. Percent mortality in the control was 6.7%.

Mean mortalities of 58.3% were observed in the reference item group.

**Table 10.3.2.1/02-1: Mortality of adult wasps 48 hours post-exposure**

| Treatment | g a.s./ha | g 2,4-D sodium salt/ha | Mean mortality1 (%) |
| --- | --- | --- | --- |
| Control | 0 | 0 | 6.7 |
| 2,4-D 95 SP | 62.5 | 73.8 | 10.0 |
| 125 | 147.6 | 11.7 |
| 250 | 295.2 | 6.7 |
| 500 | 590.3 | 13.3 |
| 1000 | 1180.6 | 10.0 |
| Dimethoate | 5 | - | 58.3 |
| 1 mean of three replicates | | | |

**Conclusion**

This study was conducted in order to assess the influence of 2,4-D 95 SP on mortality of the predacious mite *Typhlodromus pyri* in an acute glass-plate test over 7 days.

Mean mortalities of 10.0, 11.7, 6.7, 13.3 and 10.0% were observed at test concentrations 32.5, 125, 250, 500 and 1000 g a.s./ha. Percent mortality in the control was 6.7%.

The NOER was determined to be 1000.0 g a.s./ha, equivalent to 1180.6 g 2,4-D sodium salt/ha.

|  |
| --- |
| **Assessment and conclusion by applicant:**  This is a new study that has not been previously evaluated.  Validity criteria according to the Blümel *et al*. (2000) were met. In the control, the following criteria were met:   * Mean adult mortality <20% at the end of the test (actual: 6.7%); * Mean mortality in the reference item to be >50% after 7 days exposure (actual: 58.3%).   The study is therefore considered acceptable.  Mean mortalities of 10.0, 11.7, 6.7, 13.3 and 10.0% were observed at test concentrations 32.5, 125, 250, 500 and 1000 g a.s./ha. Percent mortality in the control was 6.7%.  The NOER was determined to be 1000.0 g a.s./ha, equivalent to 1180.6 g 2,4-D sodium salt/ha. |

* 1. KCP 10.4 Effects on non-target soil meso- and macrofauna
     1. KCP 10.4.1 Earthworms
        1. KCP 10.4.1.1 Earthworms - sub-lethal effects

|  |  |
| --- | --- |
| Comments of zRMS: | Study with the metabolite 4-Chlorophenol was submitted as part of a precedent Central Zone dRR ‘Tricera’ with PL as the zRMS finalised in May 2022 (formulation code ADM.3304.H.1.A).  The study is considered acceptable with the following endpoints relevant for the risk assessment:  56 d NOEC (reproduction, mortality, biomass) ≥ 10 mg pm/kg dw soil  As no effects >10% were observed, the ECx value could not be determined. |
| Reference: | KCP 10.4.1.1/01 |
| Report: | 4-chlorophenol: Sublethal Toxicity to the Earthworm, *Eisenia fetida* (Annelida, Lumbricidae) in Artificial Soil with 10 % Peat.  Wagenhoff, E. (2015). S15-00154 (report number) |
| Guideline(s): | OECD 222 |
| Deviations: | - |
| GLP: | Yes |
| Acceptability: |  |

**Executive Summary**

This study was conducted in order to assess the influence of 4-chlorophenol on the earthworm *Eisenia fetida* over 56 days.

No effects of 4-chlorophenol could be demonstrated on *E. fetida* for any of the investigated endpoints and test item concentrations including the highest tested concentration of 10 mg/kg soil dry weight in this study. Accordingly, the NOECs for mortality, body weight change and reproduction were all considered to be 10 mg/kg soil dry weight, the highest concentration tested. The EC10, EC20 and EC50 for reproduction were assumed to be greater than 10 mg/kg soil dry weight.

**Materials and Methods**

**Materials**

|  |  |  |
| --- | --- | --- |
| **Test Material:** | 4-chlorophenol | |
| **Description:** | Not specified | |
| **Lot/batch:** | TSN304318/MKBJ7452V | |
| **Concentration/Purity:** | 100 % | |
| **Stability of test compound:** | Re-certification date: 26 January 2017 | |
|  |  | |
| **Vehicle and/or control:** | Vehicle control: deionized water  Positive control: Twist WP 60 % w/w (active ingredient: carbendazim) | |
|  |  | |
| **Test animals (Species):** | *Eisenia fetida* | |
| **Age:** | They were at least two months old, but not older than one year with a clitellum (age of the worms did not differ by more than four weeks) | |
| **Mean weight:** | Body weight at test start: 366 to 600 mg | |
| **Source:** | Bred under standardised conditions at the test facility | |
| **Feeding:** | Feeding: weekly up to the 28-day assessment with dried and finely ground cow manure | |
| **Acclimation period:** | One day before exposure, the adult earthworms were selected and transferred from the rearing medium into moist, untreated artificial soil for acclimatisation. | |
| **Animals per test concentration:** | See below | |
| **Number of replicates:** | Four replicates/test substance treatment and eight replicates/control. 10 earthworms per replicate | |
| **Artificial soil components:** | Artificial soil, percentage distribution on dry weight basis):   * Sphagnum peat: 10% * Kaolin clay (kaolinite content > 30%): 20% * Fine quartz sand (> 50% particles of 50 – 200 µm): 69.5% * < 1% calcium carbonate – precipitated extra pure (the soil pH is adjusted to 6.0 ± 0.5 at the start of the test before the addition of the test item)   The dry components were blended and mixed thoroughly in an electric mixer. After mixing the mean maximum water holding capacity (WHCmax.) was determined to be 48.92 % and the pH value was 5.9. | |
| **Test unit:** | Immediately after mixing, the test substrate of each treatment group was split and 634.6 g (corresponding to 500 g dry substrate) were placed into the test units (BELAPLAST, 17 cm × 12.5 cm × 6 cm; 1000 cm3, filling height approximately 5 cm).  The individual weights of the earthworms were recorded after washing them shortly before use. Ten randomly selected earthworms were placed onto the soil surface of each test container in a manner which ensures that they are homogenously distributed throughout the treatment groups with regard to the mean body weight per replicate. The test containers were closed with a perforated lid to allow gaseous exchange between the medium and the atmosphere. | |
| **Untreated variant:** | The control substrate was left untreated | |
| **Reference standard:** | Twist WP 60 % w/w (active ingredient: carbendazim) | |
|  |  | |
| **Environmental conditions** | | |
| **Temperature:** | 20.0 – 23.9 °C (the temperature was outside the required range of 20 ± 2 °C for approx. 20 minutes only; this should have had no impact on the outcome and integrity of the study) | |
| **pH:** | pH at initiation: 5.8 to 5.9  pH at termination: 6.0 | |
| **Humidity (Moisture content of the soil):** | Water content at initiation: 28.3 – 28.9 %  Water content at termination: 28.9 – 30.6 % | |
| **Photoperiod:** | 16 hour light to 8 hour dark photoperiod | |
| **Light intensity:** | 600 – 700 lux | |
|  | |

**Study Design**

Adult earthworms were exposed to artificial soil mixed with the test substance at five concentrations or remaining untreated (control) for a period of four weeks. After this period, the adults were removed from the test vessels and their survival, behavioural effects and growth (body weight change) were determined. The cocoons and juvenile earthworms remained in the test vessels for additional four weeks. The reproduction rate was determined by counting the number of offspring hatched from the cocoons after this additional test period of four weeks.

The test substance was applied to the artificial soil via deionized water at test concentrations 0.625, 1.25, 2.50, 5.00, 10.0 mg test item/kg soil dry weight and a control.

Observations were recorded over 8 weeks (4 weeks adult mortality; 4 weeks juvenile development).

Parameters reported are mortality, body weight change and reproduction. Earthworm body weight was recorded individually at test initiation and per replicate after four weeks of exposure. Reproduction was evaluated as the number of juveniles per replicate and the mean number per treatment group after eight weeks.

The pH and water of the soil was measured for all treatment groups and the control at the start and at the end of the test.

**Results and Discussion**

All validity criteria according to OECD 222 were met in the control:

* Adult mortality required ≤: 10%. An adult mortality of 2.5 % was observed in the test.
* Mean number of juveniles per vessel required ≥ 30. A mean of 312.9 juveniles was obtained in the test.
* Coefficient of variation (CV) of reproduction required ≤ 30 %. The CV in the test was 20.9 %.

There were no pathological symptoms of the adult earthworms observed during the first four weeks of exposure to the test item. Food consumption of the adult earthworms was estimated to be similar in all treatment groups compared to the control group during the first four weeks of the study.

Exposure of *E. fetida* to 4-chlorophenol had no effect on mortality, body weight or reproduction. Therefore, the LOEC for all measurement endpoints was greater than 10 mg/kg soil dry weight, and the NOEC was 10 mg/kg soil dry weight, the highest concentration tested. Since there was no dose-response relationship, and as the effect on reproduction was below 10 % for each of the concentrations tested, the EC10, EC20 and EC50 for reproduction were assumed to be greater than 10 mg/kg soil dry weight, the highest concentration tested in this study.

