Draft Renewal Assessment Report
under Regulation (EC) 1107/2009

# CLOPYRALID

## Volume 3 – B.9 (PPP) – GF-1374

RMS: Finland
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# Version History

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# B.9. ECOTOXICOLOGY DATA AND ASSESSMENT OF RISKS FOR NON-TARGET SPECIES

In this section only ecotoxicological studies with the new representative formulation GF-1374 are presented, which have not been evaluated before in the context of EU renewal of approval of clopyralid. In the DAR (2003) the representative formulation was different, and the product studies evaluated in the DAR and considered in the EFSA conclusion on clopyralid (EFSA Scientific Report (2005) 50, 1-65) are no longer relevant for the ecotoxicological risk assessment of clopyralid, resulting from the current GAP of the product GF-1374.

GF-1374 is an emulsifiable concentrate containing the active substance clopyralid at 80 g ae/L and mixing partners namely fluroxypyr-meptyl 144 g/L (100 g ae/L) and florasulam 2.5 g/L. The representative uses of the formulation GF-1374 to be evaluated include two uses evaluated during the first approval (cereals and pasture) which also reflect changes in dosage of clopyralid containing products as doses have been reduced since the first approval. Currently the intended use of the product GF-1374 results in the environmental load of 80 – 120 g clopyralid + 2.5 – 3.75 g florasulam + 144 – 0.216 g fluroxypyr-meptyl per hectare, sprayed once per season on cereals or established pasture. Only this GAP is considered in the ecotoxicological risk assessment in this chapter.

In case of a need to consider the data about the former representative formulation previously evaluated during the first approval of clopyralid, please see the DAR (2003) with addenda, and the EFSA conclusion on clopyralid.

## B.9.1. EFFECTS ON BIRDS AND OTHER TERRESTRIAL VERTEBRATES

### B.9.1.1. Effects on birds

The Notifier submitted one study not previously evaluated in the context of approval of clopyralid on the effects of the new representative formulation GF-1374 to bobwhite quail (CP 10.1.1.1; [redacted] and [redacted] 2005; DAS Study ID 040261). The submission of this vertebrate study was justified since it was conducted after the previous Annex I inclusion and is conducted with the new lead formulation, although the endpoint from this study is not a critical endpoint and is not used for the risk assessment.

**CP. 10.1.1.1/1. Acute effects of GF-1374 in birds**

| | |
|---|---|
| Report: | [redacted] (2005). |
| Title: | GF-1374: an acute oral toxicity study with the northern bobwhite |
| Document No: | Dow AgroSciences Study Number: 040261 |
| Guidelines: | OPPTS Number 850.2100 and FIFRA Subdivision E, Section 71-1 |
| GLP | Yes |

**Methodology:** Aim of the study was to determine the acute toxicity of GF-1374 to the bobwhite quail (*Colinus virginianus*). GF-1374 (Lot No. 187/76-A) is an emulsion concentrate (EC) nominally containing fluroxypyr-meptyl at 144 g/L (142 g/L, actual), clopyralid at 80 g/L (86 g/L, actual) and florasulam at 2.5 g/L (2.5 g/L, actual). Sixty quail (5 males and 5 females per treatment or control group), *ca.* 18 weeks of age and weighing 174 to 236 g at study initiation, were administered the formulated product dispersed in deionised water at nominal concentrations of 0 (control), 292, 486, 810, 1350 and 2250 mg GF-1374 per kilogram of body weight. Dosages were based on individual body weights and were given by oral

intubation directly into the crop or proventriculus. The control group was treated with an equivalent dosage volume (4 mL/kg-body weight) of water only. Birds were not fed for approximately 17 hours prior to dosing. After dosing, male and female birds were segregated by sex in groups of 5 birds per pen and held at 23.3°C ± 0.3°C (SD) at a mean daily relative humidity of 72% ± 6% (SD) under a photoperiod of 8 h light (239 lux): 16 h dark.

The birds were observed daily for 14 days post-dosing. Body weights were measured individually on Days 0 (immediately prior to dosing), 3, 7 and 14. Average food consumption was estimated by pen for each treatment and control group for the periods Day 0 to 3, 4 to 7 and 8 to 14.

**Findings:**

No mortalities occurred during the observation period in any treatment or control group. One male bird in the control was noted to be head picked. All other control birds were normal in behaviour and appearance. Abnormal appearance and behaviour were observed in all treatment levels and included ruffled appearance, reduced reaction to external stimuli, lethargy, gaping, depression, wing droop, anorexia and loss of coordination.

No apparent differences in body weight, compared to control, were detected in males and females at 292, 486 and 810 mg/kg or in males at 1350 mg/kg. Reductions in mean body weight gain were observed, however, in females at 1350 mg/kg and in both males and females at 2250 mg/kg during the period Days 0-3. At least partial compensatory gains in bodyweight were evident in birds thereafter. Statistical analyses of the data indicated significant differences compared to controls for the males in the 292, 810 and 1350 mg/kg treatments. However these differences were primarily due to the exceptional mean body weight gain observed in the control males and were not considered being treatment related. When the data was compared to body weight gains more typical for bobwhite quail, a slight reduction in body weight gain was still observed for males in the 292 mg/kg treatment and for females in the 486 mg/kg treatment from Day 0 to 3. Since in both cases the difference can be attributed to a single bird in the group and these two birds had head lesions, these results are not considered to be treatment related.

During the period from Day 0 to Day 3, an apparent related reduction in feed consumption was observed for males at 810, 1350 and 2250 mg/kg, when compared to control. No other adverse effects on food consumption were apparent during the study. The effects are summarized in Table 9.1.1.

**Table 9.1.1. Effects of GF-1374 on bobwhite quail**

| Treatment (mg product/kg) | | Mortality (%) | Mean body weight (g) | | | | Mean group food consumption (g/bird/day) | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Day 0-14 | Day 0 | Day 3 | Day 7 | Day 14 | Day 0 to 3 | Day 4-7 | Day 8-14 |
| Control | Males | 0 | 191 | 205 | 207 | 200 | 28 | 20 | 18 |
| | Females | 0 | 197 | 204 | 204 | 198 | 23 | 23 | 18 |
| 292 | Males | 0 | 195 | 196 | 201 | 198 | 29 | 26 | 21 |
| | Females | 0 | 187 | 191 | 193 | 191 | 23 | 20 | 18 |
| 486 | Males | 0 | 204 | 212 | 211 | 209 | 29 | 26 | 21 |
| | Females | 0 | 194 | 192 | 197 | 196 | 27 | 26 | 23 |
| 810 | Males | 0 | 203 | 208 | 211 | 207 | 19 | 21 | 18 |
| | Females | 0 | 193 | 196 | 200 | 198 | 20 | 16 | 18 |
| 1350 | Males | 0 | 189 | 190 | 193 | 177 | 17 | 17 | 13 |
| | Females | 0 | 197 | 197 | 202 | 203 | 25 | 22 | 20 |
| 2250 | Males | 0 | 207 | 204 | 209 | 209 | 15 | 20 | 18 |
| | Females | 0 | 195 | 192 | 196 | 196 | 26 | 25 | 23 |

Since no mortalities were observed during the study, the $LD_{50}$ could not be determined but must be in excess of 2250 mg product/kg body weight, the highest treatment level tested, as summarized in Table 9.1.2. below.

**Table 9.1.2. Summary of the acute oral toxicity of GF-1374 to bobwhite quail.**

| End-point | mg GF-1374/kg bw |
|---|---|
| $LD_{50}$ (95% c.l.) | >2250 (-) |

**Conclusions:** The acute oral $LD_{50}$ of the product GF-1374 to the bobwhite quail *Colinus virginianus* has been shown to be >2250 mg product/kg body weight.

RMS comments and evaluation:

The avian oral acute toxicity study with the representative formulation GF-1374 submitted by the Notifier was well performed and reported, according to the test guidelines and the GLP, and acceptable. The outcome is appropriate to be used in the risk assessment of GF-1374 to birds, as presented in Chapter 9.2.1. below.The formulated product does not indicate a higher toxicity to birds than clopyralid as active substance. Thus the endpoint for the formulated product does not change the overall conclusion of low risk to avian species from exposure to clopyralid, as derived from the active substance studies already available in the original DAR in 2002 and the EFSA conclusions in 2005. The data requirement is fulfilled and no further studies are required to support the renewal for the approval of clopyralid.

## B.9.1.2. Effects on terrestrial vertebrates other than birds

An acute oral toxicity study has been conducted with the formulated product, as presented in the mammalian toxicity section for study report.

**CP 7.1.1. / CP 10.1.2.1/1. GF-1374: Acute oral toxicity up and down procedure in rats**

| | |
|---|---|
| Report: | [redacted] (2005) |
| Title: | GF-1374: Acute oral toxicity up and down procedure in rats. |
| Document No: | Dow AgroSciences Study Number: 040251 |
| Guidelines: | OECD 423 |
| GLP | Yes |

The acute oral toxicity study with GF-1374 was conducted using an "up and down" method, which tests the effects of the test substance at several doses, exposing one animal per dose at the time. Based on the results observed, more individuals may be exposed at the doses surrounding the expected $LD_{50}$ until a computer program, specifically designed for this type of tests, has sufficient information to estimate the $LD_{50}$ value. In the study for GF-1374 a total of 1, 1, 5 and 7 rats were exposed at the concentrations of 175, 550, 1750 and 5000 mg GF-1374/kg bw, respectively. No mortality was observed at the doses up to 1750 mg/kg bw, while 85.7% mortality was observed at the highest tested dose. The $LD_{50}$ indicated in the study report is equivalent to 5000 mg/kg bw. However, based on the results obtained during the study, the real $LD_{50}$ for GF-1374 appears to lie between 1750 and 5000 mg/kg bw. The original results have therefore been re-evaluated using a linear interpolation of the arcsine transformed data between the doses of 1750 and 5000 mg/kg bw. The results of this analysis estimate the acute oral $LD_{50}$ of GF-1374 to be equivalent to 3378 mg/kg bw. This estimated value has been used in the present risk assessment.

RMS comments and evaluation:

The mammalian oral acute toxicity study with the new representative formulation GF-1374 submitted by the Notifier was evaluated in more detail in the mammalian toxicity chapter. Overall, the study was assessed as well performed and reported, according to the test guidelines and the GLP, and acceptable. The formulated product does not indicate a significantly higher toxicity to mammals than clopyralid as active substance. The outcome is appropriate to be used in the ecotocicological risk assessment of GF-1374 to wild mammals, as presented in Chapter 9.2.2. below. Thus the acute endpoint for the new representative formulation does not change the overall conclusion of low risk to mammals from the exposure to clopyralid, as derived from the active substance studies already available in the original DAR in 2002 and the EFSA conclusions in 2005. The data requirement is fulfilled and no further vertebrate studies are required to support the renewal for the approval of clopyralid.

## B.9.2. RISK ASSESSMENT FOR BIRDS AND OTHER TERRESTRIAL VERTEBRATES

### B.9.2.1. Risk Assessment for Birds

A risk assessment for birds, specific to GF-1374 uses, was not evaluated as part of the Active Approval therefore all relevant information is provided.

#### B.9.2.1.1. Toxicity endpoints used in the risk assessment

The critical endpoints employed in the risk assessment for birds are indicated in bold in the tables below. Detailed descriptions of ecotoxicological studies with birds are given under point 8.1 in the Annex II dossier of clopyralid.

**Table 9.2.1. EU Endpoints - Toxicity of fluroxypyr to birds**

| Compound | Test species | Endpoint | EU agreed endpoints* | Endpoint used in risk assessment** | |
|---|---|---|---|---|---|
| | | | Value | Value | Ref |
| Fluroxypyr-meptyl | Bobwhite quail | Acute oral $LD_{50}$ | >2000 mg/kg bw | Same as EU | N/A |
| Fluroxypyr | Bobwhite quail | Acute oral $LD_{50}$ | >2000 mg/kg bw | Same as EU | N/A |
| Fluroxypyr-meptyl | Mallard duck | Reproduction NOEL | 57.8 mg/kg bw/day[a] | Same as EU | N/A |
| Fluroxypyr | Mallard duck | Reproduction NOEL | 40.1 mg/kg bw/day[b] | Same as EU | N/A |

* EFSA Journal 2011;9(3):2091
** Since Annex I inclusion new studies have been performed providing new end-points which are used in the risk assessment.
[a]: The endpoint is equivalent to 500 mg/kg diet
[b:] Endpoint obtained by recalculation of the long term fluroxypyr-meptyl endpoints
N/A: not applicable.

**Table 9.2.2. EU Endpoints - Toxicity of clopyralid to birds**

| Compound | Test species | Endpoint | EU agreed endpoints* | Endpoint used in risk assessment** | |
|---|---|---|---|---|---|
| | | | Value | Value | Ref |
| Clopyralid | Mallard duck | Acute oral $LD_{50}$ | **1465** mg/kg bw | Same as EU | EFSA 2005 |
| Clopyralid | Mallard duck | Reproduction NOEL | **118** mg/kg diet[a] | Same as EU | EFSA 2005 |

* EFSA Scientific Report (2005) 50, 1-65
** Since Annex I inclusion new studies have been performed providing new end-points which are used in the risk assessment.
[a]: The endpoint is equivalent to 1000 mg/kg bw/day.
N/A: not applicable.

**Table 9.2.3. EU Endpoints - Toxicity of florasulam to birds**

| Compound | Test species | Endpoint | EU agreed endpoints* | Endpoint used in risk assessment** | |
|---|---|---|---|---|---|
| | | | Value | Value | Ref |
| Florasulam | Japanese quail | Acute oral $LD_{50}$ | 1046 mg/kg bw | Same as EU | N/A |
| Florasulam | Bobwhite quail | Reproduction NOEL | ≥162 mg/kg bw/day[a] | Same as EU | N/A |

* SANCO/1406/2001
** Since Annex I inclusion new studies have been performed providing new end-points which are used in the risk assessment.
[a]: converted from the NOEC value of ≥1500 mg/kg diet considering the measured food consumption (21.9 g/bird/d) and the mean body weight (203 g) of birds exposed to 1500 mg/kg diet.
N/A: not applicable

The available data for GF-1374 is summarised in the following table.

**Table 9.2.4. Toxicity of GF-1374 to birds**

| Compound | Test species | Endpoint | EU agreed endpoints* | Endpoint used in risk assessment** | |
|---|---|---|---|---|---|
| | | | Value | Value | Ref |
| GF-1374 | Bobwhite quail | Acute oral $LD_{50}$ | N/A | > **2250** mg/kg bw | [redacted] (2005) DAS Study ID 040261 |

* GF-1374 is a formulation which was not evaluated at EU level.
** Studies for GF-1374 have been performed providing end-points which are used in the risk assessment.
N/A: not applicable

Even though an acute oral toxicity study with birds exposed to GF-1374 is available, the dose additivity principle has been used to derive the theoretical acute $LD_{50}$ of GF-1374 to birds according to the equation (EFSA Journal 2009; 7(12): 1438):

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

where: $X(a.s._i)$ is the fraction of the active substance *i* in the formulation;
$LD_{50}(a.s._i)$ is the acute toxicity for the active substance *i*.

Considering the lowest $LD_{50}$ values determined for clopyralid, florasulam and fluroxypyr-meptyl of 1465, 1046 and >2000 mg/kg bw, respectively, and their nominal concentrations in GF-1374 (7.69% w/w, 0.24% w/w and 13.85% w/w for clopyralid, florasulam and fluroxypyr-meptyl, respectively), the resulting $LD_{50}$ value for GF-1374 is equivalent to 1756 mg/kg bw thus the experimental value of GF-1374 has been used in the $TER_A$ calculation along with the $LD_{50}$ values from the single active substance clopyralid.

According to the appendix B of the EFSA birds and mammals guidance document (EFSA Journal 2009; 7(12): 1438) when the $LD_{50}$ of a formulated product with more than one active substance is available, this value should be compared with the predicted mixture toxicity assuming dose additivity The resulted values indicated that the measured toxicity of a formulation is lower than predicted. Based on the EFSA birds and mammals guidance document the predicted mixture toxicity should be used in the first-tier risk assessment.

As stated in the EFSA birds and mammals guidance document (EFSA Journal 2009; 7(12): 1438), it is currently not recommended to predict toxicity values for long-term reproductive effects of formulations containing more than one active substance.

**B.9.2.1.2. Exposure**

Birds are typically exposed to dry residues on their food items following the dilution and spraying of the formulated product. During these processes, much of the formulation constituents are likely to be lost by volatilisation. Since dietary exposure is the main route of exposure, toxicity data for the active substances are used in preference to data from tests with the formulated material.

Exposure of birds will be predominantly dietary, through the consumption of residues on food items. Direct exposure of birds to GF-1374 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 risk to birds from metabolites formed in plants and animals is considered negligible. There were not any observable, plant or animal, metabolites of concern for clopyralid. During the plant metabolism studies, only clopyralid and glucose conjugates were identified

(see Table 9.2.5 for study specifics). No extensive metabolism occurred in crop studies and clopyralid was found to be the major component of residue (EFSA 2005). Furthermore, only clopyralid and glycine conjugates were observed during hen metabolism study suggesting clopyralid does not have any plant or animal metabolites, in this instance avian metabolites, of concern (Table 9.2.6) as stated by EFSA. Please see table below for summary of clopyralid plant and animal metabolism studies.