Table 10.4.1.1/01-1: Effects of 4-chlorophenol on earthworm survival and biomass and reproduction

| **Test concentrations**  **(mg/kg soil dry weight)** | **% Mortality after 28 days** | **% Bodyweight change after 28 days** | **Mean no. of juveniles at day 56** | **% Reduction in number of juveniles compared to control 1)** |
| --- | --- | --- | --- | --- |
| Control | 2.5 | -8.6 | 312.9 | -- |
| 0.625 | 2.5 | -11.3 | 304.8 | 2.6 |
| 1.25 | 0.0 | -6.9 | 367.8 | -17.5 |
| 2.50 | 0.0 | -8.1 | 357.5 | -14.3 |
| 5.00 | 5.0 | -7.4 | 342.8 | -9.6 |
| 10.0 | 0.0 | -9.6 | 340.3 | -8.8 |

\* Statistically different from the control.

1) Negative values indicate higher reproduction compared to the control.

**Conclusion**

This study was conducted in order to assess the influence of 4-chlorophenol on the earthworm *Eisenia fetida* over 56 days.

No effects of 4-chlorophenol could be demonstrated on *E. fetida* for any of the investigated endpoints and test item concentrations including the highest tested concentration of 10 mg/kg soil dry weight in this study. Accordingly, the NOECs for mortality, body weight change and reproduction were all considered to be 10 mg/kg soil dry weight, the highest concentration tested. The EC10, EC20 and EC50 for reproduction were assumed to be greater than 10 mg/kg soil dry weight.

|  |
| --- |
| **Assessment and conclusion by applicant:**  This is a new study that has not been previously evaluated.  All validity criteria according to OECD 222 were met in the control:   * Adult mortality required ≤: 10%. An adult mortality of 2.5 % was observed in the test. * Mean number of juveniles per vessel required ≥ 30. A mean of 312.9 juveniles was obtained in the test. * Coefficient of variation (CV) of reproduction required ≤ 30 %. The CV in the test was 20.9 %.   The study is therefore considered acceptable.  No effects of 4-chlorophenol could be demonstrated on *E. fetida* for any of the investigated endpoints and test item concentrations including the highest tested concentration of 10 mg/kg soil dry weight in this study. Accordingly, the NOECs for mortality, body weight change and reproduction were all considered to be 10 mg/kg soil dry weight, the highest concentration tested. The EC10, EC20 and EC50 for reproduction were assumed to be greater than 10 mg/kg soil dry weight. |

* + - 1. KCP 10.4.1.2 Earthworms - field studies
    1. KCP 10.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)
       1. KCP 10.4.2.1 Species level testing
       2. KCP 10.4.2.2 Higher tier testing
  1. KCP 10.5 Effects on soil nitrogen transformation
  2. KCP 10.6 Effects on terrestrial non-target higher plants
     1. KCP 10.6.1 Summary of screening data
     2. KCP 10.6.2 Testing on non-target plants

|  |  |
| --- | --- |
| Comments of zRMS: | The effect of 2,4-D sodium salt on seedling emergence, survival, and early growth of non-target terrestrial plant species was investigated in turnip, lettuce, sugar beet, onion and ryegrass. A species *Daucus carota* (carrot) was also tested, however due to three failed tests, where control emergence validity criteria were not met, this species was replaced with *Brassica napus* (oilseed rape).  The study was performed in glasshouse cubicles, equipped with artificial lighting to supplement natural light (16 hours per day) and temperature control. The study was carried out in four trials.  Trial 1 was stopped due to biological issues and the test not meeting validity criteria. Germination was poor for all species with only two species reaching the required 70% control emergence (*Beta vulgaris* and *Brassica rapa*). In Trial 2, all validity criteria were met for all species, except for *Daucus carota,* which failed to meet the validity criterion for control as only 40% emergence observed. In Trial 3, *Daucus carota* was retested as before. This trail was not deemed valid as only 63% emergence observed. Thus, in Trial 4, it was decided to replace *Daucus carota* by *Brassica napus,* as carrot was deemed a non-viable test species.  The test item had a distinct visual effect on the treated plants. It appeared to delay emergence and cause a retardation of both growth and development, when compared to the controls. In mild cases, affected plants appeared slightly smaller than the controls. In moderate cases, seedling emergence appeared to be delayed, when compared to the controls with affected seedlings appearing smaller and less developed than the controls. In severe cases, an affected seedlings struggled to break through the soil surface. At harvest these seedlings had poorly developed roots systems, grew very slowly and by day 14 had developed yellowing and dead leaf tissue.  The most sensitive species in terms of dry weight was *Brassica rapa* (turnip) with NOER and ER50 values of 7.5 and 28.69 g a.s./ha, respectively.  The study is considered to be reliable and suitable for the risk assessment. |
| Reference: | KCP 10.6.2/01 |
| Report: | 2,4-D 95 SP: Terrestrial Plant Seedling Emergence Test  Jarratt, N. (2023a), Report no.: FR/002603-08, Document no.: 000109115 |
| Guideline(s): | OECD 208 (2006) |
| Deviations: | - |
| GLP: | Yes |
| Acceptability: | - |

**Executive Summary**

The effects of exposure to 2,4-D 95 SP on the seedling emergence, survival, and early growth of six non-target plant species was assessed over 14 days.

Test concentrations were 1.875, 3.75, 7.5, 15.0, 30.0, 60.0 and 120.0 g a.s./ha for *Brassica rapa* (turnip), *Lactuca sativa* (lettuce), *Beta vulgaris* (sugar beet), *Daucus carota* (carrot) and *Allium cepa* (onion), 3.75, 7.5, 15.0, 30.0, 60.0, 120.0 and 240.0 g a.s./ha for *Brassica napus* (oilseed rape) and 30.0, 60.0, 120.0, 240.0, 480.0, 960.0 and 1920.0 g a.s./ha for *Lolium perenne* (ryegrass).

After 14 days exposure the most sensitive parameter was shoot dry weight. The most sensitive species in terms of dry weight was *Brassica rapa* (turnip) with NOER and ER50 values of 7.5 and 28.69 g a.s./ha, respectively.

**Materials and Methods**

**Materials**

|  |  |
| --- | --- |
| **Test Material** | 2,4-D 95 SP |
| **Lot/Batch #:** | 2111140274 |
| **Purity:** | 95.1% |
| **Description:** | White/beige powder |
| **Stability of test compound:** | Not reported |
| **Reanalysis/Expiry date:** | 1st November 2023 |
| **Density:** | Not reported |
| **Treatments** |  |
| **Test rates:** | 1.875, 3.75, 7.5, 15.0, 30.0, 60.0 and 120.0 g a.s./ha for *Brassica rapa* (turnip), *Lactuca sativa* (lettuce), *Beta vulgaris* (sugar beet), *Daucus carota* (carrot) and *Allium cepa* (onion)  3.75, 7.5, 15.0, 30.0, 60.0, 120.0 and 240.0 g a.s./ha for *Brassica napus* (oilseed rape),  30.0, 60.0, 120.0, 240.0, 480.0, 960.0 and 1920.0 g a.s./ha for *Lolium perenne* (ryegrass). |
| **Solvent/vehicle:** | Deionised water |
| **Test organisms** |  |
| **Species:** | Four dicotyledonous species *Brassica rapa* (turnip), *Lactuca sativa* (lettuce), *Beta vulgaris* (sugar beet), *Brassica napus* (Oilseed rape), *Daucus carota* (carrot)  Two monocotyledonous *Allium cepa* (onion) and *Lolium perenne* (ryegrass) |
| **Source:** | E.W. King & Co. Ltd, CO5 9PG.  Cotswold Grass Seeds Direct, GL56 0JQ. Team PPPF (glasshouses), Fera Science Ltd. |
| **Test design** |  |
| **Test vessel:** | 11.0 x 8.6 cm plastic pots in a glasshouse |
| **Replication:** | 30 plants per species. Ten replicate pots of three plants for *Daucus carota, Brassica napus, Allium cepa* and *Lolium perenne*. Fifteen replicate pots of two plants for *Lactuca sativa, Brassica rapa* and *Beta vulgaris*. |
| **Duration of test:** | 14 days |
| **Environmental test conditions** |  |
| **Temperature:** | 17.0 – 26.5°C |
| **Relative humidity** | 16.4 – 81.6% |
| **Photoperiod:** | 16 hours light : 8 hours darkness at ≥200 µmol/m/s |

**Study Design**

The effects of exposure to 2,4-D 95 SP on the seedling emergence, survival, and early growth of six non-target plant species was assessed over 14 days.

Plants from four dicotyledonous (*Brassica rapa* (turnip), *Lactuca sativa* (lettuce), *Beta vulgaris* (sugar beet), *Brassica napus* (Oilseed rape), *Daucus carota* (carrot)) and two monocotyledonous (*Allium cepa* (onion), *Lolium perenne* (ryegrass)) species were tested. The study was split into four trials, of which trials one and three were stopped prior to completion due to issues with emergence. The results of trials two and four are therefore reported.

The test substance was applied to surface soil at nominal treatment rates of 1.875, 3.75, 7.5, 15.0, 30.0, 60.0 and 120.0 g a.s./ha for *Brassica rapa* (turnip), *Lactuca sativa* (lettuce), *Beta vulgaris* (sugar beet), *Daucus carota* (carrot) and *Allium cepa* (onion), 3.75, 7.5, 15.0, 30.0, 60.0, 120.0 and 240.0 g a.s./ha for *Brassica napus* (oilseed rape) and 30.0, 60.0, 120.0, 240.0, 480.0, 960.0 and 1920.0 g a.s./ha for *Lolium perenne* (ryegrass).

Plants were allowed to emerge and grow for 14 days following 50% emergence of the control plants under glasshouse conditions. Test units were supplied with water as required. The tests were performed in glasshouse cubicles equipped with artificial lighting (16 hours per day) and temperature control. Test units were placed randomly at the beginning of the test.

After 7 and 14 days following the emergence of at least 50% of the controls, seedlings were evaluated visually.

Final visual assessments and harvest to determine shoot length and shoot dry weight of the seedlings took place on day 14.

The concentration and homogeneity of 2,4-D in the final application vehicle (test solution) was determined by analysis of the test solutions applied to the test systems with the highest and lowest treatment rates. This was done for both trials. An assessment of stability was made based on expected and measured concentrations.

Light intensities in the glasshouse cubicles, were ≥200 μ mol m-2s-1. The photoperiod was 16 hours (±10 minutes) light and 8 hours (±10 minutes) darkness.

**Glasshouse Cubicle 34DG01 - Onion**

Air temperature in 34DG01 were within target range of 22 ± 10 °C (Mean: 20.0°C, Max: 23.6°C,

Min: 15.8 °C). Relative humidity was also recorded in 34DG01 (Mean: 67.0%, Max: 91.5%, Min:

44.2%). Light intensities ranged between 280 - 491 μ mol m-2s-1.

**Glasshouse Cubicle 34DG02 - Lettuce & Turnip**

Air temperature in 34DG02 were within target range of 22 ± 10 °C (Mean: 22.6°C, Max: 25.6°C,

Min: 19.2°C). Relative humidity was also recorded in 34DG02 (Mean: 62.4%, Max: 86.0%, Min:

43.8%). Light intensities ranged between 202 - 525 μ mol m-2s-1.

**Glasshouse Cubicle 34DG07 – Oilseed rape**

Air temperature in 34DG08 were within target range of 22 ± 10 °C (Max: 29.9°C, Min: 17.0°C).

Relative humidity was also recorded in 34DG08 (Max: 81.6%, Min:16.4 %). Light intensities ranged between 309 - 661 μ mol m-2s-1.

**Glasshouse Cubicle 34DG08 – Rye grass and Sugar beet**

Air temperature in 34DG08 were within target range of 22 ± 10 °C (Mean: 20.3°C, Max: 26.5°C,

Min: 15.5°C). Relative humidity was also recorded in 34DG08 (Mean: 66.9%, Max: 96.3%, Min:

31.1%). Light intensities ranged between 221 - 655 μ mol m-2s-1.

**Results and Discussion**

Validity criteria according to OECD 208 (2006) were met:

*Trial 2*

* Control seedling emergence from to be ≥70% (actual: 90 to 100%).
* The mean survival of seedlings to be ≥90% for the duration of the experiment (actual: 93 to 100%).

*Trial 4*

* Control seedling emergence from to be ≥70% (actual: 93.3%).
* The mean survival of seedlings to be ≥90% for the duration of the experiment (actual: 96.4%).

The control seedlings did not exhibit visible phytotoxic effects, apart from normal variation in growth or morphology associated with a species.

Environmental conditions for a species were identical and the growing media contained the same amount of soil matrix, support media or substrate from the same source.

The mean analytically determined concentrations of 2,4-D in the highest and lowest treatment solutions from trial 2 were 102.1 % and 90.9 % of nominal with a procedural recovery of 102.8%.

The mean analytically determined concentrations of 2,4-D in the highest and lowest treatment solutions from trial 4 were 94.6 % and 99.0 % of nominal with a procedural recovery of 101.4%.

After 14-days exposure, the following effects were observed:

**Table 10.6.2/01-1**: **Summary of *Allium cepa* (Onion) emergence and survival after 14 days exposure**

|  |  |  |  |
| --- | --- | --- | --- |
| Nominal treatment rate (g a.s./ha) | Total emergence | Total emergence  (% of seeds sown) | Survival (% of emerged seedlings) |
| Control | 28 | 93.33 | 92.86 |
| 1.875 | 25 | 83.33 | 100.0 |
| 3.75 | 28 | 93.33 | 100.0 |
| 7.5 | 21 | 70.0\* | 95.24 |
| 15.0 | 24 | 80.0\* | 91.67 |
| 30.0 | 23 | 76.67\* | 91.30 |
| 60.0 | 24 | 80.0\* | 91.67 |
| 120.0 | 18 | 60.0\* | 66.67\* |

\* Statistically significant effect (p<0.05)

**Table 10.6.2/01-2**: **Summary of *Allium cepa* (Onion) mean shoot fresh weights, % survival and visual injury scores after 14 days exposure**

|  |  |  |  |
| --- | --- | --- | --- |
| Nominal treatment rate (g a.s./ha) | Mean shoot length (mm) (± SD) | Mean seedling dry weight (g) (± SD) | Mean visual injury score (± SD) |
| Control | 107.5 ± 48.7 | 0.0056 ± 0.0023 | 20.7 ± 36.4 |
| 1.875 | 121.6 ± 45.0 | 0.0073 ± 0.0038 | 24.0 ± 38.6 |
| 3.75 | 110.3 ± 44.2 | 0.0059 ± 0.0015 | 17.7 ± 30.5 |
| 7.5 | 106.1 ± 48.7 | 0.0071 ± 0.0042 | 45.2 ± 43.3 |
| 15.0 | 111.7 ± 47.2 | 0.0061 ± 0.0026 | 44.3 ± 37.0 |
| 30.0 | 108.9 ± 54.7 | 0.0060 ± 0.0035 | 49.0 ± 39.9 |
| 60.0 | 97.0 ± 55.1 | 0.0058 ± 0.0034 | 52.3 ± 34.3 |
| 120.0 | 61.2 ± 31.6\* | 0.0025 ± 0.0005\* | 82.5 ± 23.4 |

\* Statistically significant effect (p<0.05)

Visual injury scale: 0 = no effect, 1-39.9 = slight effect, 40-69.9 moderate effect and 70-99.9 = severe effect with 100 = all plants dead

**Table 10.6.2/01-3: Summary of *Lolium perenne* (Ryegrass) emergence and survival after 14 days exposure**

|  |  |  |  |
| --- | --- | --- | --- |
| Nominal treatment rate (g a.s./ha) | Total emergence | Total emergence  (% of seeds sown) | Survival (% of emerged seedlings) |
| Control | 28 | 93.33 | 96.43 |
| 30.0 | 26 | 86.7 | 100.0 |
| 60.0 | 25 | 83.33 | 96.0 |
| 120.0 | 26 | 86.67 | 96.15 |
| 240.0 | 20 | 66.67\* | 100.0 |
| 480.0 | 23 | 76.67\* | 95.65 |
| 960.0 | 21 | 70.0\* | 90.48 |
| 1920.0 | 21 | 70.0\* | 95.24 |

\* Statistically significant effect (p<0.05)

**Table 10.6.2/01-4**: **Summary of *Lolium perenne* (Ryegrass) mean shoot fresh weights, % survival and visual injury scores after 14 days exposure**

|  |  |  |  |
| --- | --- | --- | --- |
| Nominal treatment rate (g a.s./ha) | Mean shoot length (mm) (± SD) | Mean seedling dry weight (g) (± SD) | Mean visual injury score (± SD) |
| Control | 149.0 ± 51.3 | 0.0091 ± 0.0042 | 17.5 ± 35.7 |
| 30.0 | 134.8 ± 39.9 | 0.0087 ± 0.0037 | 18.7 ± 36.6 |
| 60.0 | 132.1 ± 47.3 | 0.0087 ± 0.0034 | 29.5 ± 42.5 |
| 120.0 | 134.5 ± 49.4 | 0.0084 ± 0.0026\* | 25.2 ± 40.6 |
| 240.0 | 132.3 ± 45.6 | 0.0063 ± 0.0034\* | 46.3 ± 43.0 |
| 480.0 | 131.5 ± 56.8 | 0.0060 ± 0.0037\* | 50.5 ± 37.2 |
| 960.0 | 90.9 ± 45.2\* | 0.0026 ± 0.0011\* | 68.5 ± 27.7 |
| 1920.0 | 81.8 ± 49.7\* | 0.0020 ± 0.0013\* | 72.5 ± 25.6 |

\* Statistically significant effect (p<0.05)

Visual injury scale: 0 = no effect, 1-39.9 = slight effect, 40-69.9 moderate effect and 70-99.9 = severe effect with 100 = all plants dead