**Table 9.2.5. Summary of Plant Nature of Residue (NOR) studies.**

| crop | Cabbage | Pasture Grass | Sugarbeet | Oilseed rape |
|---|---|---|---|---|
| Study ID/accession # | GH-C-4289<br>48060 | GH-C 1424 | GHE-P-9939 | GHE-P-9938<br>101948 |
| Year completed | 1996 | 1981 | 2002 | 2002 |
| Guideline | 171-4 (a)(2) | | EC 91/414/EEC | EC 91/414/EEC |
| GLP? (yes or no) | Yes | No | Yes | Yes |
| Crop grouping | Leafy vegetable | Cereal/grass crop | Root crop | Pulses and oilseeds |
| Application route (e.g., foliar or soil) | Foliar | Foliar | Foliar and Soil | Foliar |
| Test compound | 14C-clopyralid | 14C-3,6-Dichloropicolinic acid | 14C-clopyralid | 14C-clopyralid |
| Radiolabel position | 2,6 position on Pyridine ring | 2,4 position on Pyridine ring | 2,6 position on Pyridine ring | 2,6 position on Pyridine ring |
| Application rate (g ai/h) | 0.1875 lbs a.i./acre | 1 lb ae/acre | 1 lb ae/acre | 300 g a.s/ha |
| # applications | 1 | 1 | 1 | 1 |
| Application timing (growth stage) | 31 | Long established Pasture grass | 8 | Growth stage 36 |
| PHI (pre-harvest interval) days from last application until harvest | 0, 5, and 38d | 2h, growth stage 36, 43 & 49 | 28, 62d | 1h, growth stage 36, 69 and 85 |
| Interval between applications (days) | N/A | N/A | N/A | N/A |
| Extraction solvent | 0.125M $NaOH/CH_3OH$ | 0.5N NaOH & Methanol | Acetonitrile:water | 1:1 Acetonitrile:water |
| Analytical techniques | HPLC | TLC | HPLC | HPLC |
| Surface residues? | None | None | N/A | N/A |
| Photolysis important? | N/A | N/A | N/A | N/A |
| Translocation to grain/fruit? | None | None | None | None |
| Primary residues | Clopyralid | clopyralid | clopyralid | Clopyralid and conjugates of clopyralid |
| Metabolite ID | TLC | GC-MS | TLC | TLC |

| crop | Cabbage | Pasture Grass | Sugarbeet | Oilseed rape |
|---|---|---|---|---|
| Complete characterization? | Yes | Yes | Yes | Yes |

**Table 9.2.6. Summary of animal metabolism studies**

| Animal | Hen |
|---|---|
| Study ID/accession # | 2024323 |
| Year completed | 2014 |
| Guideline | OECD 503 |
| GLP? (yes or no) | Yes |
| Test compound | 14C-clopyralid |
| Radiolabel position | 2,6 position on Pyridine ring |
| Dose rate | 10 ppm |
| # of dose days | 7 |
| Extraction solvent | 80/20 acetonitrile/water |
| Analytical techniques | HPLC |
| Primary residues | Clopyralid |
| Metabolite ID | LC/MS |
| Complete characterization? | Yes |

The critical GAP is summarised in Table 9.2.7. The maximum application rates considered are 216 g a.s./ha for fluroxypyr-meptyl, 150 g a.e./ha for fluroxypyr, 120 g a.s./ha for clopyralid and 3.75 g a.s./ha for florasulam. The worst-case actual application rate of 1.5 L/ha has been used for the formulation GF-1374 applied to pasture and 1.0 L/ha for applications to cereals.

**Table 9.2.7. Critical GAP to assess the risk of GF-1374 applications to grassland and cereals**

| **Crop** | **Time of application** | **No. of applications per year** | **Maximum rate per application** |
|---|---|---|---|
| Cereals | BBCH 13 – 39<br>February to June 30 | 1 | 1.0 L GF-1374/ha (or 1040[a] g/ha)<br>equivalent to:<br>fluroxypyr-meptyl 144 g/ha (or 100 g a.e./ha);<br>florasulam 2.5 g/ha;<br>clopyralid 80 g/ha |
| Pasture | Feb 01 to September 30 | | 1.5 L GF-1374/ha (or 1560[a] g/ha)<br>equivalent to:<br>fluroxypyr-meptyl 216 g/ha (or 150 g a.e./ha);<br>florasulam 3.75 g/ha;<br>clopyralid 120 g/ha |

[a]: based on a nominal formulation density of 1.04 g/mL.

Since the plant protection product is to be applied post-emergence to cereals and pasture, a small omnivorous and a large herbivorous bird have been considered as models in the initial screening risk assessment, respectively. These models have been taken from the standard scenarios for the screening risk assessment described in the EFSA birds and mammals guidance document (EFSA Journal 2009; 7(12): 1438, 26 July 2010 update). Estimates of the Daily Dietary Dose (DDD) have been determined using the default shortcut values for acute and long-term screening risk assessment also given in this guidance document.

The EFSA birds and mammals guidance document (EFSA Journal 2009; 7(12): 1438, 26 July 2010 update) requires the risk of exposure from drinking water in axils of leaves and from puddles to be considered. For applications to cereals and grassland only the "puddle scenario" requires consideration. The EFSA guidance states that, when the "puddle scenario" is relevant and the ratio of the effective application rate (g/ha) to the relevant endpoint is either <50 for substances with $K_{oc}$ values <500 L/kg or <3000 for substances with $K_{oc}$ values ≥500 L/kg, then an assessment of the risk to birds from drinking water is not required.

The artithmetic mean of $K_{oc}$ values for clopyralid in nine soils is 1.41 (see Section 5, Table 8.2.13), so the approach above for less sorptive substances can be applied to this active substance. The maximum application rate for clopyralid is 120 g/ha and the relevant endpoint for the avian risk assessment is the reproduction NOEL of 118 mg/kg bw/d. The ratio of these figures is 1.02, which is lower than 50, so a formal assessment of the risk to birds from exposure to clopyralid through drinking water is not necessary. The risk via consumption of contaminated water was assessed to be low with florasulam (EFSA 2015). A low risk was identified for birds which may be exposed to fluroxypyr-meptyl or fluroxypyr by the consumption of contaminated water (EFSA 2011).

#### B.9.2.1.3. Acute screening risk assessment to birds

The acute screening risk assessment for birds is summarised in the following table.

For all representative uses for fluroxypyr, which are included in the GAP, the acute and long term risk was low for large and medium birds and insectivorous birds exposed to fluroxypyr-meptyl and fluroxypyr through the diet (EFSA 2011). The acute risk to birds via dietary exposure were assessed as low during the screening steps for all the representative uses that include the GAP presented in Table 10.1.1-7 for florasulam (EFSA 2015).

As stated previously, fluroxypyr and florasulam have recently been granted Annex I inclusion. For fluroxypyr, the conclusions from the peer review process were published in 2011 (EFSA 2011). For florasulam, conclusions from the peer review process were published in 2015 (EFSA 2015). A risk envelope approach is being applied for the risk assessment of fluroxpyr and florasulam. Within the scope of this assessment, one application of fluroxypyr at 200 g a.s./ha was determined to be a safe use on both cereals and grasslands. Also, one application of flurosulam at 6.25 g a.s./ha was determine to be a safe use on cereals and grasslands. Therefore, for the below assessments, it is justified to refer to fluroxypyr and florasulam data wherever appropriate. Specific risk assessments for these two active substances are not necessary to defend the Annex I listing of clopyralid since the proposed use rate falls within their safe use. Please refer to the EFSA conclusion documents for fluroxypyr and florasulam for specific risk assessment details.

**Table 9.2.8. Acute screening risk assessment for clopyralid following applications of GF-1374 to pasture and amenity grasslands as well as cereals.**

| Substance | Indicator bird | App. rate (kg/ha) | Shortcut value (acute) | MAF[a] | DDD (mg/kg bw) | $LD_{50}$ (mg/kg bw) | $TER_A$ |
|---|---|---|---|---|---|---|---|
| ***Pasture and amenity grassland (1.5 L GF-1374/ha)*** | | | | | | | |
| Clopyralid | Large herbivorous | 0.12 | 30.5 | n.a. | 3.66 | 1465 | 400 |
| GF-1374 | | 1.56 | | n.a. | 47.6 | > 1756 | 36.9 |
| ***Cereal (1 L GF-1374/ha)*** | | | | | | | |
| Clopyralid | Small omnivorous | 0.08 | 158.8 | n.a. | 12.7 | 1465 | 115 |
| GF-1374 | | 1.04 | | n.a. | 165 | > 1756 | 10.6 |

[a]n.a. not applicable.

Considering the worst-case shortcut values of the screening risk assessment, all the $TER_A$ values calculated above are higher than the Annex VI trigger of 10, indicating a low acute risk to birds from the use of GF-1374 when applied at maximum rates up to 0.12 kg a.s/ha clopyralid to pasture and 0.08 kg clopyralid/ha on cereals.

RMS comments and evaluation:

The acute screening risk assessment to birds presented by the Notifier was conducted acceptably according to the current guidance given by EFSA and indicates that the use of GF-1374, the representative formulation of clopyralid, does not pose an unacceptable acute risk to birds if the product is used according to the GAP. No higher tier risk assessment is required.

#### B.9.2.1.4. Long-term screening risk on birds

The long-term screening dietary risk for birds is summarised in the following table for grassland and cereals.

For all representative uses for fluroxypyr, which are within the GAP evaluated during this risk assessment, the long term risk was low for large and medium birds and insectivorous birds exposed to fluroxypyr-meptyl and fluroxypyr through the diet (EFSA 2011).

The chronic risk to birds via dietary exposure were assessed as low with the screening steps for all the representative uses of florasulam (EFSA 2015).

**Table 9.2.9. Long-term avian screening risk assessment for clopyralid following applications to pasture and cereals.**

| Substance | Indicator bird | App. rate (kg/ha) | Shortcut value (long-term) | $f_{TWA}$ | MAF[a] | DDD (mg/kg bw) | NOEC (mg/kg bw/day) | $TER_{LT}$ |
|---|---|---|---|---|---|---|---|---|
| ***Pasture and amenity grassland (1.5 L GF-1374/ha)*** | | | | | | | | |
| Clopyralid | Large herbivorous | 0.12 | 16.2 | 0.53 | n.a | 1.03 | 118[b] | 115 |
| ***Cereals (1.0 L GF-1374/ha)*** | | | | | | | | |
| Clopyralid | Small omnivorous | 0.08 | 64.8 | 0.53 | n.a | 2.75 | 118[b] | 42.9 |

[a] n.a. not applicable
[b] NOEC value for clopyralid based on 1000 mg/kg diet

The $TER_{LT}$ values calculated above are greater than the Annex VI trigger of 5 indicating low long-term risk to birds from the use of GF-1374, at proposed use rates, on pasture and cereals.

**Considering the worst-case shortcut values of the screening risk assessment, all $TER_{LT}$ values are in excess of the Annex VI trigger of 5, indicating an acceptable long-term risk to birds after application of GF-1374 at rates up to 1.5 L product/ha on pasture and amenity grassland and 1.0 L product/ha on cereals.**

RMS comments and evaluation:

The long term risk assessment to birds presented by the Notifier was conducted acceptably according to the current guidance given by EFSA and indicates that the use of GF-1374, the representative formulation of clopyralid, does not pose an unacceptable long term risk to birds if the product is used according to the GAP.

## B.9.2.2. Risk assessment for other terrestrial vertebrates

### B.9.2.2.1. Toxicity endpoints used in the risk assessment

The critical endpoints employed in the risk assessment for mammals are indicated in bold in the tables below.

**Table 9.2.10. Acute and long-term toxicity endpoints of fluroxypyr meptyl, fluroxypyr, florasulam, clopyralid to mammals.**

| Species | Test substance | Endpoint | Value | Reference |
|---|---|---|---|---|
| Rat | Fluroxypyr meptyl | Acute oral $LD_{50}$ | **> 2000 mg/kg bw** | EFSA 2011 |
| Rat | Fluroxypyr | Acute oral $LD_{50}$ | **> 2000 mg/kg bw** | EFSA 2011 |
| Rabbit developmental | Fluroxypyr | NOEC | **100 mg/kg bw/day** | EFSA 2011 |
| Mouse | Florasulam | Acute oral $LD_{50}$ | **> 5000** | EFSA 2015 |
| Rat | Florasulam | NOAEL | **> 100** | EFSA 2015 |
| Rat | Clopyralid | Acute oral $LD_{50}$ | **> 5000** | EFSA 2005 |
| Rat | Clopyralid | NOEC | **50 mg/kg bw/day** | EFSA 2005 |

The available data for GF-1374 is summarised in the following table. The acute oral toxicity study with GF-1374 was conducted using an “up and down” method, which tests the effects of the test substance at several doses, exposing one animal per dose at the time. Based on the results observed, more individuals may be exposed at the doses surrounding the expected $LD_{50}$ until a computer program, specifically designed for this type of tests, has sufficient information to estimate the $LD_{50}$ value. In the study for GF-1374 a total of 1, 1, 5 and 7 rats were exposed at the concentrations of 175, 550, 1750 and 5000 mg GF-1374/kg bw, respectively. No mortality was observed at the doses up to 1750 mg/kg bw, while 85.7% mortality was observed at the highest tested dose. The $LD_{50}$ indicated in the study report is equivalent to 5000 mg/kg bw. However, based on the results obtained during the study, the real $LD_{50}$ for GF-1374 appears to lie between 1750 and 5000 mg/kg bw. The original results have therefore been re-evaluated using a linear interpolation of the arcsine transformed data between the doses of 1750 and 5000 mg/kg bw. The results of this analysis estimate the acute oral $LD_{50}$ of GF-1374 to be equivalent to 3378 mg/kg bw. This estimated value has been used in the present risk assessment.

**Table 9.2.11. Toxicity of GF-1374 to mammals**

| Compound | Test species | Endpoint | EU agreed endpoints* | Endpoint used in risk assessment** | |
|---|---|---|---|---|---|
| | | | Value | Value | Ref |
| GF-1374 | Rat | Acute oral $LD_{50}$ | N/A | 3378 mg/kg bw | [redacted] (2005) DAS Study ID 040251 |

* GF-1374 is a formulation which was not evaluated at EU level.
** Studies for GF-1374 have been performed providing end-points which are used in the risk assessment.
N/A: not applicable

Even though an acute oral toxicity study for mammals exposed to GF-1374 is available, the dose additivity principle has been used to derive the theoretical acute $LD_{50}$ of GF-1374 to mammals according to the equation:

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

where: $X(a.s._i)$ is the fraction of the active substance *i* in the formulation;
$LD_{50}(a.s._i)$ is the acute toxicity for the active substance *i*.

Considering the lowest $LD_{50}$ values determined for clopyralid, florasulam and fluroxypyr-meptyl of >5000, >5000 and >2000 mg/kg bw, respectively, and their nominal concentrations in GF-1374 (7.69% w/w, 0.24% w/w and 13.85% w/w for clopyralid, florasulam and fluroxypyr-meptyl, respectively), the resulting $LD_{50}$ value for GF-1374 is equivalent to 2559 mg/kg bw. This estimated value is in line with the experimental $LD_{50}$.

According to the appendix B of the EFSA birds and mammals guidance document (EFSA Journal 2009; 7(12): 1438) when the $LD_{50}$ of a formulated product with more than one active substance is available, this value should be compared with the predicted mixture toxicity assuming dose additivity. The resulted values indicated that the measured toxicity of a formulation is lower than predicted. Based on the EFSA birds and mammals guidance document the predicted mixture toxicity should be used in the first-tier risk assessment.

As stated in the EFSA birds and mammals guidance document (EFSA Journal 2009; 7(12): 1438), it is currently not recommended to predict toxicity values for long-term reproductive effects of formulations containing more than one active substance.

**B.9.2.2.2. Exposure**

Mammals are typically exposed to dry residues on their food items following the dilution and spraying of the formulated product. During these processes, much of the formulation constituents are likely to be lost by volatilisation. Since dietary exposure is the main route of exposure, toxicity data for the active substances are used in preference to data from tests with the formulated material.

Exposure of mammals will be predominantly dietary, through the consumption of residues on food items. Direct exposure of birds to GF-1374 applications is considered unlikely since, at the time of application and for a short period thereafter, most mammals will leave the immediate vicinity of spray operations in response to the human disturbance. The risk to mammals from metabolites formed in plants and animals is considered negligible. There were not any observable, plant or animal, metabolites of concern for clopyralid. During the plant metabolism studies, only clopyralid and glucose conjugates were identified. No extensive metabolism occurred in crop study and clopyralid was found to be the major component of residue (EFSA 2005). Furthermore, only clopyralid and glycine conjugates were

observed during hen metabolism study suggesting clopyralid does not have any plant or animal metabolites of concern as stated by EFSA. Tables 9.2.13. and 9.2.14. below summarize the plant and animal metabolism studies on clopyralid.

**Table 9.2.13. Summary of Plant Nature of Residue (NOR) studies.**

| crop | Cabbage | Pasteur Grass | Sugarbeet | Oilseed rape |
|---|---|---|---|---|
| Study ID/accession # | GH-C-4289<br>48060 | GH-C 1424 | GHE-P-9939 | GHE-P-9938<br>101948 |
| Year completed | 1996 | 1981 | 2002 | 2002 |
| Guideline | 171-4 (a)(2) | | EC 91/414/EEC | EC 91/414/EEC |
| GLP? (yes or no) | Yes | No | Yes | Yes |
| Crop grouping | Leafy vegetable | Cereal/grass crop | Root crop | Pulses and oilseeds |
| Application route (e.g., foliar or soil) | Foliar | Foliar | Foliar and Soil | Foliar |
| Test compound | 14C-clopyralid | 14C-3,6-Dichloropicolinic acid | 14C-clopyralid | 14C-clopyralid |
| Radiolabel position | 2,6 position on Pyridine ring | 2,4 position on Pyridine ring | 2,6 position on Pyridine ring | 2,6 position on Pyridine ring |
| Application rate (g ai/h) | 0.1875 lbs a.i./acre | 1 lb ae/acre | 1 lb ae/acre | 300 g a.s/ha |
| # applications | 1 | 1 | 1 | 1 |
| Application timing (growth stage) | 31 | Long established Pasteur grass | 8 | Growth stage 36 |
| PHI (pre-harvest interval) days from last application until harvest | 0, 5, and 38d | 2h, growth stage 36, 43 & 49 | 28, 62d | 1h, growth stage 36, 69 and 85 |
| Interval between applications (days) | N/A | N/A | N/A | N/A |
| Extraction solvent | 0.125M NaOH/$CH_3OH$ | 0.5N NaOH & Methanol | Acetonitrile:water | 1:1 Acetonitrile:water |
| Analytical techniques | HPLC | TLC | HPLC | HPLC |
| Surface residues? | None | None | N/A | N/A |
| Photolysis important? | N/A | N/A | N/A | N/A |
| Translocation to grain/fruit? | None | None | None | None |
| Primary residues | Clopyralid | clopyralid | clopyralid | Clopyralid and conjugates of clopyralid |
| Metabolite ID | TLC | GC-MS | TLC | TLC |
| Complete characterization? | Yes | Yes | Yes | Yes |

**Table 9.2.14. Summary of animal metabolism studies**

| Annex II point | Species | Route | Study | Report ref. |
|---|---|---|---|---|
| 5.1.1 | Sprague Dawley Rat | Oral: single dose, 10 mg/kg bw | Measure time course of radioactivity in plasma, urine, faeces, expired air and tissues. | [redacted] *et al*., 1975. **H01** |
| 5.1.1 | Fischer 344 Rat | Oral: single dose, 5 or 150 mg/kg bw;<br>IV: single dose, 5 mg/kg bw;<br>Oral: repeat dose, 5 mg/kg bw | Measure time course of radioactivity in plasma, urine, faeces, expired air and tissues. | [redacted] 1991. **H07** |

Following oral administration in the rat, clopyralid was rapidly and completely (>80%) absorbed and excreted quantitatively unchanged in the urine. There were no differences in distribution of radioactivity between dose rates, sex, route (oral vs. iv) or frequency of administration. Clopyralid was not metabolised in the rat.