**Table 10.6.2/01-5**: **Summary of *Lactuca sativa* (Lettuce) emergence and survival after 14 days exposure**

|  |  |  |  |
| --- | --- | --- | --- |
| Nominal treatment rate (g a.s./ha) | Total emergence | Total emergence  (% of seeds sown) | Survival (% of emerged seedlings) |
| Control | 27 | 90.0 | 100.0 |
| 1.875 | 25 | 83.33 | 100.0 |
| 3.75 | 26 | 86.67 | 96.15 |
| 7.5 | 24 | 80.0 | 100.0 |
| 15.0 | 21 | 70.0\* | 90.48\* |
| 30.0 | 19 | 63.33\* | 94.74\* |
| 60.0 | 18 | 60.0\* | 88.89\* |
| 120.0 | 16 | 53.33\* | 75.0\* |
| Toxic reference | 26 | 86.67\* | 0.0 |

\* Statistically significant effect (p<0.05)

**Table 10.6.2/01-6**: **Summary of *Lactuca sativa* (Lettuce) mean shoot fresh weights, % survival and visual injury scores after 14 days exposure**

|  |  |  |  |
| --- | --- | --- | --- |
| Nominal treatment rate (g a.s./ha) | Mean shoot length (mm) (± SD) | Mean seedling dry weight (g) (± SD) | Mean visual injury score (± SD) |
| Control | 61.8 ± 18.5 | 0.0625 ± 0.0366 | 18.0 ± 32.7 |
| 1.875 | 69.8 ± 13.0 | 0.0784 ± 0.0274 | 19.2 ± 38.4 |
| 3.75 | 73.2 ± 10.7 | 0.0812 ± 0.0366 | 22.7 ± 36.9 |
| 7.5 | 68.7 ± 10.9 | 0.0907 ± 0.0505 | 25.7 ± 38.5 |
| 15.0 | 66.4 ± 8.8 | 0.0609 ± 0.0344 | 51.0 ± 39.9 |
| 30.0 | 52.3 ± 17.1\* | 0.0493 ± 0.0233 | 62.5 ± 34.8 |
| 60.0 | 39.8 ± 15.0\* | 0.0191 ± 0.0155\* | 77.3 ± 23.6 |
| 120.0 | 24.9 ± 9.5\* | 0.0077 ± 0.0078\* | 86.8 ± 17.5 |
| Toxic reference | - | - | 100.0 |

\* Statistically significant effect (p<0.05)

Visual injury scale: 0 = no effect, 1-39.9 = slight effect, 40-69.9 moderate effect and 70-99.9 = severe effect with 100 = all plants dead

- No seedlings at harvest

**Table 10.6.2/01-7**: **Summary of *Brassica rapa* (Turnip) emergence and survival after 14 days exposure**

|  |  |  |  |
| --- | --- | --- | --- |
| Nominal treatment rate (g a.s./ha) | Total emergence | Total emergence  (% of seeds sown) | Survival (% of emerged seedlings) |
| Control | 29 | 96.67 | 100.0 |
| 1.875 | 29 | 96.67 | 100.0 |
| 3.75 | 29 | 96.67 | 100.0 |
| 7.5 | 28 | 93.33 | 100.0 |
| 15.0 | 30 | 100.0 | 90.0\* |
| 30.0 | 29 | 96.67 | 93.10\* |
| 60.0 | 28 | 93.33 | 92.86\* |
| 120.0 | 26 | 86.67 | 88.46\* |

\* Statistically significant effect (p<0.05)

**Table 10.6.2/01-8**: **Summary of *Brassica rapa* (Turnip) mean shoot fresh weights, % survival and visual injury scores after 14 days exposure**

|  |  |  |  |
| --- | --- | --- | --- |
| Nominal treatment rate (g a.s./ha) | Mean shoot length (mm) (± SD) | Mean seedling dry weight (g) (± SD) | Mean visual injury score (± SD) |
| Control | 141.2 ± 24.8 | 0.2342 ± 0.1084 | 6.0 ± 23.0 |
| 1.875 | 138.7 ± 14.2 | 0.1974 ± 0.0826 | 3.3 ± 18.3 |
| 3.75 | 142.0 ± 16.3 | 0.2374 ± 0.1246 | 3.3 ± 18.3 |
| 7.5 | 126.6 ± 21.9 | 0.1761 ± 0.0763 | 12.0 ± 26.2 |
| 15.0 | 130.7 ± 22.2 | 0.1121 ± 0.0700\* | 25.7 ± 28.2 |
| 30.0 | 128.4 ± 21.3 | 0.1530 ± 0.0752\* | 31.0 ± 25.6 |
| 60.0 | 100.0 ± 30.6\* | 0.0598 ± 0.0319\* | 54.3 ± 24.2 |
| 120.0 | 85.0 ± 40.5\* | 0.0581 ± 0.0673\* | 65.7 ± 27.7 |

\* Statistically significant effect (p<0.05)

Visual injury scale: 0 = no effect, 1-39.9 = slight effect, 40-69.9 moderate effect and 70-99.9 = severe effect with 100 = all plants dead

**Table 10.6.2/01-9**: **Summary of *Beta vulgaris* (Sugar beet) emergence and survival after 14 days exposure**

|  |  |  |  |
| --- | --- | --- | --- |
| Nominal treatment rate (g a.s./ha) | Total emergence | Total emergence  (% of seeds sown) | Survival (% of emerged seedlings) |
| Control | 30 | 100.0 | 93.33 |
| 1.875 | 28 | 93.33 | 92.86 |
| 3.75 | 27 | 90.0 | 96.30 |
| 7.5 | 27 | 90.0 | 96.30 |
| 15.0 | 28 | 93.33 | 92.86 |
| 30.0 | 26 | 86.67 | 88.46 |
| 60.0 | 30 | 100.0 | 90.0 |
| 120.0 | 27 | 90.0 | 100.0 |

**Table 10.6.2/01-10**: **Summary of *Beta vulgaris* (Sugar beet) mean shoot fresh weights, % survival and visual injury scores after 14 days exposure**

|  |  |  |  |
| --- | --- | --- | --- |
| Nominal treatment rate (g a.s./ha) | Mean shoot length (mm) (± SD) | Mean seedling dry weight (g) (± SD) | Mean visual injury score (± SD) |
| Control | 103.6 ± 12.0 | 0.1598 ± 0.0466 | 6.7 ± 25.4 |
| 1.875 | 96.6 ± 25.8 | 0.1404 ± 0.0574 | 15.0 ± 34.7 |
| 3.75 | 91.3 ± 23.8 | 0.1463 ± 0.0340 | 13.8 ± 34.4 |
| 7.5 | 94.2 ± 17.4 | 0.1602 ± 0.0726 | 15.8 ± 34.4 |
| 15.0 | 106.1 ± 17.6 | 0.1683 ± 0.0659 | 18.0 ± 35.9 |
| 30.0 | 94.7 ± 15.5 | 0.1450 ± 0.0402 | 31.3 ± 40.3 |
| 60.0 | 79.1 ± 28.8\* | 0.1483 ± 0.1954\* | 44.5 ± 32.7 |
| 120.0 | 69.1 ± 26.1\* | 0.0629 ± 0.0520\* | 52.2 ± 30.5 |

\* Statistically significant effect (p<0.05)

Visual injury scale: 0 = no effect, 1-39.9 = slight effect, 40-69.9 moderate effect and 70-99.9 = severe effect with 100 = all plants dead

**Table 10.6.2/01-11**: **Summary of *Brassica napus* (Oilseed rape) emergence and survival after 14 days exposure**

|  |  |  |  |
| --- | --- | --- | --- |
| Nominal treatment rate (g a.s./ha) | Total emergence | Total emergence  (% of seeds sown) | Survival (% of emerged seedlings) |
| Control | 28 | 93.33 | 96.43 |
| 3.75 | 28 | 93.33 | 100.0 |
| 7.5 | 29 | 96.67 | 100.0 |
| 15.0 | 28 | 93.33 | 100.0 |
| 30.0 | 27 | 90.0 | 100.0 |
| 60.0 | 25 | 83.33 | 92.0 |
| 120.0 | 24 | 80.0\* | 100.0 |
| 240.0 | 21 | 70.0\* | 95.24 |
| Toxic reference | 30 | 100.0 | 0.0\* |

\* Statistically significant effect (p<0.05)

**Table 10.6.2/01-12**: **Summary of *Brassica napus* (Oilseed rape) mean shoot fresh weights, % survival and visual injury scores after 14 days exposure**

|  |  |  |  |
| --- | --- | --- | --- |
| Nominal treatment rate (g a.s./ha) | Mean shoot length (mm) (± SD) | Mean seedling dry weight (g) (± SD) | Mean visual injury score (± SD) |
| Control | 124.3 ± 20.0 | 0.2497 ± 0.0898 | 16.3 ± 32.3 |
| 3.75 | 127.2 ± 10.8 | 0.2349 ± 0.0381 | 7.3 ± 25.3 |
| 7.5 | 121.0 ± 16.9 | 0.2216 ± 0.0622 | 14.0 ± 23.4 |
| 15.0 | 126.8 ± 13.3 | 0.2299 ± 0.0588 | 15.7 ± 25.6 |
| 30.0 | 105.0 ± 43.9\* | 0.1710 ± 0.0812\* | 29.0 ± 38.4 |
| 60.0 | 85.8 ± 48.2\* | 0.1109 ± 0.0816\* | 57.0 ± 36.2 |
| 120.0 | 415 ± 31.9\* | 0.0232 ± 0.0162\* | 85.7 ± 11.9 |
| 240.0 | 30.5 ± 27.8\* | 0.0174 ± 0.0182\* | 90.7 ± 8.7 |
| Toxic reference | - | - | 100.0 |

\* Statistically significant effect (p<0.05)

Visual injury scale: 0 = no effect, 1-39.9 = slight effect, 40-69.9 moderate effect and 70-99.9 = severe effect with 100 = all plants dead

Test item related effects were evident in the species tested, to varying degrees. The test item appeared to cause a retardation of both growth and development, when compared to the controls.

The severity of the growth and development effects appeared to increase with concentration, however certain species were more sensitive to the effects than others.

The test item had a significant effect (p<0.05) on shoot length development of *Allium cepa*, *Lolium perenne, Lactuca sativa, Brassica rapa, Beta vulgaris* and *Brassica napus.* NOER values for thesespecies were 60.0, 480.0, 15.0, 30.0, 30.0 and 15.0 g a.s./ha in 400 L/ha, respectively.

The ER10 values for shoot length ranged between 18.62 – 479.27 g a.s./ha in 400 L/ha

The ER20 values for shoot length ranged between 30.41 – 788.27 g a.s./ha in 400 L/ha

*Lactuca sativa* and *Brassica napus* were the only species that reliable ER50 value were calculated.

The ER50 values were 77.74 and 84.86 g a.s./ha in 400 L/ha, respectively.

The test item had a significant effect (p<0.05) on shoot dry weight of *Allium cepa*, *Lolium perenne, Lactuca sativa, Brassica rapa****,*** *Beta vulgaris* and *Brassica napus.* NOER values for these specieswere 60.0, 120.0, 30.0, 7.5, 30.0 and 15.0 g a.s./ha in 400 L/ha, respectively.

The ER10 values for shoot dry weight ranged between 2.73 – 103.71 g a.s./ha in 400 L/ha

The ER20 values for shoot dry weight ranged between 6.12 – 191.28 g a.s./ha in 400 L/ha

The ER50 values for shoot dry weight ranged between 28.69 – 616.95 g a.s./ha in 400 L/ha

It was not possible to calculate ERx values for *Allium cepa.*

The most sensitive endpoint was shoot dry weight. Related NOEC and ECx values for this parameter are presented below.

Table 10.6.2/01-13*:* Summary of effects on shoot dry weight after 14-day exposure to 2.4-D 95 SP (g a.s./ha in 400 L/ha).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Species | Endpoint (g a.s./ha) with 95% confidence intervals | | | |
| NOER | ER10 | ER20 | ER50 |
| *Allium cepa* (onion) | 60 | >120.0 a | >120.0 a | >120.0 a |
| *Lolium perenne* (ryegrass) | 120 | 103.71 (28.21 – 381.24) | 191.28 (71.90 – 508.85) | 616.95 (359.40 – 1059.06) |
| *Beta vulgaris* (sugar beet) | 30 | 30.79 (17.23 – 55.03) | 44.81 (29.17 – 68.84) | 91.89 (66.79 – 126.42) |
| *Lactuca sativa* (lettuce) | 30 | 13.78 (7.14 – 26.60) | 19.37 (11.63 – 32.27) | 37.18 (25.50 – 54.22) |
| *Brassica rapa* (turnip) | 7.5 | 2.73 (0.61 – 12.29) | 6.12 (1.94 – 19.28) | 28.69 (15.24 – 54.04) |
| *Brassica napus* (oilseed rape) | 15.0 | 18.31 (9.92 – 33.83) | 26.14 (16.39 – 41.70) | 51.64 (39.55 – 67.43) |

Values in parentheses indicate 95% confidence intervals

**Conclusion**

The effects of exposure to 2,4-D 95 SP on the seedling emergence, survival, and early growth of six non-target plant species was assessed over 14 days.

Test concentrations were 1.875, 3.75, 7.5, 15.0, 30.0, 60.0 and 120.0 g a.s./ha for *Brassica rapa* (turnip), *Lactuca sativa* (lettuce), *Beta vulgaris* (sugar beet), *Daucus carota* (carrot) and *Allium cepa* (onion), 3.75, 7.5, 15.0, 30.0, 60.0, 120.0 and 240.0 g a.s./ha for *Brassica napus* (oilseed rape) and 30.0, 60.0, 120.0, 240.0, 480.0, 960.0 and 1920.0 g a.s./ha for *Lolium perenne* (ryegrass).

After 14 days exposure the most sensitive parameter was shoot dry weight. The most sensitive species in terms of dry weight was *Brassica rapa* (turnip) with NOER and ER50 values of 7.5 and 28.69 g a.s./ha, respectively.

|  |
| --- |
| **Assessment and conclusion by applicant:**  This is a new study that has not been previously evaluated.  Validity criteria according to OECD 208 (2006) were met:  *Trial 2*   * Control seedling emergence from to be ≥70% (actual: 90 to 100%). * The mean survival of seedlings to be ≥90% for the duration of the experiment (actual: 93 to 100%).   *Trial 4*   * Control seedling emergence from to be ≥70% (actual: 93.3%). * The mean survival of seedlings to be ≥90% for the duration of the experiment (actual: 96.4%).   The control seedlings did not exhibit visible phytotoxic effects, apart from normal variation in growth or morphology associated with a species.  Environmental conditions for a species were identical and the growing media contained the same amount of soil matrix, support media or substrate from the same source.  The temperature and humidity of test units could not be maintained to guideline recommendation due to the glasshouse test design, however as all validity criteria were met and control plant performance was acceptable this is not considered to have an adverse effect on the study.  The study is therefore considered acceptable.  After 14 days exposure the most sensitive parameter was shoot dry weight. The most sensitive species in terms of dry weight was *Brassica rapa* (turnip) with NOER and ER50 values of 7.5 and 28.69 g a.s./ha, respectively. |

|  |  |
| --- | --- |
| Comments of zRMS: | The effect of 2,4-D sodium salt on n vigour and growth of non-target terrestrial plant species was investigated in carrot, lettuce, turnip, sugar beet, cucumber, soya bean, broad bean, tomato, onion and ryegrass.  The study was performed in glasshouse cubicles, equipped with artificial lighting to supplement natural light (16 hours per day) and temperature control.  The test item had a distinct visual effect on the treated plants causing abnormal growth and development. This included twisted stems, blister like growths on stems of affected plants and curled, abnormal shaped leaves. The test item also caused retardation of both growth and development, when compared to the controls.  In mild cases, affected plants appeared slightly smaller than the controls, had slight leaf deformities, which curled at the edges and some leaf discoloration. In moderate cases, affected plants appeared noticeably smaller than the controls, had abnormal shaped leaves, which curled at the edges, with some wilting tissue death. There was also some blister like structures on some of the affected plants. In severe cases, affected plants were noticeably smaller and less developed than the controls. Plants were deformed with twisted stems, which spiralled rather than growing upwards. Stems had blister like structures and showed signs of structural damage include splitting at points where the stems had twisted. This led to the stems of plants affected rotting in some cases. The leaves of affected plants were deformed (small curled, abnormal shape) and either wilted or dead.  Shoot fresh weight was the most sensitive endpoint tested. The most sensitive species in terms of fresh weight was *Brassica rapa* (turnip) with NOER and ER50 values of 6.25 and 29.4 g a.s./ha, respectively.  The study is considered to be reliable and suitable for the risk assessment. |
| Reference: | KCP 10.6.2/02 |
| Report: | 2,4-D 95 SP: Terrestrial Plant Test: Vegetative Test  Jarratt, N. (2023b), Report no.: FR/002603-09, Document no.: 000109114 |
| Guideline(s): | OECD 208 (2006) |
| Deviations: | Minor deviations to recommended temperature and humidity due to glasshouse test design. Not considered to have negatively affected the study. |
| GLP: | Yes |
| Acceptability: | - |

**Executive Summary**

The effects of exposure to 2,4-D 95 SP on the vegetative vigour, survival, height and fresh weight of ten non-target plant species was assessed over 21 days.

Test concentrations were 6.25, 12.5, 25.0, 50.0, 100.0, 200, 400,0, 800.0, 1600 and 3200.0 g a.s./ha to *Glycine max, Cucumis sativus, Allium cepa* and *Lolium perenne* and 3.13, 6.25, 12.5, 25.0, 50.0, 100.0, 200, 400,0, 800.0 and 1600.0 g a.s./ha to *Daucus carota, Beta vulgaris, Vica faba, Lactuca sativa, Brassica rapa* and *Solanum lycopersicum*.

Phytotoxic effects such as abnormal growth and development were observed with *Glycine max, Cucumis sativus, Daucus carota, Beta vulgaris, Vica faba, Lactuca sativa, Brassica rapa* and *Solanum lycopersicum*, with severity increasing with dose.

After 21-days exposure the most sensitive parameter was shoot fresh weight. The most sensitive species in terms of fresh weight was *Brassica rapa* (turnip) with NOER and ER50 values of 6.25 and 29.4 g a.s./ha, respectively.