The GAPs and rationale for the risk envelope assessed for wild mammals are provided in Table 9.2.15. below. The approach taken in this dossier was to consider the GAP for fluroxypyr, clopyralid and florasulam across a number of formulations and to select the maximum application rate as a worst case. The maximum application rates considered is 120 g a.s./ha for clopyralid and application rate of 1.5 L/ha has been used for the formulation GF-1374.

The EFSA birds and mammals guidance document (EFSA Journal 2009; 7(12): 1438, 26 July 2010 update) requires the risk of exposure from drinking water in axils of leaves and from puddles to be considered. For applications to cereals and grassland only the "puddle scenario" requires consideration. The EFSA guidance states that, when the "puddle scenario" is relevant and the ratio of the effective application rate (g/ha) to the relevant endpoint is either <50 for substances with $K_{oc}$ values <500 L/kg or <3000 for substances with $K_{oc}$ values ≥500 L/kg, then an assessment of the risk to birds from drinking water is not required.

The $K_{oc}$ for clopyralid is 1.41 (Table 8.2.11), so the approach above for less sorptive substances can be applied to this active substance. The maximum application rate for clopyralid is 120 g/ha and the relevant endpoint for the mammalian risk assessment is the reproduction NOEL of 50 mg/kg bw/d. The ratio of these figures is 2.14, which is lower than 50, so a formal assessment of the risk to mammals from exposure to clopyralid through drinking water is not necessary.

The risk via consumption of contaminated water was assessed to be low with florasulam (EFSA 2015). A low risk was identified for birds which may be exposed to fluroxypyr-meptyl or fluroxypyr by the consumption of contaminated water (EFSA 2011).

The following risk assessment has been based on realistic worst-case scenarios for the applications summarised in the table below.

**Table 9.2.15. Critical GAP to assess the risk of GF-1374 applications to grassland and cereals**

| Crop | Time of application | No. of applications per year | Maximum rate per application |
|---|---|---|---|
| Cereals | BBCH 13 – 39<br>February to June 30 | 1 | 1.0 L GF-1374/ha (or 1040[a] g/ha)<br>equivalent to:<br>fluroxypyr-meptyl 144 g/ha (or 100 g a.e./ha);<br>florasulam 2.5 g/ha;<br>clopyralid 80 g/ha |
| Pasture | Feb 01 to August 31 | | 1.5 L GF-1374/ha (or 1560[a] g/ha)<br>equivalent to:<br>fluroxypyr-meptyl 216 g/ha (or 150 g a.e./ha);<br>florasulam 3.75 g/ha;<br>clopyralid 120 g/ha |

[a]: based on a nominal formulation density of 1.04 g/mL.

Since the plant protection product is to be applied to range and pasture and cereals, the small herbivorous mammal was selected as the model species in the initial screening risk assessment. The model mammals were taken from the standard scenarios for the screening assessment described in the European Food Safety Authority Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438). Estimates of the Daily Dietary Dose (DDD) have been determined using the default shortcut values for acute and long-term screening risk assessments described in the guidance document.

#### B.9.2.2.3. Acute screening risk assessment for mammals

The acute screening risk assessment for mammals is summarised in the following table.

The acute risk was assessed as low for small and medium herbivourous mammals and insectivourous mammals for both fluroxypyr. The acute first tier risk was slightly below the trigger of Annex VI for small herbivorous mammals, indicating potential risk but a TER was achieved above the trigger value based on further calculations based on the guidance document (EFSA 2011).

The acute risk to mammals via dietary exposure was considered low eith the screening steps for all the respresentative uses of florasulam which include the GAP being evaluated in this dossier (EFSA 2015).

Due to these facts, only the risk assessment with clopyralid and the formulated product are presented below.

**Table 9.2.16. Acute screening risk assessment (using small herbivorous mammal as screening species) for GF-1374 after applications to pasture and cereals.**

| Substance | App. rate (kg/ha) | Shortcut value (acute) | DDD (mg/kg bw) | $LD_{50}$ (mg/kg bw) | $TER_A$ |
|---|---|---|---|---|---|
| **Cereals (1.0 L/ha rate)** | | | | | |
| Clopyralid | 0.08 | 118.4 | 9.47 | > 5000 | 528 |
| GF-1374 | 1.040 | | 123 | 2559 | 20.8 |
| **Grassland (1.5 L/ha rate)** | | | | | |
| Clopyralid | 0.120 | 136.4 | 16.4 | >5000 | 305 |
| GF-1374 | 1.560 | | 213 | 2559 | 12.0 |

Calculated $TER_A$ values are higher than the Annex VI trigger of 10 for clopyralid and GF-1374 indicating a low acute risk to mammals from the use of GF-1374 on pasture and cereals.

**Considering the worst-case shortcut values of the screening risk assessment, all $TER_A$ values are in excess of the Annex VI trigger of 10 for clopyralid and GF-1374 indicating acceptable acute risk to mammals following applications of GF-1374 to pasture and amenity grasslands as well as cereals.**

RMS comments and evaluation:

The acute oral risk assessment to wild mammals, as presented by the Notifier, was conducted acceptably according to the guidance given by EFSA, and indicates that the use of GF-1374, the representative formulation of clopyralid, does not pose an unacceptable acute risk to wild mammals, if the product is used according to the GAP.

Similarly to birds, the risk assessment to mammals of the product GF-1374 was recalculated by the RMS evaluator based on dose additivity, as this approach gave the lower $LD_{50}$ estimation of 2559 mg/kg bw. The recalculated values are presented in Table 9.2.16.

Neverthless, the risk to wild mammals is acceptable regardless of which toxicity values are used.

#### B.9.2.2.4. Long Term Reproductive Risk Assessment

The long term and reproductive toxicity of mammals to clopyralid was considered under points CA 5.5 and 5.6. The long-term screening long term risk for mammals is summarised in the following table for range/pasture and amenity grassland as well as applications to cereals.

**Table 9.2.17. Long-term mammalian screening risk assessment for GF-1374 applications to grassland and cereals using the small herbivorous mammal as indicator species.**

| Indicator mammal | App. rate (kg/ha) | Shortcut value (long-term) | $f_{TWA}$ | MAF | DDD (mg/kg bw) | NOEC (mg/kg bw/day) | $TER_{LT}$ |
|---|---|---|---|---|---|---|---|
| **Cereals** | | | | | | | |
| Clopyralid | 0.08 | 48.3 | 0.53 | n.a | 2.05 | 50 | 24.4 |
| **Grassland** | | | | | | | |
| Clopyralid | 0.120 | 72.3 | 0.53 | n.a | 4.60 | 50 | 10.9 |

n.a. not applicable.

Considering the worst-case shortcut values of the screening risk assessment, all $TER_{LT}$ values are greater than the Annex VI trigger value of 5, indicating low long term risk to mammals for fluroxypyr, florasulam, and clopyralid for both crop scenarios.

RMS comments and evaluation:

The long term risk assessment to wild mammals, as presented by the Notifier, is acceptable and indicates that the use of GF-1374, the representative formulation of clopyralid, does not pose an unacceptable risk to wild mammals if the product is used according to the GAP.

**B.9.2.2.5. Risk from Secondary Poisoning**

The EFSA birds and mammals guidance document (EFSA Journal 2009; 7(12): 1438) states that a log $K_{ow}$ ≥3 is used to indicate that there might be a potential for bioaccumulation (see Section 5.6 Bioaccumulation and food chain behaviour).

Although the log $P_{OW}$ of fluroxypyr-meptyl is greater than 3, the risk assessment for secondary poisoning of birds and mammals was not conducted because fluroxypyr-meptyl rapidly hydrolyses to fluroxypyr in the environment and it does not bioaccumulate in fish (measured BCF = 26). Due to the log $P_{ow}$ = 3.09 the risk assessment from secondary poisoning was provided for the metabolite fluroxypyr methodypyridine. No chronic toxicity data were available fore this metabolite therefore it was assumed to be ten times more toxic than the parent. Moreover, to cover the lack of an experimental measured BCF value on fish, a QSAR approach was used to attain an estimated BCF. On the basis of these assumptions the risk for earthworm and fish-eating birds and mammals was assessed as low. A low risk was identified for birds and mammals which may be exposed to fluroxypyr-meptyl or fluroxypyr by the consumption of contaminated water (EFSA 2011). Secondary poisoning for florasulam was not triggered (EFSA 2015).

Clopyralid is not expected to bioaccumulate in animal tissues as indicated by a log $P_{ow}$ of -2.63 and a fish BCF < 1.

RMS comments and evaluation:

The data provided by the Notifier are adequate and acceptable to conclude that the risk of secondary poisoning of wild birds and mammals is low, if the representative formulation of clopyralid GF-1374 is used according to the GAP.

### B.9.2.3. Effects on terrestrial vertebrate wildlife (reptiles and amphibians)

In absence of standard guidelines and validated methods for amphibian and reptiles, the assessment should be based on any existing relevant information. As such, there are not any publications or studies available in the literature pertaining to the toxicity of clopyralid or the product GF-1374 on amphibians and reptiles.

RMS comments and evaluation:

The explanation presented by the Notifier is acceptable. As the risk assessment to birds and mammals as well as to aquatic organisms indicates a low risk to both terrestrial and aquatic vertebrates from the use of GF-1374, the representative formulation of clopyralid, no further vertebrate studies are warranted. It can be concluded that the risk is most likely low also to reptiles and amphibians.

## B.9.3. EFFECTS ON AQUATIC ORGANISMS

### B.9.3.1. Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes

#### B.9.3.1.1. Acute toxicity of GF-1374 to fish

The following fish acute toxicity study performed on GF-1374 is provided in support of the assessment and has not been previously evaluated in the context of EU approval of clopyralid.

**CP 10.2.1.4/1 GF-1374 Acute Study with Rainbow Trout (*Oncorhynchus mykiss*)**

| | |
|---|---|
| Report: | [redacted] (2005a). |
| Title: | GF-1374: Acute toxicity to rainbow trout (*Oncorhynchus mykiss*) under flow-through conditions. |
| Document No: | Dow AgroSciences Study Number: 040342<br>[redacted] Study Number: 12550.6349 |
| Guidelines: | OECD Guideline No. 203,<br>EC Guideline L383A - Method C.1,<br>OPPTS Draft Guideline 850.1075. |
| GLP | Yes |

**Methodology:** Aim of the study was to determine the acute toxicity of GF-1374 to the rainbow trout (*Oncorhynchus mykiss*) under flow-through conditions. GF-1374 (Lot No. 187/76-A) is an emulsion concentrate (EC) nominally containing fluroxypyr-meptyl at 144 g/L (142 g/L, actual), clopyralid at 80 g/L (86 g/L, actual) and florasulam at 2.5 g/L (2.5 g/L, actual). The acute lethal toxicity of GF-1374 to rainbow trout of mean weight 0.73 g and mean total length of 4.1 cm was assessed under continuous flow exposure conditions over a period of 96 hours. Ten fish (one replicate per treatment) were exposed to GF-1374 at nominal concentrations of 0, 1.3, 2.5, 5.0, 10 and 20 mg product/L, delivered by an intermittent flow proportional diluter at a flow rate of 6.0 volume exchanges per 24 hours.

The test vessels were glass aquaria containing 6.5 litres media at a standing loading rate of 1.12 g body weight/L (0.187 g body weight/L/24 hours). The test was conducted at 13 to 14°C under a 16-h light:8-h dark photoperiod (310 to 520 lux) in well water with a hardness of 38-40 mg/L as $CaCO_3$. Dissolved oxygen levels were 6.2 to 8.5 mg $O_2$/L (60% to 81% air saturation value) during the study and pH values ranged from 6.8 to 7.1.

Exposure levels in the test media were monitored by measuring the concentrations of fluroxypyr-methylheptyl and its hydrolysis product, fluroxypyr acid, at 0 and 96 hours using an HPLC/UV method of analysis. Fluroxypyr-methylheptyl concentrations were converted to acid equivalents and added to the measured fluroxypyr acid concentrations to calculate the overall concentration in acid equivalents.

**Findings:** Mean measured concentrations of fluroxypyr as total acid (acid + methylheptyl converted to acid equivalents) were 75, 84, 80, 84 and 80% of nominal for the 1.3, 2.5, 5.0, 10 and 20 mg GF-1374/L treatments. Although the mean recovery for the lowest concentration was below 80%, the test concentrations defining the dose-response were ≥80% throughout the study.

The ester form generally represented the majority of the fluroxypyr measured in the test vessels. Low levels of fluroxypyr acid (up to 11% of nominal) were detected at the start of the study in the 1.3 and 2.5 mg/L treatments. At the end of the study fluroxypyr acid was detected at concentrations between 10 and 25% of nominal in all GF-1374 treatments with the exception of the highest one (20 mg/L).

Following 96 hours exposure, 100% mortality was observed at the two highest exposure levels of 10 and 20 mg/L. No mortality occurred in any other treatment or control group.

Sublethal signs of toxicity were observed at 2.5, 5.0, 10 and 20 mg/L and included partial or complete loss of equilibrium and lying on the bottom of the test vessel.

The effects of GF-1374 to rainbow trout are summarized in Table 9.3.1. below.

**Table 9.3.1. Effects of GF-1374 on *Oncorhynchus mykiss***

| Nominal concentration (mg GF-1374/L) | Sublethal observations (10 fish/vessel)[a] | | | | | | Mean cumulative mortality (%) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **4 h** | **6 h** | **24 h** | **48 h** | **72 h** | **96 h** | **4 h** | **6 h** | **24 h** | **48 h** | **72 h** | **96 h** |
| Control | None | None | None | None | None | None | 0 | 0 | 0 | 0 | 0 | 0 |
| 1.3 | None | None | None | None | None | None | 0 | 0 | 0 | 0 | 0 | 0 |
| 2.5 | None | None | None | 1 pE | 1 pE | 2 pE | 0 | 0 | 0 | 0 | 0 | 0 |
| 5.0 | 1 pE | 1 pE | sev. pE<br>2 cE | sev. pE<br>sev. cE | sev. pE<br>2 cE | sev. pE<br>2 cE | 0 | 0 | 0 | 0 | 0 | 0 |
| 10 | 10 cE<br>10 B | 10 cE<br>10 B | 4 cE<br>4 B | 2 cE | -- | -- | 0 | 0 | 60 | 80 | 100 | 100 |
| 20 | 10 cE<br>10 B | 10 cE<br>10 B | -- | -- | -- | -- | 0 | 0 | 100 | 100 | 100 | 100 |

[a] explanations to abbreviations: pE: Partial loss of equilibrium; cE: Complete loss of equilibrium; B: Fish on bottom of test vessel; sev: several.

In view of the incidence of mortality and sub-lethal effects at 2.5 mg product/L and above, the NOEC was considered to be 1.3 mg product/L. Analysis of the mortality data (binomial probability method) gave the following results, as presented in Table 9.3.2. below.

**Table 9.3.2. Results**

| End-point | mg GF-1374/L, nominal |
|---|---|
| 24-h $LC_{50}$ (95% c.l.) | 9.2 (5.0 - 20) |
| 48-h $LC_{50}$ (95% c.l.) | 8.0 (5.0 - 20) |
| 72-h $LC_{50}$ (95% c.l.) | 7.1 (5.0 - 10) |
| 96-h $LC_{50}$ (95% c.l.) | 7.1 (5.0 - 10) |
| NOEC | 1.3 |

**Conclusions:** The 96-h $LC_{50}$ of GF-1374 to rainbow trout (*Oncorhynchus mykiss*) under flow-through conditions is 7.1 mg product/L, based on nominal concentrations of total formulation. The NOEC is 1.3 mg product/L.

RMS comments and evaluation:

The acute toxicity study to rainbow trout with the new representative formulation GF-1374 was well performed and reported, according to the test guideline and GLP, except the routine water and food contaminant analyses that were conducted in another laboratory using validated standard methods. This deviation was considered to have no impact on the results.

In the analytical part of the study, the measured concentrations of test substance relied on measurements of only one of the active substances in the formulation, fluroxypyr, although the test substance GF-1374 contained three active substances. Thus the clopyralid concentrations are nominal only.

Slight deviations to study protocol were reported: calibration of the dilution system was not performed at the termination, slight deviations of the temperature range ($\pm$2 ºC instead of $\pm$1 ºC) and some variation in dissolved oxygen concentrations (60-81% of saturation). However, these deviations were within the ranges of test guidelines, and were assessed as not having negative impact on the quality of the study.