**Materials and Methods**

**Materials**

|  |  |
| --- | --- |
| **Test Material** | 2,4-D 95 SP |
| **Lot/Batch #:** | 2111140274 |
| **Purity:** | 95.1% |
| **Description:** | White/beige powder |
| **Stability of test compound:** | Not reported |
| **Reanalysis/Expiry date:** | 1st November 2023 |
| **Density:** | Not reported |
| **Treatments** |  |
| **Test rates:** | 6.25, 12.5, 25.0, 50.0, 100.0, 200, 400,0, 800.0, 1600 and 3200.0 g a.s./ha to *Glycine max, Cucumis sativus, Allium* *cepa* and *Lolium perenne.*  3.13, 6.25, 12.5, 25.0, 50.0, 100.0, 200, 400,0, 800.0 and 1600.0 g a.s./ha to *Daucus carota,* *Beta vulgaris, Vica* *faba, Lactuca sativa,* *Brassica rapa* and *Solanum lycopersicum.* |
| **Solvent/vehicle:** | Deionised water |
| **Test organisms** |  |
| **Species:** | Eight dicotyledonous *(Daucus carota, Lactuca sativa, Brassica rapa, Beta vulgaris, Cucumis sativus, Glycine max, Vica faba, Solanum lycopersicum)*  Two monocotyledonous *(Allium cepa, Lolium perenne)* |
| **Source:** | E.W. King & Co. Ltd, CO5 9PG.  Cotswold Grass Seeds Direct, GL56 0JQ. Team PPPF (glasshouses), Fera Science Ltd. Moles Seeds (UK) Ltd, CO3 8PD. |
| **Test design** |  |
| **Test vessel:** | 15 x 11.4 cm plastic pots in a glasshouse |
| **Replication:** | 30 plants per species. Six replicate pots of five plants for *Allium cepa* and *Lolium perenne*. Ten replicate pots of three plants for *Daucus carota*. Fifteen replicate pots of two plants for *Glycine max*, *Cucumis sativa*, *Solanum lycopersicum*, *Vica faba*, *Beta vulgaris*, *Brassica rapa* and *Lactuca sativa*. |
| **Duration of test:** | 21 days |
| **Environmental test conditions** |  |
| **Temperature:** | 16.5 - 36.6°C |
| **Relative humidity** | 50.4 – 93.6% |
| **Photoperiod:** | 16 hours light : 8 hours darkness |

**Study Design**

This study was conducted in order to investigate the effect of exposure to 2,4-D 95 SP on the growth and vegetative vigour of ten non-target terrestrial plant species.

Plants from eight dicotyledonous (*Daucus carota, Lactuca sativa, Brassica rapa, Beta vulgaris, Cucumis sativus, Glycine max, Vica faba, Solanum lycopersicum*) and 2 monocotyledonous (*Allium cepa, Lolium perenne*) species were tested. The study was split into two trials of five species each due to limited space in the glasshouse.

Plants were grown from seed to two to four true leaves (BBCH 12-14) for all species. The test solutions were diluted in deionised water and applied using a laboratory track sprayer calibrated to deliver 400 L/ha to the plants of the treatment.

The test substance was sprayed on the plant and leaf surfaces at nominal treatment rates of 6.25, 12.5, 25.0, 50.0, 100.0, 200, 400,0, 800.0, 1600 and 3200.0 g a.s./ha to *Glycine max*, *Cucumis sativus*, *Allium cepa* and *Lolium perenne*. Treatment rates of 3.13, 6.25, 12.5, 25.0, 50.0, 100.0, 200, 400,0, 800.0 and 1600.0 g a.s./ha to *Daucus carota*, *Beta vulgaris*, *Vica faba*, *Lactuca sativa*, *Brassica rapa* and *Solanum lycopersicum*..

Plants were evaluated after 7, 14 and 21 days. Endpoints were shoot fresh weight, survival and any visual detrimental effects such as chlorosis or other development abnormalities assessed according to a numbered scoring system.

The concentration and homogeneity of 2,4-D in the final application vehicle (test solution) was determined by analysis of the test solutions applied to the test systems with the highest and lowest treatment rates. This was done for both trials. An assessment of stability was made based on expected and measured concentrations.

**Results and Discussion**

Validity criteria according to OECD 227 (2006) were met:

*Trial 1*

* Control seedling emergence from to be ≥70% (actual: 85 to 100%).
* The mean survival of seedlings to be ≥90% for the duration of the experiment (actual: 100%).

*Trial 2*

* Control seedling emergence from to be ≥70% (actual: 83 to 97%).
* The mean survival of seedlings to be ≥90% for the duration of the experiment (actual: 100%).

The control seedlings did not exhibit visible phytotoxic effects, apart from normal variation in growth or morphology associated with a species.

Environmental conditions for a species were identical and the growing media contained the same amount of soil matrix, support media or substrate from the same source.

The mean analytically determined concentrations of 2,4-D in the highest and lowest treatment solutions were 97.45% and 79.90% of nominal in the first trial, with a procedural recovery of 94.75%.

The mean analytically determined concentrations of 2,4-D in the highest and lowest treatment solutions were 101.32% and 90.13% of nominal in the second trial, with a procedural recovery of 101.87%.

After 21-days exposure, the following effects were observed:

**Table 10.6.2/02-1**: **Summary of *Allium cepa* (Onion) mean shoot fresh weights, % survival and visual injury scores (±SD)**

|  |  |  |  |
| --- | --- | --- | --- |
| Nominal treatment rate (g a.s./ha) | Mean foliar fresh weight (g) Day 21 (± SD) | Survival Day 21 (%) | Mean visual injury score Day 21  (± SD) |
| Control | 5.6758 ± 1.4010 | 100 | 0.7 ± 2.2 |
| 6.25 | 6.1752 ± 0.7689 | 100 | 1.2 ± 2.2 |
| 12.5 | 5.3553 ± 0.9305 | 100 | 0.8 ± 1.9 |
| 25.0 | 7.4916 ± 0.8700 | 100 | 0.3 ± 1.3 |
| 50.0 | 6.7278 ± 1.1733 | 100 | 0.2 ± 0.9 |
| 100.0 | 4.7370 ± 1.0661 | 100 | 0.5 ± 1.5 |
| 200.0 | 5.7259 ± 0.8943 | 100 | 0.3 ± 1.3 |
| 400.0 | 5.1250 ± 0.9754 | 100 | 1.0 ± 2.0 |
| 800.0 | 5.9877 ± 1.0220 | 100 | 0.5 ± 1.5 |
| 1600.0 | 4.8024 ± 0.7627 | 100 | 0.0 |
| 3200.0 | 4.4451 ± 0.6459\* | 86.7\* | 14.2 ± 34.3 |

\* Statistically significant effect (p<0.05)

Visual injury scale: 0 = no effect, 1-39.9 = slight effect, 40-69.9 moderate effect and 70-99.9 = severe effect with 100 = all plants dead

**Table 10.6.2/02-2**: **Summary of *Lolium perenne* (Ryegrass) mean shoot fresh weights, % survival and visual injury scores (±SD)**

|  |  |  |  |
| --- | --- | --- | --- |
| Nominal treatment rate (g a.s./ha) | Mean foliar fresh weight (g) Day 21 (± SD) | Survival Day 21 (%) | Mean visual injury score Day 21  (± SD) |
| Control | 3.1645 ± 0.8566 | 100 | 0.0 |
| 6.25 | 3.4350 ± 0.7884 | 100 | 10.0 ± 0 |
| 12.5 | 4.7464 ± 1.1932 | 100 | 0.0 |
| 25.0 | 4.2533 ± 0.6085 | 100 | 0.0 |
| 50.0 | 3.9039 ± 0.4446 | 100 | 0.0 |
| 100.0 | 3.8930 ± 0.4342 | 100 | 0.0 |
| 200.0 | 3.4002 ± 0.3472 | 100 | 0.2 ± 0.9 |
| 400.0 | 3.8903 ± 0.5998 | 100 | 0.0 |
| 800.0 | 4.5946 ± 0.5832 | 100 | 0.0 |
| 1600.0 | 3.7090 ± 0.6082 | 100 | 0.0 |
| 3200.0 | 3.2164 ± 0.4941 | 100 | 0.2 ± 0.9 |

Visual injury scale: 0 = no effect, 1-39.9 = slight effect, 40-69.9 moderate effect and 70-99.9 = severe effect with 100 = all plants dead

**Table 10.6.2/02-3**: **Summary of *Cucumis sativa* (Cucumber) mean shoot fresh weights, % survival and visual injury scores (±SD)**

|  |  |  |  |
| --- | --- | --- | --- |
| Nominal treatment rate (g a.s./ha) | Mean foliar fresh weight (g) Day 21 (± SD) | Survival Day 21 (%) | Mean visual injury score Day 21  (± SD) |
| Control | 54.24 ± 5.11 | 100 | 0.0 |
| 6.25 | 56.80 ± 6.51 | 100 | 0.0 |
| 12.5 | 61.65 ± 7.39 | 100 | 1.0 ± 3.1 |
| 25.0 | 54.83 ± 5.60 | 100 | 0.3 ± 1.8 |
| 50.0 | 59.87 ± 10.05 | 100 | 2.0 ± 4.8 |
| 100.0 | 61.88 ± 7.23 | 100 | 2.0 ± 7.6 |
| 200.0 | 56.36 ± 9.43 | 100 | 13.2 ± 17.9 |
| 400.0 | 52.45 ± 5.67 | 100 | 26.7 ± 17.6 |
| 800.0 | 51.93 ± 8.62 | 100 | 42.0 ± 16.9 |
| 1600.0 | 46.49 ± 6.22\* | 100 | 63.8 ± 10.1 |
| 3200.0 | 36.99 ± 12.38\* | 66.7\* | 85.2 ± 12.1 |