Otherwise the study was of good quality and valid. The data is acceptable and the result can be used in the risk assessment. No further aquatic vertebrate data on the product GF-1374 are required.

**B.9.3.1.2. Acute toxicity of GF-1374 to Daphnia**

The following *Daphnia* acute toxicity study performed on GF-1374 is provided in support of the assessment and has not been previously evaluated in the context of EU approval of clopyralid.

**CP 10.2.1.4/2 GF-1374 Acute Study with Daphnia (*Daphnia magna*)**

| | |
|---|---|
| Report: | KIIIA1 10.2.2.2/01, Sayers, L.E. (2005b) |
| Title: | GF-1374: Acute toxicity to water fleas (*Daphnia magna*) under static conditions. |
| Document No: | Dow AgroSciences Study Number: 040343<br>Springborn Smithers Study No. 12550.6348 |
| Guidelines: | OECD Guideline No. 202, |

| | |
|---|---|
| | EC Guideline L383A - Method C.2,<br>FIFRA Guideline 72-2. |
| GLP | Yes |

**Methodology:** Aim of the study was to determine the acute toxicity of GF-1374 to water fleas (*Daphnia magna*) under static conditions. GF-1374 (Lot No. 187/76-A) is an emulsion concentrate (EC) nominally containing fluroxypyr-meptyl at 144 g/L (142 g/L, actual), clopyralid at 80 g/L (86 g/L, actual) and florasulam at 2.5 g/L (2.5 g/L, actual). Four groups of five, 1st instar *Daphnia* (less than 24 hours old) were exposed to nominal concentrations of 0.63, 1.3, 2.5, 5.0 and 10 mg product/L. The test vessels were 250 mL glass beakers containing 200 mL media. The test was conducted at 19 to 20°C under a 16-h light:8-h dark photoperiod (55 to 66 footcandles) in resin filtered fortified well water with a hardness of 190 mg/L as $CaCO_3$. Dissolved oxygen levels were 8.6 to 9.3 $mgO_2$/L during the study and pH was 8.4.

Exposure levels in the test media were monitored by measuring the concentrations of fluroxypyr-methylheptyl and its hydrolysis product, fluroxypyr acid, at 0 and 48 hours using an HPLC/UV method of analysis. Fluroxypyr-methylheptyl concentrations were converted to acid equivalents and added to the measured fluroxypyr acid concentrations to calculate the overall concentration in acid equivalents.

**Findings:** At the beginning of the study measured concentrations of fluroxypyr as total acid (acid + methylheptyl converted to acid equivalents) were 83, 92, 92, 97 and 94% of nominal for the 0.63, 1.3, 2.5, 5.0 and 10 mg GF-1374/L treatments. Fluroxypyr acid was not detected at the start of the study. Over the course of the study the concentrations of the ester form decreased, while those of the acid form increased. By the end of the study fluroxypyr acid was detected at concentrations between 11 and 36% of nominal in all GF-1374 treatments with the exception of the highest one (10 mg/L). Mean measured concentrations of fluroxypyr as total acid ranged between 78 and 89% over course of the study.

Following 48 hours exposure, immobilisation ranged from 10% at 2.5 mg/L to 95% at 10 mg/L. No immobilisation occurred at concentrations lower than or equal to 1.3 mg/L.

**Table 9.3.3. Sublethal observations and immobilisation data**

| Nominal concentration (mg GF-1374/L) | Sublethal observations (20 daphnid/treatment)[a] | | Mean cumulative immobilisation (%) | |
|---|---|---|---|---|
| | 24 h | 48 h | 24 h | 48 h |
| Control | None | None | 0 | 0 |
| 0.63 | None | None | 0 | 0 |
| 1.3 | None | None | 0 | 0 |
| 2.5 | None | None | 0 | 10 |
| 5.0 | 20 P; 20 L | 18 P; 18 L | 0 | 10 |
| 10 | 10 P; 10 L | 1 P, 1 L | 50 | 95 |

[a] explanations: P: Pale; L: lethargic.

In view of the incidence of immobilisation at 2.5 mg product/L and above, the NOEC was considered to be 1.3 mg product/L. Analysis of the immobilisation data (binomial probability method) gave the following results, as presented in Table 9.3.4.

**Table 9.3.4. Results**

| End-point | mg GF-1374/L, nominal |
|---|---|
| 24-h $EC_{50}$ (95% c.l.) | 10 (5.0 – n.a.)[a] |
| 48-h $EC_{50}$ (95% c.l.) | 6.9 (5.0 - 10) |
| NOEC | 1.3 |

[a]: n.a.: not applicable.

**Conclusions:** The 48-h $EC_{50}$ of GF-1374 to *Daphnia magna* under static conditions is 6.9 mg product/L, based on nominal concentrations of total formulation. The NOEC is 1.3 mg product/L.

RMS comments and evaluation:

The acute toxicity study to waterflea with the new representative formulation GF-1374 was well performed and reported, according to the test guideline and GLP, except the routine water and food contaminant analyses that were conducted in another laboratory using validated standard methods. This deviation was considered to have no impact on the results.

In the analytical part of the study, the measured concentrations of test substance relied on measurements of only one of the active substances in the formulation, fluroxypyr, although the test substance GF-1374 contained three active substances. Thus the clopyralid concentrations are nominal only.

One slight deviation to study protocol was reported in temperature regime at the end of the study, when the temperature was measured to be slightly below the constant 20 °C, obviously due to not resetting the thermometer during biological observations. All readings in the exposure solutions during the exposure were within the acceptable temperature range. This deviation was within the range of test guidelines, and did not have negative impact on the quality of the study.

Otherwise the study was of good quality and valid. The data is acceptable and the result can be used in the risk assessment. No further data on aquatic invertebrates on the product GF-1374 are required.

**B.9.3.1.3. Algal Growth Inhibition Tests with GF-1374**

Two algal growth inhibition studies with the new representative formulation GF-1374 were submitted, which have not been evaluated before in the context of renewal of the approval of clopyralid. Therefore the studies are evaluated below.

**CP 10.2.1.4/3: GF-1374 – 72-Hour acute toxicity test with the freshwater green alga *Pseudokirchneriella subcapitata***

| | |
|---|---|
| Report: | Hoberg, J. R. (2005a) |
| Title: | GF-1374 – 72-Hour acute toxicity test with the freshwater green alga *Pseudokirchneriella subcapitata*. |
| Document No: | Dow AgroSciences Study Number: 040270 |

| | |
|---|---|
| | Springborn Smithers Study Number: 12550.6338 |
| Guidelines: | OECD Guideline No. 201<br>EC Guideline L383A - Method C.3. |
| GLP | Yes |

**Methodology:** The inhibitory effect of GF-1374 on the growth of the unicellular green alga *Pseudokirchneriella subcapitata* was determined in a 72 hour study conducted to OECD Guideline No. 201 in accordance with GLP. GF-1374 (Lot No. 187/76-A) is an emulsion concentrate (EC) nominally containing fluroxypyr-meptyl at 144 g/L (142 g/L, actual), clopyralid at 80 g/L (86 g/L, actual) and florasulam at 2.5 g/L (2.5 g/L, actual). Three replicated 100 mL cultures of *P. subcapitata* in sterile enriched AAP medium, at an initial cell density of *ca*. $1.0 \times 10^4$ cells/mL, were exposed to a nominal test concentration series of 0.0056, 0.018, 0.057, 0.18, 0.59, 1.9 and 6.0 mg product/L. Three replicate controls were also prepared plus one additional replicate flask at 0.59 mg/L which was not inoculated. Finally one 1000-mL flask was prepared for each of the 0, 0.0056 and 0.018 mg/L treatments and was used for analytical purposes only. The cultures were incubated for 72 hours at 23 to 24°C under continuous illumination of 7000 to 7800 lux with constant stirring at 100 rpm. The pH of the test solutions ranged from 7.0 to 7.2 at the start of exposure and from 7.4 to 9.7 at the end of the test.

Samples were taken daily from each flask and the cell density measured using a haemacytometer. Exposure levels in the test media were monitored by measuring the concentrations of fluroxypyr-meptyl and its hydrolysis product, fluroxypyr, at 0 and 72 hours using an HPLC/UV method of analysis. Fluroxypyr-methylheptyl concentrations were converted to acid equivalents and added to the measured fluroxypyr acid concentrations to calculate the overall concentration in acid equivalents. Since the toxicity of GF-1374 cannot be attributed to any one or more of the active ingredients present in the product, results are expressed in terms of nominal concentration of total formulation.

After 72 hours of exposure, the replicate vessels of each treatment were pooled. Measured volumes of each composite solution were transferred to flasks containing 100 mL of untreated AAP medium to obtain an initial cell density of approximately $0.005 \times 10^4$ cells/mL. These cultures were incubated for a further 72 hours to determine if the effects of GF-1374 were algistatic or algicidal.

**Findings:** At the beginning of the study measured concentrations of fluroxypyr as total acid (acid + methylheptyl converted to acid equivalents) were 110, 110, 110, 99, 94, 93 and 97% of nominal for the 0.0056, 0.018, 0.057, 0.18, 0.59, 1.9 and 6.0 mg GF-1374/L treatments. Fluroxypyr acid was not detected at the start of the study. Over the course of the study the concentrations of the ester form decreased, while those of the acid form increased. By the end of the study the fluroxypyr ester was not detected in the treatments up to and including 0.18 mg GF-1374/L, but it was detected at concentrations between 11 and 69% of nominal in the three higher GF-1374 treatments. Mean measured concentrations of fluroxypyr as total acid ranged between 80 and 100% over course of the study.

Algal cells in the control cultures increased from *ca*. $1 \times 10^4$ cells/mL to an average of *ca*. $136.39 \times 10^4$ cells/mL during the 72 hour test period. Exposure of *P. subcapitata* to GF-1374 resulted in significant inhibition in growth rates at nominal concentrations of 0.59 mg product/L and above when compared to

control cultures (Williams' test, p = 0.05). The NOEC for growth rate was therefore determined to be 0.18 mg product/L. For the area under the growth curve, no statistically significant differences were determined up to the highest concentration tested of 6.0 mg GF-1374/L (Kruskal-Wallis' test). The NOEC was empirically estimated to be 0.057 mg GF-1374/L, the highest concentration at which less than 10% inhibition compared to the control was observed.

During the 72 hour recovery phase, algal cells previously exposed to concentrations ≤ 1.9 mg product/L yielded cell densities similar to or greater than the control, thereby indicating that the inhibitory effect of GF-1374 at these concentrations is algistatic rather than algicidal.

**Table 9.3.5. Inhibition of growth**

| Nominal concentration (mg GF-1374/L) | Area under growth curve at 72 h ($\times 10^4$) | | Average specific growth rate 0-72-h ($d^{-1}$) | |
|---|---|---|---|---|
| | Mean | % inhibition | Mean | % inhibition |
| Control | 84.31 | -- | 1.69 | -- |
| 0.0056 | 75.22 | 11 | 1.62 | 4 |
| 0.018 | 85.54 | -1 | 1.61 | 5 |
| 0.057 | 83.92 | 0 | 1.64 | 3 |
| 0.18 | 71.64 | 15 | 1.66 | 2 |
| 0.59 | 55.49 | 34 | 1.57* | 7 |
| 1.9 | 20.53 | 76 | 1.20* | 29 |
| 6.0 | -0.89 | 101 | -0.10* | 106 |

* Significantly reduced compared to control

**Table 9.3.6. Recovery phase after the 72 hour exposure phase**

| Nominal concentration in exposure phase (mg GF-1374/L) | Mean cell density ($\times 10^4$ cells/mL) | | | |
|---|---|---|---|---|
| | Estimated initial density | 24 h | 48 h | 72 h |
| Control | 0.0055 | 2.00 | 29.33 | 169.42 |
| 0.0056 | 0.0050 | 2.25 | 25.50 | 120.33 |
| 0.018 | 0.0050 | 2.25 | 29.67 | 173.64 |
| 0.057 | 0.0056 | 3.08 | 35.17 | 158.53 |
| 0.18 | 0.0050 | 2.00 | 32.17 | 204.06 |
| 0.59 | 0.0050 | 3.42 | 30.67 | 147.58 |
| 1.9 | 0.0050 | 2.00 | 24.50 | 120.50 |
| 6.0 | 0.0050 | 0.00 | 0.00 | 0.08 |

Analysis of the "area under the growth curve" and the "average specific growth rate" data gave the $E_bC_{50}$ and $E_rC_{50}$ values summarised in the following Table 9.3.7.

**Table 9.3.7. Results**

| End-point | mg GF-1374/L, nominal |
|---|---|
| 72-h $E_bC_{50}$ (95% c.l.) | 1.1 (0.92-1.2) |
| 72-h $E_rC_{50}$ (95% c.l.) | 3.1 (3.0-3.2) |
| $NOEC_b$ | 0.057 (estimated) |
| $NOEC_r$ | 0.18 |

**Conclusions:** The 72-h $E_bC_{50}$ and $E_rC_{50}$ values for GF-1374 to the green alga *Pseudokirchneriella subcapitata* are 1.1 and 3.1 mg product/L, respectively, based on nominal concentrations of the formulation. The NOEC for biomass has been estimated to be 0.057 mg product/L, being this the highest concentration at which an inhibition lower than 10% was observed. The NOEC for growth rate was 0.18 mg GF-1374/L.

RMS comments and evaluation:

The algal growth inhibition study with the new representative formulation GF-1374 was well performed and reported, according to the test guideline and GLP, except the routine water contaminant analyses that were conducted in another laboratory using validated standard methods. This deviation was considered to have no impact on the results. In the analytical part of the study, the measured concentrations of test substance relied on measurements of only one of the active substances in the formulation, fluroxypyr, although the test substance GF-1374 contained three active substances.

A few deviations to study protocol were reported. The number of control vessels was less than stated in the study protocol. During the study the pH of the control solutions increased by 2.6 units. However, this deviation was not considered to have impacted the study results, because the increase in solution pH was due to photosynthesis by the algae and is representative of rapid cell growth. Despite the increase in pH, the growth curve for the control indicates the culture was in log phase growth, demonstrating the conditions were still optimal for growth and not limiting during the 72-hour test.

At the initiation of the recovery phase, a calculation error in cell densities happened between the treated and control samples. However, at the termination of the recovery phase, the mean growth rates for the treatments, with the exception of the highest treatment, 6.0 mg formulation/L, deviated <3% from the mean control value, indicating that growth recovery could not be distinguished.

These deviations were not considered to have negative impact on the results. Otherwise the study was of good quality and valid. The data is acceptable and the result can be used in the risk assessment.

Furthermore, one study with diatoms as a second taxonomic group of algae was submitted by the Notifier, and not evaluated before, and is therefore presented below.

**CP 10.2.1.4/4: GF-1374 – Acute toxicity to the freshwater diatom *Navicula pelliculosa***

| | |
|---|---|
| Report: | Hoberg, J. R. (2005b) |
| Title: | GF-1374 – Acute toxicity to the freshwater diatom *Navicula pelliculosa*. |
| Document No: | Dow Agrosciences Study Number: 040272<br>Springborn Smithers Study Number: 12550.6340 |
| Guidelines: | OECD Guideline No. 201<br>EC Guideline L383A - Method C.3. |
| GLP | Yes |

**Methodology:** The inhibitory effect of GF-1374 on the growth of the diatom *Navicula pelliculosa* was determined in a 96 hour study conducted to OECD Guideline No. 201 in accordance with GLP. GF-1374 (Lot No. 187/76-A) is an emulsion concentrate (EC) nominally containing fluroxypyr-meptyl at 144 g/L (142 g/L, actual), clopyralid at 80 g/L (86 g/L, actual) and florasulam at 2.5 g/L (2.5 g/L, actual). Three replicated 100 mL cultures of *N. pelliculosa* in sterile enriched AAP medium, at an initial cell density of *ca*. $1.0 \times 10^4$ cells/mL, were exposed to a nominal test concentration series of 0.31, 0.63, 1.3, 2.5, 5.0 and 10 mg product/L. Six replicate controls were also prepared plus one additional replicate flask at 2.5 mg/L which was not inoculated. The cultures were incubated for 96 hours at 24°C under continuous illumination of 3200 to 5400 lux with constant stirring at 100 rpm. The pH of the test solutions ranged from 7.0 to 7.2 at the start of exposure and from 6.7 to 8.7 at the end of the test.

Samples were taken daily from each flask and the cell density measured using a haemacytometer. Exposure levels in the test media were monitored by measuring the concentrations of fluroxypyr-meptyl and its hydrolysis product, fluroxypyr, at 0 and 96 hours using an HPLC/UV method of analysis. Fluroxypyr-methylheptyl concentrations were converted to acid equivalents and added to the measured fluroxypyr acid concentrations to calculate the overall concentration in acid equivalents. Since the toxicity of GF-1374 cannot be attributed to any one or more of the active ingredients present in the product, results are expressed in terms of nominal concentration of total formulation.

**Findings:** At the beginning of the study measured concentrations of fluroxypyr as total acid (acid + methylheptyl converted to acid equivalents) were 93, 89, 88, 89, 91 and 92% of nominal for the 0.31, 0.63, 1.3, 2.5, 5.0 and 10 mg GF-1374/L treatments. Fluroxypyr acid was not detected at the start of the study. Over the course of the study the concentrations of the ester form decreased, while those of the acid form increased. By the end of the study the fluroxypyr ester was not detected in the treatments up to and including 1.3 mg GF-1374/L, but it was detected at concentrations between 30 and 38% of nominal in the three higher GF-1374 treatments. Mean measured concentrations of fluroxypyr as total acid ranged between 74 and 84% over course of the study.

Algal cells in the control cultures increased from *ca*. $1 \times 10^4$ cells/mL to an average of *ca*.$78.71 \times 10^4$ cells/mL during the 96 hour test period. After 72 hours of exposure the mean cell density in the controls vessels was $30.50 \times 10^4$ cells/mL and it was, therefore, increased at least 16 times since the study initiation. However, due to the analytical laboratory schedule, the test was continued up to 96 hours of exposure.

Exposure of *N. pelliculosa* to GF-1374 resulted in significant inhibition in biomass and growth rates, respectively, at nominal concentrations of 1.3 and 2.5 mg product/L and above when compared to control cultures (Williams' test, $p = 0.05$). The NOEC for biomass and growth rate were therefore determined to be 0.63 and 1.3 mg product/L, respectively.

**Table 9.3.8. Inhibition of growth**

| Nominal concentration (mg GF-1374/L) | Area under growth curve at 72 h ($\times 10^4$) | | Average specific growth rate 0-72-h ($d^{-1}$) | |
|---|---|---|---|---|
| | Mean | % inhibition | Mean | % inhibition |
| Control | 19.71 | -- | 1.17 | -- |

| | | | | |
|---|---|---|---|---|
| 0.31 | 21.70 | -10 | 1.22 | -4 |
| 0.63 | 15.78 | 20 | 1.09 | 7 |
| 1.3 | 11.29* | 43 | 1.00 | 15 |
| 2.5 | -0.89* | 105 | -0.10* | 109 |
| 5.0 | -0.90* | 105 | -0.24* | 121 |
| 10 | -1.53* | 108 | -0.23* | 120 |

* Significantly reduced compared to control

Analysis of the "area under the growth curve" and the "average specific growth rate" considering the cell count data gave the $E_bC_{50}$ and $E_rC_{50}$ values summarised in the following table.

**Table 9.3.9. Results**

| End-point | mg GF-1374/L, nominal |
|---|---|
| 72-h $E_bC_{50}$ (95% c.l.) | 1.4 (1.0-1.7) |
| 72-h $E_rC_{50}$ (95% c.l.) | 1.7 (1.7-2.0) |
| $NOEC_b$ | 0.63 |
| $NOEC_r$ | 1.3 |

**Conclusions:** The 72-h $E_bC_{50}$ and $E_rC_{50}$ values for GF-1374 to the diatom *Navicula pelliculosa* are 1.4 and 1.7 mg product/L, respectively, based on nominal concentrations of the formulation. The NOEC for biomass and growth rate have been estimated to be 0.63 and 1.3 mg GF-1374/L, respectively.

RMS comments and evaluation:

The second algal growth inhibition study with the new representative formulation GF-1374 was well performed and reported, according to the test guideline and GLP, except the routine water contaminant analyses that were conducted in another laboratory using validated standard methods. This deviation was considered to have no impact on the results. In the analytical part of the study, the measured concentrations of test substance relied on measurements of only one of the active substances in the formulation, fluroxypyr, although the test substance GF-1374 contained three active substances. Therefore the concentrations of clopyralid are nominal only.

The 72-hour mean control cell density ($30.5 \times 10^4$ cells/mL) exceeded the 16 times increase required by the protocol. Due to the analytical laboratory schedule, the test was however not terminated and the analytical samples were not collected until 96 hours. The biological endpoints for this study are based on 72-hour cell counts and analytical results are representative of the 96-hour test solutions. This deviation was not considered to have negative impact on the outcome since the results are based on nominal concentrations of formulation. Otherwise the study was of good quality and valid.

The data is acceptable and the result can be used in the risk assessment. No further algal studies with the product GF-1374 are required.

**B.9.3.1.4. Toxicity of GF-1374 to aquatic plants**

The Notifier submitted two studies on the toxicity of the new representative formulation GF-1374 to aquatic plants. First, the following *Lemna* toxicity study performed on GF-1374 is provided in support of the assessment and has not been previously evaluated.

**CP 10.2.1.3/5. GF-1374 - Toxicity to duckweed, *Lemna gibba***

| | |
|---|---|
| Report: | Hoberg, J. R. 2005c |
| Title: | GF-1374 – Toxicity to duckweed, *Lemna gibba*. |
| Document No: | Dow Agrosciences Study Number: 040271<br>Springborn Smithers Study Number: 12550.6339 |
| Guidelines: | OECD proposed Guideline No. 221<br>U.S. EPA FIFRA Guidelines 122-2 and 123-2 |
| GLP | Yes |

**Methodology:** The inhibitory effect of GF-1374 on the growth of the duckweed *Lemna gibba* was determined in a 7 day semi-static study conducted to OECD 221 proposed guideline in accordance with GLP. GF-1374 (Lot No. 187/76-A) is an emulsion concentrate (EC) nominally containing fluroxypyr-meptyl at 144 g/L (142 g/L, actual), clopyralid at 80 g/L (86 g/L, actual) and florasulam at 2.5 g/L (2.5 g/L, actual). Six replicated 100 mL cultures of *Lemna gibba* in sterile 20X-AAP medium, at an initial density of five plants each with three fronds, were exposed to a nominal test concentration series of 0.010, 0.032, 0.10, 0.32 and 1.0 mg product/L. Six replicate controls were also prepared. Finally one additional replicate was prepared for the control and the 0.010 mg/L treatments and was used for analytical purposes on Day 3. Test solutions were renewed on Days 3 and 5 of the study. The cultures were incubated for a maximum of 7 days at 23 to 24°C under continuous illumination of 8100-9100 lux. The pH in the vessels ranged from 7.5 to 8.0 in the newly prepared solutions to 8.3 to 8.9 in the aged test solutions.

Fronds were counted and observations made on Days 3, 5 and 7 until the end of the exposure period. On Day 7, after frond density determinations and removal of the necessary fronds for initiation of the recovery phase, the remaining fronds were dried to determine their dry weight.

Exposure levels in the test media were monitored by measuring the concentrations of fluroxypyr-meptyl and its hydrolysis product, fluroxypyr, on Days 0 and 3 using an HPLC/UV method of analysis. Fluroxypyr-methylheptyl concentrations were converted to acid equivalents and added to the measured fluroxypyr acid concentrations to calculate the overall concentration in acid equivalents. Since the toxicity of GF-1374 cannot be attributed to any one or more of the active ingredients present in the product, results are expressed in terms of nominal concentration of total formulation.

After 3 and 7 days of exposure, a set of three exposure vessels for each treatment was terminated and five plants with three fronds each were transferred from each of the three replicates into fresh medium. The fronds from Day 3 and 7 were monitored for 11 and 14 days post-exposure, respectively, to determine if the inhibitory effects of GF-1374 were reversible. Test conditions

were consistent with those of the exposure phase. At the end of each of the recovery phases, frond dry weight was determined.

**Findings:** At the beginning of the study measured concentrations of fluroxypyr as total acid (acid + methylheptyl converted to acid equivalents) were 120, 110, 100, 140 and 49% of nominal for the 0.010, 0.032, 0.10, 0.32 and 1.0 mg GF-1374/L treatments. Fluroxypyr acid was not detected at the start of the study. During the first three days of the study the concentrations of the ester form decreased, while those of the acid form increased. On Day 3, the fluroxypyr ester was detected at concentrations between 5.6 and 26% of nominal in the GF-1374 treatments. Mean measured concentrations of fluroxypyr as total acid ranged between 62 and 110% between Days 0 and 3. The highest test concentration was adjusted to 0.62 mg GF-1374/L based on the percent recovery of fluroxypyr mean acid equivalents in the 1.0 mg GF-1374/L treatment. Results for this treatment are therefore not based on nominal formulation concentrations.

Frond counts in the control cultures increased from 15 fronds/vessel to a mean of 407.67 fronds/vessel during the 7 day test period. On Day 7, fronds exposed to the 0.32 and 1.0 mg GF-1374/L treatments were slightly chlorotic. Fronds from lower treatments and the control appeared to be normal. Exposure of *L. gibba* to GF-1374 resulted in significant inhibition in frond production at, or above, concentrations of 0.10 mg product/L, when compared to control cultures (William's test, $p \leq 0.05$). The NOEC for frond production was therefore determined to be 0.032 mg product/L.

During both the 11-day and 14-day recovery phases for fronds exposed, respectively, for 3 and 7 days to the test substance, no significant reduction in frond density was observed in any of the treatments relative to the controls. Consequently the inhibitory effect on growth was reversible. In the 14-day recovery phase, the dry weight of fronds previously exposed for 7 days to 0.32 and 0.62 mg GF-1374/L differed significantly from that of the control (William's test, $p \leq 0.05$).

**Table 9.3.10. Inhibition of growth**

| Nominal concentration (mg GF-1374/L) | Frond production 0-7 d | | Average growth rate 0-7 d ($d^{-1}$) | | Frond dry weight after 7 d (g) | |
|---|---|---|---|---|---|---|
| | Mean | % inhibition | Mean | % inhibition | Mean | % inhibition |
| Control | 407.67 | -- | 0.47 | -- | 0.0539 | -- |
| 0.010 | 396.00 | 2.9 | 0.47 | 0 | 0.0492 | 9 |
| 0.032 | 393.00 | 3.6 | 0.47 | 0 | 0.0455* | 16 |
| 0.10 | 317.00* | 22 | 0.44* | 6 | 0.0402* | 25 |
| 0.32 | 134.30* | 67 | 0.31* | 34 | 0.0272* | 50 |
| 0.62 [a] | 28.70* | 93 | 0.09* | 81 | 0.0088* | 84 |

[a] The nominal concentration of 1.0 mg GF-1374/L has been adjusted to 0.62 mg/L, based on the total recovery of fluroxypyr acid in this treatment level. * Significantly reduced compared to control.

**Table 9.3.11. 11-day and 14-day recovery phases after 3 and 7 days of exposure**

| Nominal concentration (mg GF-1374/L) | 11-day recovery after 3-day exposure | | 14-day recovery after 7-day exposure | |
|---|---|---|---|---|
| | Frond production 0-11 d | Frond dry weight after 11 d (g) | Frond production 0-14 d | Frond dry weight after 14 d (g) |

| | **Mean** | **% inhibition** | **Mean** | **% inhibition** | **Mean** | **% inhibition** | **Mean** | **% inhibition** |
|---|---|---|---|---|---|---|---|---|
| Control | 597.00 | -- | 0.0722 | -- | 683.00 | -- | 0.1351 | -- |
| 0.010 | 689.00 | -15 | 0.0929 | -29 | 727.67 | -6.5 | 0.1208 | 11 |
| 0.032 | 602.00 | -0.84 | 0.0789 | -9 | 873.67 | -28 | 0.1131 | 16 |
| 0.10 | 597.67 | 0.0 | 0.0786 | -9 | 770.33 | -13 | 0.1172 | 13 |
| 0.32 | 663.67 | -11 | 0.0759 | -5 | 829.33 | -21 | 0.0986* | 27 |
| 0.62 [a] | 544.33 | 8.8 | 0.0736 | -2 | 612.00 | 10 | 0.0815* | 40 |

[a] The nominal concentration of 1.0 mg GF-1374/L has been adjusted to 0.62 mg/L, based on the total recovery of fluroxypyr acid in this treatment level.
* Significantly reduced compared to control.

The calculated $EC_{50}$ values are summarised in the following table.

**Table 9.3.12. Results**

| **End-point** | **mg GF-1374/L, nominal** |
|---|---|
| 7-d $EC_{50}$ (95% c.l.) | 0.24 (0.22-0.25) |
| 7-d $E_rC_{50}$ (95% c.l.) | 0.42 (0.41-0.43) |
| 7-d $E_bC_{50}$ (95% c.l.) | 0.32 (0.29-0.35) |
| NOEC | 0.032 |
| $NOEC_r$ | 0.032 |
| $NOEC_b$ | 0.010 |

Biomass results are based on frond dry weight results.

**Conclusions:** The 7-day $EC_{50}$ value for GF-1374 to the duckweed *Lemna gibba* is 0.24 mg product/L, based on nominal concentrations of total formulation. The NOEC is 0.032 mg product/L. The 7-day $EC_{50}$ values for growth rate and frond dry weight are 0.42 and 0.32 mg GF-1374/L, respectively.

RMS comments and evaluation:

The growth inhibition study on duckweed with the new representative formulation GF-1374 was well performed and reported, according to the test guideline and GLP, except the routine water contaminant analyses that were conducted in another laboratory using validated standard methods. This deviation was considered to have no impact on the results.

In the analytical part of the study, the measured concentrations of test substance relied on measurements of only one of the active substances in the formulation, fluroxypyr, although the test substance GF-1374 contained three active substances. Therefore the concentrations of clopyralid are nominal only. Otherwise the study was of good quality and valid. The data is acceptable and the result can be used in the risk assessment.

Second, the following *Myriophyllum spicatum* toxicity study with the representative formulation GF-1374 was submitted in support of the renewal of approval of clopyralid, which has not been previously evaluated.

**CP 10.2.1.3/6. GF-1374 - Growth Inhibition of *Myriophyllum spicatum* in a Water/Sediment System**

| | |
|---|---|
| Report: | Gonsior, G, 2013 |
| Title: | GF-1374 - Growth Inhibition of *Myriophyllum spicatum* in a Water/Sediment System. |
| Document No: | Dow AgroSciences Study Number: 121208<br>Eurofins Agroscience Services EcoChem GmbH: S13-00058-L1_AAMs |
| Guidelines: | Draft AMRAP Method (Maltby et al. 2010):<br>Growth Inhibition Test for the Rooted Aquatic Macrophyte, *Myriophyllum* sp.<br>Submitted to OECD for Evaluation, 2011 |
| GLP | Yes |

## Test material

| | |
|---|---|
| Name: | GF-1374 |
| Test item code: | 2013-000180 |
| Test substance no.: | TSN303060 |
| Batch/Lot no.: | 1C03150102 |
| Active substances, CAS-numbers and their content (analysed): | Clopyralid, 1702-17-6, 7.84 % w/w<br>Florasulam, 145701-23-1, 0.23 % w/w<br>Fluroxypyr-meptyl, 81406-37-3, 13.9 % w/w |
| Appearance/colour: | liquid/brown |
| Certificate of analysis: | 01 May 2012 |
| Expiration date: | 18 April 2014 |
| Storage conditions: | 5°C to ambient |

## Test system

| | |
|---|---|
| Organism (*Species*): | *Myriophyllum spicatum* L. |
| Study Type: | Growth Inhibition of *Myriophyllum spicatum* in a Water/Sediment System |
| GLP Status: | GLP |
| Guidelines followed: | Based on the draft AMRAP Method (MALTBY et al. 2010): Growth Inhibition Test for the Rooted Aquatic Macrophyte, *Myriophyllum sp*. which was submitted to OECD for evaluation, 2011. |
| Guideline deviations reported by Study Director: | Minor deviations (more replicates, plant material for testing was cut to a shoot length of 5 cm instead of 6 cm) |
| Protocol deviations reported by the Study Director: | None |
| Duration of study: | 14 days |
| Parameters measured: | The average pH-value was determined to be 7.98 ± 0.54, the average temperature was measured to be 20.5 ± 1.0 °C and the oxygen saturation was determined to be 94 ± 13 %. The test item had no influence on the pH-value of the test solutions. |
| Environmental conditions: | Test vessels were maintained in a controlled environment at 20.5 ± 1.0 °C under warm and/or cool white fluorescent lighting (approx. 8000 lux at the water surface) with a 16-h day-length |
| Observation intervals: | 0, 7, 14 days |
| Test concentrations: | Nominal: 2.98, 9.54, 30.5, 97.7, 313, and 1000 µg/L GF-1374.<br>The measured concentration of the test item (GF-1374) in the test vessels at test start ranged between 66 and 101 % of nominal in the overlaying water. The mean measured content for all concentrations at test start was 82 %. As the mean content of the test item was between 80 and 120 % of nominal at test start, all toxicological endpoints were evaluated using nominal concentrations of the test item (GF-1374). Due to the fast degradation of fluroxypyr-meptyl, test item concentrations were calculated based on the content of fluroxypyr-meptyl and his acid hydrolysis product.<br>After 14 days fluroxypyr-meptyl concentrations in the water and sediment were below the limit of quantification. |

| | |
|---|---|
| | In contrast the fluroxypyr acid concentration increased after 14 days. The measured concentration in the test vessels after 14 days ranged between 40 and 87 % of nominal in the overlaying water. The mean measured content at test end was 64 %. In the sediment 15 and 13% of fluroxypyr acid was detected at the two highest treatments. |
| Growth medium | SMART AND BARKO medium |
| Method of test item added to the test medium | 10 mL per 1.5 L test medium |
| No. of control replicates | 10 |
| No. of test concentration replicates | 5 |
| Analytical verification: | An analytical method for the determination of fluroxypyr-meptyl and fluroxypyr acid were validated with regard to recovery (accuracy), linearity of detector response, repeatability (precision) and specificity. The analytical system fulfils the requirements of guideline SANCO/3029/99 rev. 4, 11/07/2000. |

## Methodology

Approximately 350 g of moist sediment were transferred to the test vessels. The surface was overlaid with a thin layer of washed quartz sand to minimise displacement of the sediment when the 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 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. Ten replicates were used for the control and five for each treatment group.

On day 0 ten 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. The weight of the dry plant samples was recorded.

On day 14 plants were harvested from each treatment group for assessment of plant fresh weight, 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. Data were used to calculate the following parameters for each plant, as presented in Table 9.3.13.

**Table 9.3.13. Myriophyllum growth parameters**

| Parameter | Day 14 |
|---|---|
| growth rate for total shoot length | X |
| yield for total shoot length | X |
| growth rate for plant fresh weight | X |
| yield for plant fresh weight | X |
| growth rate for plant dry weight | X |
| yield for plant dry weight | X |

For each of these parameters, yield and growth rate $EC_{50}$ values were calculated (if possible) and in addition the NOEC and LOEC were determined, if possible.

### Statistical analysis

**LOEC/NOEC Determination:** 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 test was performed. If data were normally distributed and variance was

homogeneous a Dunnett's test was performed. If data were normally distributed, but the variance was not homogeneous a Bonferroni-Holms corrected Welch's test was performed. If Shapiro Wilks test indicated a non-normal distribution of residuals a Bonferroni-U Exact Test was performed to determine significant differences from controls (SAS® Proprietary Software 9.2).