\* Statistically significant effect (p<0.05)

Visual injury scale: 0 = no effect, 1-39.9 = slight effect, 40-69.9 moderate effect and 70-99.9 = severe effect with 100 = all plants dead

**Table 10.6.2/02-4**:**Summary of *Glycine max* (Soya bean) mean shoot fresh weights, % survival and visual injury scores (±SD)**

|  |  |  |  |
| --- | --- | --- | --- |
| Nominal treatment rate (g a.s./ha) | Mean foliar fresh weight (g) Day 21 (± SD) | Survival Day 21 (%) | Mean visual injury score Day 21  (± SD) |
| Control | 18.8413 ± 3.6708 | 100 | 0.0 |
| 6.25 | 19.2880 ± 2.2717 | 100 | 0.0 |
| 12.5 | 17.4767 ± 2.9430 | 100 | 0.0 |
| 25.0 | 16.3763 ± 3.5058 | 100 | 0.0 |
| 50.0 | 18.1490 ± 2.3930 | 100 | 0.3 ± 1.3 |
| 100.0 | 15.6457 ± 3.0945\* | 100 | 5.5 ± 5.3 |
| 200.0 | 13.6840 ± 2.9028\* | 100 | 18.8 ± 8.7 |
| 400.0 | 12.6490 ± 2.8116\* | 96.7 | 32.3 ± 16.5 |
| 800.0 | 12.2740 ± 3.4088\* | 80.0\* | 56.2 ± 27.2 |
| 1600.0 | 9.3509 ± 5.0305\* | 56.7\* | 75.8 ± 29.5 |
| 3200.0 | 7.2433 ± 3.0497\* | 16.7\* | 94.5 ± 17.7 |

\* Statistically significant effect (p<0.05)

Visual injury scale: 0 = no effect, 1-39.9 = slight effect, 40-69.9 moderate effect and 70-99.9 = severe effect with 100 = all plants dead

**Table 10.6.2/02-5**: **Summary of *Daucus carota* (Carrot) mean shoot fresh weights, % survival and visual injury scores (±SD)**

|  |  |  |  |
| --- | --- | --- | --- |
| Nominal treatment rate (g a.s./ha) | Mean foliar fresh weight (g) Day 21 (± SD) | Survival Day 21 (%) | Mean visual injury score Day 21  (± SD) |
| Control | 5.039 ± 0.937 | 100 | 0.0 |
| 3.13 | 5.344 ± 1.097 | 100 | 0.0 |
| 6.25 | 5.453 ± 0.880 | 100 | 0.0 |
| 12.5 | 4.641 ± 0.890 | 100 | 0.7 ± 2.5 |
| 25.0 | 4.209 ± 0.745\* | 96.7 | 16.7 ± 19.2 |
| 50.0 | 4.385 ± 0.870\* | 86.7\* | 27.3 ± 29.7 |
| 100.0 | 3.458 ± 1.069\* | 80.0\* | 47.7 ± 27.8 |
| 200.0 | 2.783 ± 0.644\* | 56.7\* | 69.2 ± 28.7 |
| 400.0 | 2.911 ± 1.996\* | 20.0\* | 93.2 ± 14.0 |
| 800.0 | 1.140 ± 0.054\* | 13.3\* | 96.0 ± 10.4 |
| 1600.0 | 1.188 ± 0.458\* | 13.3\* | 97.0 ± 7.8 |
| Toxic reference | 0.189 ± 0.051\* | 33.3\* | 94.0 ± 8.7 |

\* Statistically significant effect (p<0.05)

Visual injury scale: 0 = no effect, 1-39.9 = slight effect, 40-69.9 moderate effect and 70-99.9 = severe effect with 100 = all plants dead

**Table 10.6.2/02-6**: **Summary of *Beta vulgaris* (Sugar beet) mean shoot fresh weights, % survival and visual injury scores (±SD)**

|  |  |  |  |
| --- | --- | --- | --- |
| Nominal treatment rate (g a.s./ha) | Mean foliar fresh weight (g) Day 21 (± SD) | Survival Day 21 (%) | Mean visual injury score Day 21  (± SD) |
| Control | 12.823 ± 2.188 | 100 | 0 |
| 3.13 | 14.050 ± 3.066 | 100 | 0 |
| 6.25 | 12.834 ± 3.358 | 100 | 0.5 ± 1.5 |
| 12.5 | 13.237 ± 5.068 | 100 | 1.8 ± 2.8 |
| 25.0 | 11.580 ± 3.454 | 100 | 4.5 ± 7.4 |
| 50.0 | 11.924 ± 3.898 | 96.7 | 18.0 ± 19.4 |
| 100.0 | 4.154 ± 2.736\* | 60.0\* | 77.2 ± 21.4 |
| 200.0 | 2.805 ± 1.954\* | 50.0\* | 84.0 ± 17.9 |
| 400.0 | 2.389 ± 1.868\* | 13.3\* | 96.5 ± 10.6 |
| 800.0 | 1.803 ± 0.918\* | 13.3\* | 95.5 ± 11.7 |
| 1600.0 | 1.829 ± 0.518\* | 16.7\* | 95.0 ± 11.4 |

\* Statistically significant effect (p<0.05)

Visual injury scale: 0 = no effect, 1-39.9 = slight effect, 40-69.9 moderate effect and 70-99.9 = severe effect with 100 = all plants dead

**Table 10.6.2/02-7**: **Summary of *Solanum lycopersicon* (Tomato) mean shoot fresh weights, % survival and visual injury scores (±SD)**

|  |  |  |  |
| --- | --- | --- | --- |
| Nominal treatment rate (g a.s./ha) | Mean foliar fresh weight (g) Day 21 (± SD) | Survival Day 21 (%) | Mean visual injury score Day 21  (± SD) |
| Control | 37.43 ± 2.95 | 100 | 0.3 ± 1.3 |
| 3.13 | 38.49 ± 6.52 | 100 | 1.2 ± 2.2 |
| 6.25 | 38.35 ± 2.60 | 100 | 1.2 ± 2.2 |
| 12.5 | 37.74 ± 6.21 | 100 | 11.0 ± 5.5 |
| 25.0 | 39.28 ± 4.12 | 100 | 16.7 ± 6.1 |
| 50.0 | 37.99 ± 5.06 | 100 | 27.7 ± 10.1 |
| 100.0 | 31.27 ± 3.94\* | 100 | 37.0 ± 7.0 |
| 200.0 | 17.29 ± 7.19\* | 96.7 | 58.2 ± 13.5 |
| 400.0 | 8.54 ± 5.41\* | 100 | 73.2 ± 14.2 |
| 800.0 | 5.26 ± 2.84\* | 100 | 82.8 ± 7.7 |
| 1600.0 | 2.34 ± 2.08\* | 90.0\* | 90.5 ± 6.9 |

\* Statistically significant effect (p<0.05)

Visual injury scale: 0 = no effect, 1-39.9 = slight effect, 40-69.9 moderate effect and 70-99.9 = severe effect with 100 = all plants dead

**Table 10.6.2/02-8**: **Summary of *Vica* *faba* (Broad bean) mean shoot fresh weights, % survival and visual injury scores (±SD)**

|  |  |  |  |
| --- | --- | --- | --- |
| Nominal treatment rate (g a.s./ha) | Mean foliar fresh weight (g) Day 21 (± SD) | Survival Day 21 (%) | Mean visual injury score Day 21  (± SD) |
| Control | 26.44 ± 2.97 | 100 | 0.0 |
| 3.13 | 25.58 ± 4.09 | 100 | 0.0 |
| 6.25 | 26.09 ± 3.70 | 100 | 0.0 |
| 12.5 | 24.09 ± 3.75\* | 100 | 0.0 |
| 25.0 | 22.88 ± 4.51\* | 100 | 10.0 ± 0.0 |
| 50.0 | 18.74 ± 3.60\* | 100 | 20.0 ± 0.0 |
| 100.0 | 14.04 ± 3.25\* | 100 | 32.7 ± 5.8 |
| 200.0 | 11.21 ± 3.64\* | 100 | 47.3 ± 7.4 |
| 400.0 | 7.12 ± 2.20\* | 46.7\* | 84.0 ± 17.7 |
| 800.0 | 4.84 ± 2.22\* | 23.3\* | 97.2 ± 5.7 |
| 1600.0 | 5.33 ± 6.10\* | 7.7\* | 99.1 ± 3.3 |

\* Statistically significant effect (p<0.05)