**$EC_{50}$ Estimation:** The $EC_{50}$ (yield and growth rate) was calculated where possible using Probit analysis. Only concentrations within the dose response were used for calculations.

**Results**

The measured concentration of the test item (GF-1374) in the test vessels at test start ranged between 66 and 101 % of nominal in the overlaying water. The mean measured content for all concentrations at test start was 82 %. As the mean content of the test item was between 80 and 120 % of nominal at test start, all toxicological endpoints were evaluated using nominal concentrations of the test item (GF-1374). Due to the fast degradation of fluroxypyr-meptyl, test item concentrations were calculated based on the content of fluroxypyr-meptyl and his acid hydrolysis product. All values were corrected by the recovery of the specific analyte.

After 14 days fluroxypyr-meptyl concentrations in the water and sediment were below the limit of quantification. In contrast the fluroxypyr acid concentration in water increased after 14 days. The measured concentration in the test vessels after 14 days ranged between 40 and 87 % of nominal in the overlaying water. The mean measured content at test end was 64 %. In the sediment 15 and 13% of fluroxypyr acid was detected at the two highest treatments.

The control plants showed uniform growth over the test period of 14 days, with strongly growing side shoots. Over 14 days, the mean total shoot length increased more than 2.5-fold, fresh weight biomass increased more than 4.5-fold, and mean dry weight biomass increased more than 4.0-fold.

Mean total shoot lengths after 14 days, yield and growth rate for each treatment group are presented in Tables 9.3.14 to 9.3.16 below.

**Table 9.3.14. 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 | 22.3 | 14.1 | 0.0 | 0.0697 | 0.0 |
| 2.98 | 8.2 | 20.2 | 12.0 | 14.9 | 0.0633 | 9.2 |
| 9.54 | 8.2 | 22.4 | 14.2 | -0.7 | 0.0712 | -2.2 |
| 30.5 | 8.2 | 22.6 | 14.4 | -2.1 | 0.0713 | -2.3 |
| 97.7 | 8.2 | 18.8 | 10.6 | 24.8 | 0.0586 | 15.9 |
| 313 | 8.2 | 16.4 | 8.2* | 41.8* | 0.0491 | 29.6 |
| 1000 | 8.2 | 11.4 | 3.2* | 77.3* | 0.0237* | 66.0* |

* significantly different reduction compared to the control

[1)] based on 10 additional plants, representative of those used in the test

Total plant fresh weight, as well as growth rate and yield based on fresh weight increase, for each replicate of each treatment group are presented below.

**Table 9.3.15. Mean total plant fresh weight (g)**

| Nominal concentration [µg/L] | Days after application | | yield [g] | reduction in yield [%] | growth rate [1/day] | reduction in growth rate [%] |
|---|---|---|---|---|---|---|
| | 0[1)] | 14 | | | | |
| Control | 0.2469 | 1.1436 | 0.8967 | 0.0 | 0.1081 | 0.0 |
| 2.98 | 0.2469 | 1.0303 | 0.7834 | 12.6 | 0.0998 | 7.7 |
| 9.54 | 0.2469 | 0.9992 | 0.7523 | 16.1 | 0.0990 | 8.4 |
| 30.5 | 0.2469 | 1.1466 | 0.8997 | -0.3 | 0.1092 | -1.0 |
| 97.7 | 0.2469 | 0.7737 | 0.5268* | 41.3* | 0.0798* | 26.2* |
| 313 | 0.2469 | 0.5365 | 0.2896* | 67.7* | 0.0552* | 48.9* |
| 1000 | 0.2469 | 0.4478 | 0.2009* | 77.6* | 0.0422* | 61.0* |

* significantly different reduction compared to the control
1) based on 10 additional plants, representative of those used in the test

Total plant dry weight (Table 9.3.16), as well as growth rate and yield based on shoot length (Table 9.3.17) and fresh and dry weight increase (Tables 9.3.18 and 9.3.19), for each replicate of each treatment group are presented below.

**Table 9.3.16. Mean total plant dry weight (g)**

| Nominal concentration [µg/L] | Days after application | | yield [g] | reduction in yield [%] | growth rate [1/day] | reduction in growth rate [%] |
|---|---|---|---|---|---|---|
| | 0[1)] | 14 | | | | |
| Control | 0.0529 | 0.2303 | 0.1774 | 0.0 | 0.1047 | 0.0 |
| 2.98 | 0.0529 | 0.1994 | 0.1465 | 17.5 | 0.0914 | 12.7 |
| 9.54 | 0.0529 | 0.1732 | 0.1203* | 32.2* | 0.0832* | 20.5* |
| 30.5 | 0.0529 | 0.1836 | 0.1307* | 26.3 | 0.0879* | 16.0* |
| 97.7 | 0.0529 | 0.1478 | 0.0949* | 46.5* | 0.0709* | 32.3* |
| 313 | 0.0529 | 0.1191 | 0.0662* | 62.7* | 0.0578* | 44.8* |
| 1000 | 0.0529 | 0.1007 | 0.0478* | 73.1* | 0.0458* | 56.3* |

* significantly different reduction compared to the control
1) based on 10 additional plants, representative of those used in the test

**Table 9.3.17. Summary of Biological Results based on Nominal Concentrations and Total Shoot Length**

| Parameter | Growth rate (mean total shoot length in cm) [µg/L] | Yield (mean total shoot length in cm) [µg/L] |
|---|---|---|
| 14-day $EC_{50}$ | 570 | 360 |
| 95% Conf. Limits of $EC_{50}$ | 455 - 752 | 295 - 448 |
| 14-day NOEC | 313 | 97.7 |
| 14-day LOEC | 1000 | 313 |

ECx_values calculated using Probit analysis

**Table 9.3.18. Summary of Biological Results based on Nominal Concentrations and Fresh Weight**

| Parameter | Growth rate (mean total plant fresh weight in g) [µg/L] | Yield (mean total plant fresh weight in g) [µg/L] |
|---|---|---|
| 14-day $EC_{50}$ | 444 | 216 |
| 95% Conf. Limits of $EC_{50}$ | 347 – 599 | 175 - 265 |
| 14-day NOEC | 30.5 | 30.5 |
| 14-day LOEC | 97.7 | 97.7 |

ECx_values calculated using Probit analysis

**Table 9.3.19. Summary of Biological Results based on Nominal Concentrations and Dry Weight**

| Parameter | Growth rate (mean total plant dry weight in g) [µg/L] | Yield (mean total plant dry weight in g) [µg/L] |
|---|---|---|
| 14-day $EC_{50}$ | 525 | 148 |
| 95% Conf. Limits of $EC_{50}$ | 339 - 1011 | 101 - 213 |
| 14-day NOEC | 2.98 | 2.98 |
| 14-day LOEC | 9.54 | 9.54 |

ECx_values calculated using Probit analysis

## Conclusions

Following exposure of the aquatic macrophyte *Myriophyllum spicatum* to GF-1374 for 14 days, the $E_rC_{50}$ and $E_yC_{50}$ values based on total shoot length were 570 µg/L and 360 µg/L respectively. The NOEC for yield and growth rate based on total shoot length was 97.7 µg/L and 313 µg/L.

The $E_rC_{50}$ and $E_yC_{50}$ values based on biomass (fresh weight) were 444 µg/L and 216 µg/L respectively. The NOEC for yield and growth rate based on biomass (fresh weight) was 30.5 µg/L.

The $E_rC_{50}$ and $E_yC_{50}$ values based on biomass (dry weight) were observed at 525 µg/L and 148 µg/L respectively. The NOEC for growth rate and yield based on biomass (dry weight) was 2.98 µg/L.

RMS comments and evaluation:

The growth inhibition study on Myriophyllum with the new representative formulation GF-1374 was well performed and reported, according to the test guideline and GLP. In the analytical part of the study, the measured concentrations of test substance relied on measurements of only one of the active substances in the formulation, fluroxypyr, although the test substance GF-1374 contained three active substances. Therefore the concentrations of clopyralid are nominal only.

The minor deviations from the test protocol reported were considered to have no impact on the results. The validity criteria of this study are fulfilled and the results can be used in the risk assessment. The data is adequate for the risk assessment and no further studies are required.

### B.9.3.2. Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms

Chronic toxicity tests with the formulation were not performed as it is possible to extrapolate from data obtained with the active substance (see M-CA Points 8.2.2 and 8.2.5). This is confirmed by the model deviation ratio which also suggests chronic testing with the formulation were not necessary for fish or aquatic invertebrates (MDR $\leq$ 0.12) and that fluroxypyr meptyl is the toxic driver (contributes 100% of the formulation toxicity or toxic units). Please refer to EFSA conclusion 2011 for fluroxypyr risk assessment. The clopyralid risk assessment is presented in Chapter 9.4. for consideration. Overall, it was determined that formulation testing was not required and that chronic toxicity can be extrapolated from the active substances as demonstrated in Table 9.3.20. below.

**Table 9.3.20. Aquatic mixture toxicity assessment for GF-1374 for fish and Daphnia**

| Compound | Content in the formulation (%) | Fraction in mixture (%) | $LD_{50}$ (mg/L) | Fraction / LD50 | Tox per fraction | Contribution to predicted toxicity (%) |
|---|---|---|---|---|---|---|
| ***Fish*** | | | | | | |
| Clopyralid | 7.69 | 35.31 | 99.9 | 0.00353450 | 282.9417425 | 0.12 |
| Florasulam | 0.24 | 1.10 | 100 | 0.00011019 | 9075 | 0.04 |
| Fluroxypyr meptyl | 13.85 | 63.59 | 0.225 | 2.82624222 | 0.353826715 | 99.87 |
| Total | 21.78 | 100.00 | | 2.82988671 | 0.353371037 | 100.00 |
| GF-1374 | -- | -- | 7.1 (measured) | | 0.35 (predicted) | MDR = 0.05 |
| ***Daphnia*** | | | | | | |
| Clopyralid | 7.69 | 35.31 | 99.0 | 0.00353430 | 282.9417425 | 0.10 |
| Florasulam | 0.24 | 1.10 | 292 | 0.00003774 | 26499 | 0.001 |
| Fluroxypyr meptyl | 13.85 | 63.59 | 0.183 | 3.47488789 | 0.287779061 | 99.90 |
| Total | 21.78 | 100.00 | | 3.47846001 | 0.287483541 | 100.00 |
| GF-1374 | -- | -- | 6.9 (measured) | | 0.29 (predicted) | MDR = 0.04 |

RMS comments and evaluation:

The data presented by the Notifier above is acceptable and adequate to perform a long-term risk assessment to aquatic organisms. For both fish and Daphnia, the predicted mixture toxicity as calculated with the concentration addition method is higher than the measured toxicity of the product GF-1374. No further data are required.

### B.9.3.3. Further testing on aquatic organisms

No further data is presented for other aquatic organisms since low acute and chronic risks have been demonstrated for the active substance clopyralid and GF-1374 on standard aquatic species fish, daphnia, algae, aquatic plants, and chironomids. Additionally, GF-1374 is not an insecticidal formulation and is not used directly on surface waters. For study summaries with the active substances, please refer to the M-CA document (Section 8).

| RMS comments and evaluation: |
|---|
| The explanation given by the Notifier is acceptable and agreed, and no further data are required. |

## B.9.4. RISK ASSESSMENT FOR AQUATIC ORGANISMS

The following Table 9.4.1. provides a justification for the use of a different study to address effects on aquatic organisms to that evaluated for the Active Approval.

**Table 9.4.1. Justification for data used in the aquatic risk assessment**

| Data Point/Study | Rationale |
|---|---|
| CP 10.2.1-1 [redacted] (2005)<br>DAS Study ID 040342 | The study is a new representative formulation not previously reviewed for the active approval of clopyralid. |
| CP 10.2.1-2 Sayers (2005)<br>DAS Study ID 040343 | The study is a new representative formulation not previously reviewed for the active approval of clopyralid. |
| CA 8.2.6.1-2 Aufderheide (2015)<br>DAS Study ID 140515 | The study has been submitted for review in order to fulfill new data requirements Regulation (EC) No 1107/2009 and Aquatic Guidance Document EFSA Journal 2013:11(7);3290 |
| CA 8.2.6.1-3 Hoberg (2006)<br>DAS Study ID 060246 | This study was conducted for different geography and is submitted for review. |
| CP 10.2.1-3 Hoberg (2005)<br>DAS Study ID 040270 | The study is a new representative formulation not previously reviewed for the active approval of clopyralid. |
| CP 10.2.1-4 Hoberg (2005)<br>DAS Study ID 040272 | The study is a new representative formulation not previously reviewed for the active approval of clopyralid. |
| CA 8.2.7.3-1 Banman (2015)<br>DAS Study ID 140735 | The study has been submitted for review in order to fulfil new data requirements Regulation (EC) No 1107/2009 and Aquatic Guidance Document EFSA Journal 2013:11(7);3290 |
| CP 10.2.3/1-1 Hoberg (2005)<br>DAS Study ID 040271 | The study is a new representative formulation not previously reviewed for the active approval of clopyralid. |
| CP 10.2.3/1-1-2 Gonsior (2013)<br>DAS Study ID 121208 | The study is a new representative formulation not previously reviewed for the active approval of clopyralid. |

### B.9.4.1. Aquatic toxicity endpoints used in the risk assessment

The endpoints employed in the risk assessment for aquatic organisms are indicated in Tables 9.4.2, 9.4.3, 9.4.4. and 9.4.5.

**Table 9.4.2. EU Endpoints - Toxicity of clopyralid to aquatic species**

| Compound | Test species | Endpoint | EU agreed endpoints* | Endpoint used in risk assessment | |
|---|---|---|---|---|---|
| | | | Value (mg/L) | Value (mg/L) | Ref |
| ***Fish*** | | | | | |
| Clopyralid | *Oncorhynchus mykiss* | 96-h $LC_{50}$ | >99.9 | Same as EU | EFSA 2005 |
| Clopyralid | *Pimephales promelas* | 34-d NOEC | 10.8 | Same as EU | EFSA 2005 |
| ***Invertebrates*** | | | | | |
| Clopyralid | *Daphnia magna* | 48-h $EC_{50}$ | >99.0 | Same as EU | EFSA 2005 |
| Clopyralid | *Daphnia magna* | 21-d NOEC | 17 | Same as EU | EFSA 2005 |
| Clopyralid | *Chironomus riparius* | 28-d NOEC | 50 | Same as EU | EFSA 2005 |
| ***Algae*** | | | | | |
| Clopyralid | *P. subcapitata* | 72-h $E_rC_{50}$ | 30.0 | Same as EU | EFSA 2005 |
| Clopyralid | *Navicula pelliculosa* | 72-h $E_rC_{50}$ | N/A | 31.3 | Aufderheide (2015) DAS Study ID 140515 |
| Clopyralid | *Anabaena flos-aquae* | 72-h $E_rC_{50}$ | N/A | 22 | Hoberg (2006) DAS Study ID 060246 |
| ***Macrophytes*** | | | | | |
| Clopyralid | *Lemna gibba* | 7-d $EC_{50}$ | 89 | Same as EU | EFSA 2005 |
| Clopyralid | *Myriophyllum spicatum* | 14-d $E_rC_{50}$ | > 3 | N/A | Banman (2015) DAS Study ID140735 |

* EFSA Scientific Report (2005) 50, 1-65

**Table 9.4.3. EU Endpoints - Toxicity of florasulam to aquatic species**

| Compound | Test species | Endpoint | EU agreed endpoints* | Endpoint used in risk assessment | |
|---|---|---|---|---|---|
| | | | Value (mg/L) | Value (mg/L) | Ref |
| ***Fish*** | | | | | |
| Florasulam | *Oncorhynchus mykiss* | 96-h $LC_{50}$ | >100 | Same as EU | EFSA 2015 |
| 5-OH-florasulam | *Oncorhynchus mykiss* | 96-h $LC_{50}$ | >91 | Same as EU | EFSA 2015 |
| Florasulam | *Oncorhynchus mykiss* | 28-d NOEC | 119 | Same as EU | EFSA 2015 |
| Florasulam | *Pimephales promelas* | 33-d NOEC | 2.9 | Same as EU | EFSA 2015 |

| Compound | Test species | Endpoint | EU agreed endpoints* | Endpoint used in risk assessment | |
|---|---|---|---|---|---|
| | | | Value (mg/L) | Value (mg/L) | Ref |
| ***Invertebrates*** | | | | | |
| Florasulam | *Daphnia magna* | 48-h $EC_{50}$ | >292 | Same as EU | EFSA 2015 |
| 5-OH-florasulam | *Daphnia magna* | 48-h $EC_{50}$ | >96.7 | Same as EU | EFSA 2015 |
| DFP-ASTCA | *Daphnia magna* | 48-h $EC_{50}$ | >0.030 | Same as EU | EFSA 2015 |
| ASTCA | *Daphnia magna* | 48-h $EC_{50}$ | >0.030 | Same as EU | EFSA 2015 |
| TSA | *Daphnia magna* | 48-h $EC_{50}$ | >0.030 | Same as EU | EFSA 2015 |
| Florasulam | *Daphnia magna* | 21-d NOEC | 23.4 | Same as EU | EFSA 2015 |
| Florasulam | *Chironomus riparius* | 28-d NOEC | 10 | Same as EU | EFSA 2015 |
| ***Algae*** | | | | | |
| Florasulam | *P. subcapitata* | 72-h $EC_{50}$ | 0.00894 | Same as EU | EFSA 2015 |
| 5-OH-florasulam | *P. subcapitata* | 72-h $E_bC_{50}$<br>72-h $E_rC_{50}$ | 21.32<br>21.57 | Same as EU | EFSA 2015 |
| ASTCA | *P. subcapitata* | 72-h $EC_{50}$ | >9.2 | Same as EU | EFSA 2015 |
| DFP-ASTCA | *P. subcapitata* | 72-h $E_yC_{50}$ | 96 | Same as EU | EFSA 2015 |
| TPSA | *P. subcapitata* | 72-h & 96-h, $E_yC_{50}$ & $E_rC_{50}$ | >100 | Same as EU | EFSA 2015 |
| TSA | *P. subcapitata* | 96-h $EC_{50}$, $E_yC_{50}$ & $E_rC_{50}$ | >94 | Same as EU | EFSA 2015 |
| 5-OH-ASTP | *P. subcapitata* | 72-h & 96-h, $E_yC_{50}$ & $E_rC_{50}$ | >100 | Same as EU | EFSA 2015 |
| ASTP | *P. subcapitata* | 72-h & 96-h, $E_yC_{50}$ & $E_rC_{50}$ | >100 | Same as EU | EFSA 2015 |

* EFSA Journal (2015) 13(1):3984

N/A: not applicable

**Table 9.4.4. EU Endpoints - Toxicity of fluroxypyr to aquatic species**

| Compound | Test species | Endpoint | EU agreed endpoints* | Endpoint used in risk assessment** | |
|---|---|---|---|---|---|
| | | | Value (mg/L) | Value (mg/L) | Ref |
| ***Fish*** | | | | | |
| Fluroxypyr-meptyl | *Oncorhynchus mykiss* | 96-h $LC_{50}$ | >0.225 | Same as EU | EFSA 2011 |
| Fluroxypyr | *Lepomis macrochirus* | 96-h $LC_{50}$ | 14.3 | Same as EU | EFSA 2011 |
| Fluroxypyr pyridinol | *Oncorhynchus mykiss* | 96-h $LC_{50}$ | 39 | Same as EU | EFSA 2011 |
| Fluroxypyr monochloro pyridinol | *Oncorhynchus mykiss* | 96-h $LC_{50}$ | 95.1 | Same as EU | EFSA 2011 |
| Fluroxypyr-meptyl | *Oncorhynchus mykiss* | 21-d NOEC | 0.2 | Same as EU | EFSA 2011 |
| Fluroxypyr | *Oncorhynchus mykiss* | 21-d NOEC | 100 | N/A | EFSA 2011 |
| Fluroxypyr | *Pimephales promelas* | 33-d NOEC | N/A | 3.0 | [redacted] et al., 2011 |

| Compound | Test species | Endpoint | EU agreed endpoints* | Endpoint used in risk assessment** | |
|---|---|---|---|---|---|
| | | | Value (mg/L) | Value (mg/L) | Ref |
| | | | | | Study no. 101728 |
| ***Invertebrates*** | | | | | |
| Fluroxypyr-meptyl | *Daphnia magna* | 48-h $EC_{50}$ | >0.183 | Same as EU | EFSA 2011 |
| Fluroxypyr | *Daphnia magna* | 48-h $EC_{50}$ | >100 | Same as EU | EFSA 2011 |
| Fluroxypyr pyridinol | *Daphnia magna* | 48-h $EC_{50}$ | >49 | Same as EU | EFSA 2011 |
| Fluroxypyr monochloro pyridinol | *Daphnia magna* | 48-h $EC_{50}$ | 7.56 | Same as EU | EFSA 2011 |
| Fluroxypyr-meptyl | *Daphnia magna* | 21-d NOEC | 0.0605 | Same as EU | EFSA 2011 |
| Fluroxypyr | *Daphnia magna* | 21-d NOEC | 56 | Same as EU | EFSA 2011 |
| Fluroxypyr-meptyl | *Chironomus riparius* | 28-d NOEC | 0.13 | Same as EU | EFSA 2011 |
| ***Algae*** | | | | | |
| Fluroxypyr-meptyl | *Skeletonema costatum* | 120-h $EC_{50}$ | 0.208 | Same as EU | EFSA 2011 |
| Fluroxypyr | *Navicula pelliculosa* | 72-h $E_bC_{50}$ | 26.0 | Same as EU | EFSA 2011 |
| Fluroxypyr pyridinol | *Navicula pelliculosa* | 72-h $EC_{50}$ | 0.640 | Same as EU | EFSA 2011 |
| Fluroxypyr monochloro pyridinol | *P. subcapitata* | 72-h $EC_{50}$ | 35.0 | Same as EU | EFSA 2011 |
| Fluroxypyr methoxy pyridine | *Anabaena flos-aquae* | 72-h $EC_{50}$<br>120-h $EC_{50}$ | <1.12<br>1.80 | Same as EU | EFSA 2011 |

* EFSA Journal 2011;9(3):2091

** Since Annex I inclusion new studies have been performed providing new end-points which are used in the risk assessment.

**Table 9.4.5. Toxicity of GF-1374 to aquatic species**

| Compound | Test species | Endpoint | EU agreed endpoints* | Endpoint used in risk assessment | |
|---|---|---|---|---|---|
| | | | Value (mg/L) | Value (mg/L) | Ref |
| ***Fish*** | | | | | |
| GF-1374 | *O. mykiss* | 96-h $LC_{50}$ | N/A | 7.1 | [redacted] (2005) DAS Study ID 040342 |
| ***Invertebrates*** | | | | | |
| GF-1374 | *D. magna* | 48-h $EC_{50}$ | N/A | 6.9 | Sayers (2005) DAS Study ID 040343 |
| ***Algae*** | | | | | |
| GF-1374 | *P. subcapitata* | 72-h $EC_{50}$, $E_yC_{50}$ | N/A | 1.1 | Hoberg (2005) DAS Study ID 040270 |
| GF-1374 | *N. pelliculosa* | 72-h $EC_{50}$, $E_bC_{50}$ | N/A | 1.4 | Hoberg (2005) DAS Study ID 040272 |
| ***Macrophytes*** | | | | | |
| GF-1374 | *Lemna gibba* | 7-d $E_rC_{50}$ | N/A | 0.420 | Hoberg (2005) DAS Study ID 040271 |
| GF-1374 | *Myriophyllum spicatum* | 14-d $E_rC_{50}$ | N/A | 0.440 | Gonsior (2013) DAS Study ID 121208 |

* GF-1374 is a formulation which was not evaluated at EU level.
** Studies for GF-1374 have been performed providing end-points which are used in the risk assessment.
N/A: not applicable

### B.9.4.2. Mixture toxicity of GF-1374 for aquatic organisms

Effects on aquatic organisms for GF-1374 were not evaluated as part of the EU review of fluroxypyr, clopyralid or florasulam. Data on GF-1374 is evaluated here, and risk assessments for GF-1374 with the proposed use pattern are provided below and are considered adequate.

$PEC_{SW}$ are provided in Part B, Section 5, Chapter B.8.6.2. for the active substances and their major metabolites. Full details are provided in those sections of how the $PEC_{SW}$ are calculated. The global maximum $PEC_{SW}$ was from one application and so this is used in the risk assessment.

Even though acute toxicity studies for fish, daphnia and algae exposed to GF-1374 are available, the dose additivity principle has been used to derive the theoretical acute $LC/EC_{50}$ of GF-1374 to fish, daphnia and algae according to the following equation (EFSA Journal 2013; 11(7):3290, Equation 13):

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

where: $X(a.s._i)$ is the fraction of the active substance $i$ in the formulation (with $\Sigma\ X(a.s._i)=1$); $EC_{50}(a.s._i)$ is the acute toxicity for the active substance $i$.

Considering the lowest $LC_{50}/EC_{50}$ values determined for for clopyralid, florasulam and fluroxypyr-meptyl and their nominal concentrations in GF-1374 (7.69% w/w, 0.24% w/w and 13.85% w/w for clopyralid, florasulam and fluroxypyr-meptyl, respectively), the resulting $LC_{50}/EC_{50}$ mix-CA were calculated and are given in Table 9.4.6.

**Table 9.4.6. Mixture toxicity assessment for GF-1374**

| Compound | Test species | Endpoint | Value (mg /L) | Predicted value (mg /L)[1] | MDR[2] |
|---|---|---|---|---|---|
| GF-1374 | Rainbown trout (*O. mykiss*) | 96 h-$LC_{50}$ | 7.1 | 0.29 | 0.04 |
| | *Daphnia magna* | 48 h-$EC_{50}$ | 6.9 | 0.35 | 0.05 |

[1] assuming dose additivity of the single active substances in the formulation ($ECx_{mix\text{-}CA}$ )
[2] MDR = ECxmix-CA/ECxPPP

The data in the above table confirms that the toxicity of GF-1374 is less than additive when compared with predicted critical endpoints for fish and daphnia as the MDR< 0.2 for both fish and daphnia. According to section 10.3.4 of the EFSA Guidance on Tiered Risk Assessment for Plant Protection Products for Aquatic Organisms in Edge-of-Field Surface Waters (EFSA Journal 2013; 11(7): 3290) when the MDR < 0.2 the predicted value should be used in the risk assessment of the formulated product. However, an assessment to determine the presence of a toxic driver was conducted and the results are presented below.

**Table 9.4.7. Toxic driver assessment for GF-1374 based on fish studies**

| Compound | Percent a i. (%) | Fraction (%) | $LD_{50}$ (mg/L) | Tox per fraction mix | Contribution of toxicity (%) |
|---|---|---|---|---|---|
| Clopyralid | 7.69 | 35 | 99.9 | 285 | < 0.001 |
| Florasulam | 0.24 | 1 | 1000 | 100000 | < 0.001 |
| Fluroxypyr meptyl | 13.85 | 64 | 0.225 | 0.35 | 121% |
| GF-1374 | 21.78 | 100 | 7.1 (measured) | 0.29 (predicted) | |

**Table 9.4.8. Toxic driver assessment for GF-1374 based on daphnid studies**

| Compound | Percent a i. (%) | Fraction (%) | $LD_{50}$ (mg/kg bw) | Tox per fraction mix | Contribution of toxicity (%) |
|---|---|---|---|---|---|
| Clopyralid | 7.69 | 35 | 99.0 | 283 | < 0.001 |
| Florasulam | 0.24 | 1 | 292 | 29200 | < 0.001 |
| Fluroxypyr meptyl | 13.85 | 64 | 0.183 | 0.29 | 121% |
| GF-1374 | 21.78 | 100 | 6.9 (measured) | 0.35 (predicted) | |

Overall, it appears the fluroxypyr-meptyl is the toxic driver. Please refer the EFSA 2011 conclusion on fluroxypyr for specific details regarding its assessment.

### B.9.4.3. Aquatic exposure

Following the dilution and spraying of the formulated product, much of the formulation constituents are likely to be lost by volatilisation. Therefore, shortly after application of a formulated product, aquatic organisms are mainly exposed to the single active substances present in the formulation. An evaluation

of the risk posed by the intact formulation is therefore relevant only for the acute assessment. The long-term risk will be assessed considering data for the active substance clopyralid and the formulation.

Clopyralid forms no major metabolites in surface water.

The following risk assessment has been based on realistic worst-case scenarios for the application summarised in the following table.

**Table 9.4.9. Critical GAP to assess the risk of GF-1374 applications to grassland and cereals**

| Crop | Time of application | No. of applications per year | Maximum rate per application |
|---|---|---|---|
| Cereals | BBCH 13 – 39<br>February to June 30 | 1 | 1.0 L GF-1374/ha (or 1040[a] g/ha)<br>equivalent to:<br>fluroxypyr-meptyl 144 g/ha (or 100 g a.e./ha);<br>florasulam 2.5 g/ha;<br>clopyralid 80 g/ha |
| Pasture | Feb 01 to August 31 | | 1.5 L GF-1374/ha (or 1560[a] g/ha)<br>equivalent to:<br>fluroxypyr-meptyl 216 g/ha (or 150 g a.e./ha);<br>florasulam 3.75 g/ha;<br>clopyralid 120 g/ha |

[a]: based on a nominal formulation density of 1.04 g/mL.

The risk envelope application rates considered in this section are 120 g clopyralid/ha on pasture and 80 g clopyralid/ha on cereals. The worst-case actual application rates of 1.5 L/ha and 1.0 L/ha have been used for the formulation GF-1374 for pasture and cereals, respectively.

Surface water PEC values are summarised below for GF-1374 and clopyralid. Full details of calculations are presented in Section 9, point CP 9.2.5. Results are summarised in the below tables.

**Table 9.4.10. FOCUS SW Steps 1 and 2 estimates of aquatic concentrations for clopyralid**

| | Maximum PECsw (µg/L) as calculated in FOCUS SW | | | |
|---|---|---|---|---|
| | Step 1 | | Step 2 (scenario)[#] | |
| | Pasture | Cereals | Pasture | Cereal |
| Clopyralid | 41.0 | 27.4 | 5.39 | 12.2 |

[#]worst case PECsw value for the various Step 2 scenarios

**Table 9.4.11. Estimates of aquatic concentrations for GF-1374 based on spray drift**

| Spray drift buffer (m) | Drift (%) | Initial PECsw (µg/L) | | | |
|---|---|---|---|---|---|
| | | 0% Drift red. | 50% Drift red. | 75% Drift red. | 90% Drift red. |
| 1 | 2.77 | 14.4 | 7.20 | 3.60 | 1.44 |
| 5 | 0.57 | 2.96 | 1.48 | 0.741 | 0.296 |
| 10 | 0.29 | 1.51 | 0.754 | 0.377 | 0.151 |
| 15 | 0.2 | 1.04 | 0.520 | 0.260 | 0.104 |
| 20 | 0.15 | 0.780 | 0.390 | 0.195 | 0.078 |

Fluroxypyr and florasulam have recently been granted Annex I inclusion. For fluroxypyr, the conclusions from the peer review process were published in 2011 (EFSA 2011). For florasulam, conclusions from the peer review process were published in 2015 (EFSA 2015). A risk envelope approach is being applied for the risk assessment of fluroxpyr and florasulam. Within the scope of this assessment, one application of fluroxypyr at 200 g a.s./ha was determined to be a safe use on both cereals and grasslands. Also, one application of flurosulam at 6.25 g a.s./ha was determine to be a safe use on cereals and grasslands. Therefore, for the below assessments, it is justified to refer to fluroxypyr and florasulam data wherever appropriate. Specific risk assessments for these two active substances are not necessary to defend the Annex I listing of clopyralid since the proposed use rate falls within their safe use. Please refer to the EFSA Scientific reports of florasulam and fluroxypyr to review their associated risk assessments. A brief overview of the impacts of florasulam and fluroxypyr are provided below.

Fluroxypyr meptyl is very toxic to aquatic organisms while fluroxypyr is harmful. The most sensitive endpoint was observed in a chronic study with daphnia magna with fluroxypyr-meptyl (NOEC = 0.0605 mg a.s./L). The acute risk to fish and the acute and chronic risk to aquatic invertebrates were assessed as high at FOCUS Step 1. The acute risk to aquatic invertebrates and fish was still high at FOCUS Step 2. However, the assessment based on FOCUS Step 3 indicates a low risk in all scenarios for the representative uses except for aquatic invertebrates in the scenario D2-ditch for the use in winter cereals (TER was slightly below the Annex VI trigger). Due to the mode of action of the fluroxypyr (i.e. auxin), a data gap was identified to provide a study with a second aquatic plant. The risk to aquatic organism was assessed as low at FOCUS Step 1 for water metabolites fluroxypyr pyrindinol and monchlropyridinol 3 CP. Toxicity data for the metabolite fluroxypyr methoxypyridine were only available on algae and higher aquatic plant. Based on these data, a low risk was indicated at FOCUS Step 1 (EFSA 2011).

For florasulam, several toxicity studies were available for aquatic organisms (fish, invertebrates, sediment dwelling organisms, algae, and aquatic plants). These studies cover the active substance and pertinent metabolites. Algae and plants were the most tested sensitive organisms. The lowest endpoint was derived from a 14 day study on *Lemna gibba*. The risk was low with FOCUS Step 1 for all aquatic organisms, except algae and plants for all uses. At Step 2 the risk was low for algae but Step 3 calculations were needed for aquatic plants for the representative uses in cereals, maize, and new leys applied at 6.25 g a.s./ha. At step 3, a high risk was excluded in all scenarios and all uses, except D2 and R3 for representative use in winter cereals and new leys (spring applications at 6.25 g a.s./ha). Only for the scenario R3 the risk was further addressed with Step 4 by considering mitigation measures comparable to 10 m of no-spray buffer zone and 10 m vegetated buffer strip. A data gap was identified to further address the risk for the scnearios D1 and D2). The risk from metabolites is considered low (EFSA 2015).

### B.9.4.4. Aquatic risk assessment

The initial risk assessments were carried out by comparing the PECsw values with the worst case acute endpoints. Acute exposure ratios ($TER_A$) were calculated using the following equation:

$$TER_A = \frac{RAC}{Maximum\ PEC_{SW}}$$

The long-term risk assessment typically is carried out by comparing PECsw values with the worst case long term toxicity endpoints using the following equation:

$$TER_{LT} = \frac{RAC}{Maximum\ PEC_{SW} / 21\,d\ TWA\ PEC_{SW}}$$

However, in this case the worst case scenario surface water concentration ($PEC_{sw}$) of clopyralid resulting from FOCUS Step 1 was used both for acute and long-term risk assessment of clopyralid. Estimation of spray drift from a distance of 1 meter to water bodies was used for the plant protection product GF-1374, as presented below. Risk assessments were carried out for clopyralid and GF-1374 and the worst case minimum TERs on aquatic organisms are listed below in Table 9.4.12.