Visual injury scale: 0 = no effect, 1-39.9 = slight effect, 40-69.9 moderate effect and 70-99.9 = severe effect with 100 = all plants dead

**Table 10.6.2/02-9**: **Summary of *Lactuca sativa* (Lettuce) mean shoot fresh weights, % survival and visual injury scores (±SD)**

|  |  |  |  |
| --- | --- | --- | --- |
| Nominal treatment rate (g a.s./ha) | Mean foliar fresh weight (g) Day 21 (± SD) | Survival Day 21 (%) | Mean visual injury score Day 21  (± SD) |
| Control | 38.62 ± 10.70 | 100 | 1.3 ± 3.5 |
| 3.13 | 42.74 ± 6.88 | 100 | 1.3 ± 3.5 |
| 6.25 | 43.83 ± 8.49 | 100 | 4.7 ± 6.3 |
| 12.5 | 39.85 ± 5.84 | 100 | 30.0 ± 0.0 |
| 25.0 | 41.34 ± 6.42 | 100 | 50.0 ±0.0 |
| 50.0 | 36.10 ± 5.01 | 100 | 60.0 ± 0.0 |
| 100.0 | 26.61 ± 10.00\* | 96.7 | 73.2 ± 7.1 |
| 200.0 | 23.15 ± 10.69\* | 83.3\* | 76.7 ± 11.5 |
| 400.0 | 13.79 ± 10.36\* | 56.7\* | 84.2 ± 15.9 |
| 800.0 | 8.54 ± 4.44\* | 20.0\* | 95.7 ± 9.7 |
| 1600.0 | DAH | 0 | 100 |
| Toxic reference | DAH | 0 | 100 |

DAH – Dead at harvest

\* Statistically significant effect (p<0.05)

Visual injury scale: 0 = no effect, 1-39.9 = slight effect, 40-69.9 moderate effect and 70-99.9 = severe effect with 100 = all plants dead

**Table 10.6.2/02-10**: **Summary of *Brassica rapa* (Turnip) mean shoot fresh weights, % survival and visual injury scores (±SD)**

|  |  |  |  |
| --- | --- | --- | --- |
| Nominal treatment rate (g a.s./ha) | Mean foliar fresh weight (g) Day 21 (± SD) | Survival Day 21 (%) | Mean visual injury score Day 21  (± SD) |
| Control | 14.574 ± 2.885 | 100 | 0.3 ± 1.3 |
| 3.13 | 17.318 ± 3.910 | 100 | 0.5 ± 1.5 |
| 6.25 | 13.750 ± 2.409 | 100 | 0.7 ± 2.2 |
| 12.5 | 9.342 ± 2.643\* | 100 | 9.5 ± 7.8 |
| 25.0 | 9.081 ± 2.535\* | 100 | 27.3 ± 14.8 |
| 50.0 | 5.024 ± 2.192\* | 96.7 | 52.3 ± 13.0 |
| 100.0 | 4.115 ± 1.254\* | 96.7\* | 62.5 ± 9.5 |
| 200.0 | 2.394 ± 0.602\* | 93.3\* | 73.0 ± 8.4 |
| 400.0 | 1.604 ± 0.558\* | 93.3\* | 83.0 ± 6.0 |
| 800.0 | 1.014 ± 0.865\* | 66.7\* | 90.7 ± 7.8 |
| 1600.0 | 0.579 ± 0.377\* | 36.7\* | 96.0 ± 6.4 |

\* Statistically significant effect (p<0.05)

Visual injury scale: 0 = no effect, 1-39.9 = slight effect, 40-69.9 moderate effect and 70-99.9 = severe effect with 100 = all plants dead

The most sensitive endpoint was shoot fresh weight. Related NOEC and ECx values for this parameter are presented below.

Table 10.6.2/02-11: Summary of effects on shoot fresh weight after 21-day exposure to 2.4-D 95 SP (g a.s./ha in 400 L/ha).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Species | NOER | ER10 | ER20 | ER50 |
| *Brassica rapa* (turnip) | 6.25 | 3.8  (2.4 – 5.8) | 7.6 (5.4– 10.7) | 29.4  (23.5 – 36.9) |
| *Vica* *faba* (broad bean) | 6.25 | 13.5 (8.2 – 22.2) | 29.6 (20.4 – 43.0) | 134.1  (108.9 – 165.1) |
| *Daucus carota* (carrot) | 12.5 | 15.8  (5.6 – 44.3) | 42.5  (19.8 – 91.2) | 282.6  (177.4 – 450.2) |
| *Lactuca sativa* (lettuce) | 50.0 | 34.4  (20.2 – 58.6) | 64.5  (43.5 – 95.5) | 214.7  (165.7 – 278.2 |
| *Beta vulgaris* (sugar beet) | 50.0 | 40.0  (22.0 – 72.9) | 52.7  (35.4 – 78.2) | 89.0  (71.7 – 110.5) |
| *Solanum lycopersicum* (tomato) | 50.0 | 63.4  (51.1 – 78.6) | 94.4  (80.4 – 110.9) | 202.4  (182.2 – 224.9) |
| *Glycine max* (soya bean) | 50.0 | 35.3  (10.2 – 121.7) | 132.7  (56.7 – 310.4) | 1673.8  (1012.2 – 2767.6) |
| *Cucumis sativa* (cucumber) | 800.0 | 716.4  (433.9 – 1182.7) | 1481.0  (1090.3 – 2011.8) | >3200 |
| *Allium cepa* (onion) | 1600.0 | 495.0  (79.1 – 3099.0) | 1747.1  (548.7 – 5563.2) | >3200 |
| *Lolium perenne* (ryegrass) | 3200.0 | >3200 | >3200 | >3200 |

Values in parentheses indicate 95% confidence intervals

**Temperature**

The study plan states that the temperature will be maintained at 22 ± 10°C. During trial 1 the temperature in glasshouse cubicle 34DG07 and 34DG08 went outside of this range.

In 34DG07 (*Allium cepa* and *Lolium perenne*) there were five study days when the temperature peaked above 32°C. The maximum temperatures on these days were 32.2 – 36.6°C.

In 34DG08 (carrot) there were two study days when the temperature peaked above 32°C. The maximum temperatures on these days were 33.5 – 36.1°C

These deviations from the Study Plan are not considered to have had any negative impact on the

integrity or the outcome of the study because the test conditions did not appear to have had any negative impact on the health of the control plants and all study validity criteria were met.

**Conclusion**

The effects of exposure to 2,4-D 95 SP on the vegetative vigour, survival, height and fresh weight of ten non-target plant species was assessed over 21 days.

Test concentrations were 6.25, 12.5, 25.0, 50.0, 100.0, 200, 400,0, 800.0, 1600 and 3200.0 g a.s./ha to *Glycine max, Cucumis sativus, Allium cepa* and *Lolium perenne* and 3.13, 6.25, 12.5, 25.0, 50.0, 100.0, 200, 400,0, 800.0 and 1600.0 g a.s./ha to *Daucus carota, Beta vulgaris, Vica faba, Lactuca sativa, Brassica rapa* and *Solanum lycopersicum*.

Phytotoxic effects such as abnormal growth and development were observed with *Glycine max, Cucumis sativus, Daucus carota, Beta vulgaris, Vica faba, Lactuca sativa, Brassica rapa* and *Solanum lycopersicum*, with severity increasing with dose.

After 21-days exposure the most sensitive parameter was shoot fresh weight. The most sensitive species in terms of fresh weight was *Brassica rapa* (turnip) with NOER and ER50 values of 6.25 and 29.4 g a.s./ha, respectively.

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| **Assessment and conclusion by applicant:**  This is a new study that has not been previously evaluated.  Validity criteria according to OECD 227 (2006) were met:  *Trial 1*   * Control seedling emergence from to be ≥70% (actual: 85 to 100%). * The mean survival of seedlings to be ≥90% for the duration of the experiment (actual: 100%).   *Trial 2*   * Control seedling emergence from to be ≥70% (actual: 83 to 97%). * The mean survival of seedlings to be ≥90% for the duration of the experiment (actual: 100%).   The control seedlings did not exhibit visible phytotoxic effects, apart from normal variation in growth or morphology associated with a species.  Environmental conditions for a species were identical and the growing media contained the same amount of soil matrix, support media or substrate from the same source.  The temperature and humidity of test units could not be maintained to guideline recommendation due to the glasshouse test design, however as all validity criteria were met and control plant performance was acceptable this is not considered to have an adverse effect on the study.  The study is therefore considered acceptable.  After 21-days exposure the most sensitive parameter was shoot fresh weight. The most sensitive species in terms of fresh weight was *Brassica rapa* (turnip) with NOER and ER50 values of 6.25 and 29.4 g a.s./ha, respectively. |

* + 1. KCP 10.6.3 Extended laboratory studies on non-target plants
  1. KCP 10.7 Effects on other terrestrial organisms (flora and fauna)
  2. KCP 10.8 Monitoring data

1. Maletzki, D. (2011): *Myriophyllum spicatum* toxicity test: Results of an inter-laboratory ring test using a sediment-free test system. Final report FKZ:36301294, Federal Environment Agency, Dessau, Germany [↑](#footnote-ref-1)