**Table 9.4.12. Minimum TERs for the most sensitive aquatic species after applications of GF-1374 to pasture and cereals at rates of 1.5 L GF-1374/ha and 1.0 L GF-1374/ha, respectively**

| Substance | Critical Endpoint (µg/L) | Distance from treated area | PECsw (µg/L) | RAC | TER[a] | TER trigger |
|---|---|---|---|---|---|---|
| ***Fish*** | | | | | | |
| Clopyralid | 99900 | Step 1 broadcast application Grassland | 41.0 | 999 | 24.4 | 1 |
| Clopyralid | 99900 | Step 1 broadcast application Cereals | 27.4 | 999 | 36.5 | 1 |
| GF-1374 | 7100 (measured) | Spray drift 1 m buffer 0% drift reduce nozzle | 14.4 | 71 | 4.93 | 1 |
| GF-1374 | 2900 (predicted by mixture toxicity) | Spray drift 1 m buffer 0% drift reduce nozzle | 14.4 | 29 | 2.01 | 1 |
| ***Invertebrates*** | | | | | | |
| Clopyralid | > 99000 | Step 1 broadcast application Grassland | 41.0 | 990 | 24.1 | 1 |
| | | Step 1 broadcast application Cereal | 27.4 | | 36.1 | 1 |
| GF-1374 | 6900 (measured) | Spray drift 1 m buffer 0% drift reduce nozzle | 14.4 | 69 | 4.79 | 1 |
| GF-1374 | 3500 (predicted by mixture toxicity) | Spray drift 1 m buffer 0% drift reduce nozzle | 14.4 | 35 | 2.43 | 1 |
| ***Invertebrates (Chironomus riparius)*** | | | | | | |
| Clopyralid | 50000 | Step 1 broadcast application Grassland | 41.0 | 5000 | 122 | 1 |
| | | Step 1 broadcast application Cereal | 27.4 | | 182 | 1 |

| | | | | | | |
|---|---|---|---|---|---|---|
| ***Green algae*** | | | | | | |
| Clopyralid *(S. capricornutum)* | 30000 | Step 1 broadcast application grassland | 41.0 | 3000 | 73.2 | 1 |
| | | Step 1 broadcast application cereals | 27.4 | | 110 | 1 |
| GF-1374 *(P. subcapitata)* | 1100 | Spray drift 1 m buffer zone 0% drift reduce nozzle | 14.4 | 110 | 7.64 | 1 |
| ***2nd algal species, Diatom*** | | | | | | |
| Clopyralid *(Navicula pelliculosa)* | 31300 | Step 1 broadcast application grassland | 41.0 | 3130 | 76.3 | 1 |
| | | Step 1 broadcast application cereals | 27.4 | | 114 | 1 |
| GF-1374 *(Navicula pelliculosa)* | 1400 | Spray drift 1 m buffer zone 0% drift reduce nozzle | 14.4 | 140 | 9.72 | 1 |
| ***3rd algal species, Bluegreen algae*** | | | | | | |
| Clopyralid *(Anabaena flos-aquae)* | 22000 | Step 1 broadcast application grassland | 41.0 | 2200 | 53.7 | 1 |
| | | Step 1 broadcast application cereals | 27.4 | | 80.3 | 1 |
| ***Macrophytes (Lemna gibba)*** | | | | | | |
| Clopyralid | 89000 | Step 1 broadcast application grassland | 41.0 | 8900 | 217 | 1 |
| | | Step 1 broadcast application cereals | 27.4 | | 325 | 1 |
| GF-1374 | 420 | Spray drift 1 m buffer zone 0% drift reduce nozzle | 14.4 | 42 | 2.92 | 1 |
| ***Macrophytes (Myriophyllum spicatum)*** | | | | | | |
| Clopyralid | 3000 | Step 1 broadcast application grassland | 41.0 | 300 | 7.32 | 1 |
| | | Step 1 broadcast application cereals | 27.4 | | 10.9 | 1 |
| GF-1374 | 440 | Spray drift 1 m buffer zone 0% drift reduce nozzle | 14.4 | 44 | 3.05 | 1 |
| [a]Relevant acute or long term TER value compared to the trigger value of 1 | | | | | | |

RMS comments and evaluation:

The assessment presented by the Notifier concerning the toxicity driver of GF-1374, the representative formulation of clopyralid, is acceptable. The mixture toxicity of the ingredients in the formulation is less than additive, fluroxypyr being the main driver of toxicity. The explanation of using the measured formulation toxicity values instead of the predicted values in the calculation is reasonable for the purpose of EU evaluation of clopyralid. It can be concluded that the aquatic toxicity of the formulation GF-1374 is mainly caused by another active substance fluroxypyr.

TER calculation to fish and Daphnia using the predicted mixture toxicity values were added for clarity and transparency in Table 9.4.12. by the RMS evaluator.

The PECsw values of clopyralid used in the risk assessment are in agreement with Step 1 as calculated in the Environmental Fate Section (B8), and represent the worst case of clopyralid use. TER calculations demonstrate that the acute and chronic risk assessment to all taxonomic groups of aquatic organisms (fish, aquatic invertebrates, sediment-dwellers, algae and higher aquatic plants) is acceptable from the use of clopyralid (applied as GF-1374) to cereals and pasture with one application per year without the need of specific risk mitigation even if the worst case Step 1 PECsw is used in the risk assessment, provided that the product GF-1374 is used according to the GAP at rates up to 1.5 L formulation/ha on pasture and 1.0 L formulation/ha on cereals.

However, at national level, the predicted mixture toxicity may be necessary to use for the aquatic risk assessment of formulated products with other active substances mixed with clopyralid. In vulnerable conditions possible risk mitigation may be considered for protecting aquatic organisms where appropriate, when products containing clopyralid in mixtures with other, more toxic active substances as fluroxypyr for instance, are authorized at MS level.

The ecotoxicological studies with clopyralid published in scientific literature, as referred in Vol. 3 of Active substance dossier, do not change the conclusions of the aquatic risk assessment of clopyralid conducted on Notifier-owned studies. As the data does not override the studies owned by the Notifier, their outcomes are not included in the risk assessment.

The aquatic risk assessment of clopyralid and the product GF-1374 is adequate and acceptable, and no further data are required on this issue at EU level.

## B.9.5. EFFECTS ON ARTHROPODS

The following table provides a justification for the use of a different study to address effects on arthropods to that evaluated originally in the DAR (2003) for the first Approval of clopyralid.

**Table 9.5.1. Justification for data used in the risk assessment to arthropods**

| Data Point/Study | Rationale |
|---|---|
| CP 10.3.1.1.1 Hughes (2004)<br>DAS Study ID 040260 | The study is a new representative formulation not previously reviewed for the active approval of clopyralid |
| CP 10.3.1.1.2 Hughes (2004)<br>DAS Study ID 040259 | The study is a new representative formulation not previously reviewed for the active approval of clopyralid |
| CP 10.3.2.2-1 Loose (2004)<br>DAS Study ID 040262 | The study is a new representative formulation not previously reviewed for the active approval of clopyralid |
| CP 10.3.2.2-2 Loose (2005)<br>DAS Study ID 040263R | The study is a new representative formulation not previously reviewed for the active approval of clopyralid |

| Data Point/Study | Rationale |
|---|---|
| CP 10.3.2.2-3 Loose (2004)<br>DAS Study ID 040264R | The study is a new representative formulation not previously reviewed for the active approval of clopyralid |

## B.9.5.1. Effects on bees

### B.9.5.1.1. Acute oral toxicity to honeybees

As the representative formulation of clopyralid was chanced since the first DAR, the following bee acute oral toxicity study performed on GF-1374 is provided in support of the assessment and has not been previously evaluated.

**CP 10.3.1.1.1/1 - Plant Protection Product GF-1374: Acute Oral Toxicity to Honeybees**

| | |
|---|---|
| Report: | KIIIA1 10.4.2.1/01, Hughes, C. (2004a) |
| Title: | GF-1374: Acute oral toxicity test with the honeybee (*Apis mellifera*) |
| Document No: | Dow AgroSciences Study Number: 040260<br>ABC Study No. 48945 |
| Guidelines: | OECD Guideline No. 213 (1998) |
| GLP | Yes, with the exceptions described below:<br>1) The homogeneity, concentration, and stability of the test substance in the carrier were not determined because analytical verification of the test substance concentration was not performed for this study.<br>2) The Dimethoate was not characterized under the GLP guidelines. |

**Methodology:** The acute oral toxicity of GF-1374 to the honeybee (*Apis mellifera*) workers was determined in a study conducted to OECD Guideline No. 213, in accordance with GLP. GF-1374 (Lot No. 187/76-A) is an emulsion concentrate (EC) nominally containing fluroxypyr-meptyl at 144 g/L (142 g/L, actual), clopyralid at 80 g/L (86 g/L, actual) and florasulam at 2.5 g/L (2.5 g/L, actual). Groups of bees (3 replicates of 10 bees/cage) were exposed orally to doses of GF-1374. The test material was dispersed in a 50% sucrose solution at concentrations of 0.313, 0.625, 1.25, 2.50 and 5.00 mg product/mL. One set of control bees received sucrose solution only. The doses were presented to the bees *via* feeding tubes placed in their cages, providing 20 µL solution/bee and resulting in oral doses of 6.3, 13, 25, 50 and 100 µg product/bee. After approximately 6 hours the feeding tubes were removed and weighed to determine the actual dose consumed.

Once treated, bees were kept in the dark in an incubator at 25.2-25.6°C and 49-64% relative humidity and fed *ad libitum* on a 50% sucrose solution. The bees were observed 4, 24 and 48 hours after treatment to assess any harmful effects.

In a concurrent study, bees from the same hive were treated with a toxic reference standard of dimethoate (Lot No. 322-88A with purity of 98%) which was applied orally at 0.020, 0.10 and 0.20 µg/bee using the same methods as employed for GF-1374.

**Findings:** During the treatment period, the bees consumed 100% of the control diet and from 79.8% to 100% of the treated diet, resulting in dosages of between 6.17 and 86.7 µg product/bee. No mortalities were observed in any of the treatments during the study.

**Table 9.5.2. Bee mortality data in acute oral toxicity test with GF-1374**

| Calculated dose (µg GF-1374/bee) | Cumulative mortality (%) | | |
|---|---|---|---|
| | 4-hour | 24-hour | 48-hour |
| Control A | 0 | 0 | 0 |
| Control B | 0 | 0 | 0 |
| Control C | 0 | 0 | 0 |
| 6.17 | 0 | 0 | 0 |
| 6.26 | 0 | 0 | 0 |
| 6.17 | 0 | 0 | 0 |
| 12.4 | 0 | 0 | 0 |
| 12.5 | 0 | 0 | 0 |
| 12.4 | 0 | 0 | 0 |
| 24.8 | 0 | 0 | 0 |
| 24.3 | 0 | 0 | 0 |
| 25.0 | 0 | 0 | 0 |
| 50.5 | 0 | 0 | 0 |
| 50.3 | 0 | 0 | 0 |
| 50.8 | 0 | 0 | 0 |
| 79.8 | 0 | 0 | 0 |
| 85.4 | 0 | 0 | 0 |
| 86.7 | 0 | 0 | 0 |

Since mortality did not exceed 50% in the study, the $LD_{50}$ value for the oral route of exposure was considered to be >86.7 µg GF-1374/bee. The 95% confidence limits could not be estimated.

The dimethoate 24-h $LD_{50}$ value for acute oral toxicity was calculated from the data to be 0.13 µg as/bee (95% c.l. 0.11-0.15 µg a.s./bee). This value is within the historical range for this reference material at this laboratory (0.060-0.35 µg a.s./bee) and within the range of published values (0.10-0.35 µg a.s./bee).

**Table 9.5.3. Results**

| End-point | µg GF-1374/bee |
|---|---|
| 48-h Oral $LD_{50}$ (95% c.l.) | >86.7 (-) |

The acute oral $LD_{50}$ value for GF-1374 to the honeybee (*Apis mellifera*) is >86.7 µg product/bee.

RMS comments and evaluation:

The study with the formulation GF-1374 is different from those referred to in the DAR of clopyralid (2003) and not assessed before within the context of the approval of clopyralid. Therefore the study was evaluated here.

The test was performed according to appropriate OECD test guideline except a few deviations. Though analytical confirmation of the test concentrations was not performed and the results being based on nominal concentrations, these exceptions to Good Laboratory Practices reported in the study did not adversely affect the integrity of the study or the interpretation of the test results. The absence of mortalities in any test concentrations did not allow the statistical analysis of the results. The study is well reported, of good quality and acceptable. The data requirement on the acute oral bee toxicity of the formulation GF-1374 is fulfilled.

The conclusion is that the formulation GF-1374 is of low acute oral toxicity to honeybees.

Based on the risk assessment presented by the Notifier above, the acute oral $Q_{HO}$ value to honeybees is below the trigger of 50 of the Uniform Principles of the Commission Regulation 546/2011. It indicates thus a low acute oral risk to honey bees, when the product GF-1374 is sprayed according to Good Agricultural Practice at rates of up to 1.5 L formulated product/ha on pasture and 1.0 L formulated product/ha to cereals.

**B.9.5.1.2. Acute contact toxicity to honeybees**

As the representative formulation of clopyralid was chanced since the first DAR, the following bee acute contact toxicity study performed on GF-1374 is provided in support of the assessment and has not been previously evaluated.

**CP 10.3.1.1.2/1 - Plant Protection Product GF-1374: Acute Contact Toxicity to Honeybees**

| | |
|---|---|
| Report | KIIIA1 10.4.2.2/01, Hughes, C. 2004b |
| Report title | GF-1374: Acute Contact Toxicity Test with the Honeybee *(Apis mellifera)* |
| DAS Study number | Dow AgroSciences Study Number: 040259<br>ABC Study No. 48946 |
| Guidelines | OECD Method 214 (1998) and FIFRA Guideline 141-1 |
| GLP | Yes, with the exceptions described below:<br>1) The homogeneity, concentration, and stability of the test substance in the carrier were not determined because analytical verification of the test substance concentration was not performed for this study.<br>2) The Dimethoate was not characterized under the GLP guidelines. |

**Methodology:** The acute contact toxicity of GF-1374 to the honeybee (*Apis mellifera*) workers was determined in a study conducted to OECD Guideline No. 214, in accordance with GLP. GF-1374 (Lot No. 187/76-A) is an emulsion concentrate (EC) nominally containing fluroxypyr-meptyl at 144 g/L (142 g/L, actual), clopyralid at 80 g/L (86 g/L, actual) and florasulam at 2.5 g/L (2.5 g/L, actual). Groups of bees (3 replicates of 10 bees/cage) were exposed to topically applied doses of GF-1374. The bees were anaesthetised with $CO_2$ and a 1 µL drop of the test material dispersed in deionised water was applied to the dorsal surface of the thorax of each bee using a micro-applicator. The bees were given a contact dose equivalent to 200 µg product/bee. Once treated, bees were kept in the dark in an incubator at 24.4-25.2°C and 55-79% relative humidity and fed *ad libitum* on a 50% sucrose solution. The percent relative humidity fell outside the intended range of 50 to 70%, but this deviation was not considered to have had any effect on the integrity and interpretations of the results. The bees were observed 4, 24 and 48 hours after treatment to assess any harmful effects.

In a concurrent study, bees from the same hive were treated with a toxic reference standard of dimethoate ((Lot No. 322-88A with purity of 98%) at 0.020, 0.10 and 0.20 µg a.s./bee, which was applied topically using the same methods as employed for GF-1374.

**Findings:** After 48 hours, no mortality had occurred in either the treatment or control groups and no sublethal effects were observed.

**Table 9.5.4. Bee mortality data in acute contact toxicity test with GF-1374**

| Calculated dose (µg GF-1374/bee) | Cumulative mortality (%) | | |
|---|---|---|---|
| | 4-hour | 24-hour | 48-hour |
| Control A | 0 | 0 | 0 |
| Control B | 0 | 0 | 0 |
| Control C | 0 | 0 | 0 |
| 200 A | 0 | 0 | 0 |
| 200 B | 0 | 0 | 0 |
| 200 C | 0 | 0 | 0 |

Since mortality did not exceed 50% in the study, the $LD_{50}$ value for the contact route of exposure was considered to be >200 µg product/bee. The 95% confidence limits could not be calculated.

The dimethoate 24-h $LD_{50}$ value for acute contact toxicity was calculated from the data to be 0.11 µg a.s./bee (95% c.l. 0.099-0.13 µg a.s./bee). This value is within the historical range for this reference material at this laboratory (0.055-0.15 µg a.s./bee) and within the range of published values (0.10-0.35 µg a.s./bee).

**Table 9.5.5. Results**

| End-point | µg GF-1374/bee |
|---|---|
| 48-h Contact $LD_{50}$ (95% c.l.) | >200 (-) |

**Conclusions:** The acute contact $LD_{50}$ value for GF-1374 to the honeybee (*Apis mellifera*) is >200 µg product/bee.

RMS comments and evaluation:

Because the representative formulation of clopyralid was changed, the study with the formulation GF-1374 is different from those referred to in the DAR of clopyralid (2003) and not assessed before within the context of the approval of clopyralid. Therefore the study was evaluated here.

The test was performed according to appropriate OECD test guideline except a few deviations. Though analytical confirmation of the test concentrations was not performed and the results being based on nominal concentrations, these exceptions to Good Laboratory Practices reported in the study did not adversely affect the integrity of the study or the interpretation of the test results. The absence of mortalities in any test concentrations did not allow the statistical analysis of the results. The study is well reported, of good quality and acceptable. The data requirement on acute contact bee toxicity of the formulation GF-1374 is fulfilled.

The conclusion is that the formulation GF-1374 is of low acute contact toxicity to honeybees.

Based on the risk assessment presented by the Notifier, the acute contact $Q_{HO}$ value to honeybees is below the trigger of 50 of the Uniform Principles of the Commission Regulation 546/2011. It indicates thus a low acute risk to honey bees via contact exposure, when the product GF-1374 is sprayed according to Good Agricultural Practice at rates of up to 1.5 L formulated product/ha on pasture and 1.0 L formulated product/ha to cereals.

## B.9.5.2. Risk assessment for bees

### B.9.5.2.1. Toxicity endpoints used in the risk assessment

The critical endpoints employed in the risk assessment for honeybees are indicated in bold in the below tables. During previous Annex I Review, it was concluded that clopyralid was of low toxicity to bees.

**Table 9.5.6. Toxicity of clopyralid to bees**

| Compound | Test species | Endpoint | EU agreed endpoints* | Endpoint used in risk assessment | |
|---|---|---|---|---|---|
| | | | Value (µg/bee) | Value | Ref |
| Clopyralid | *Apis mellifera* | Acute oral LD50 | >100 | Same as EU | EFSA 2005 |
| Clopyralid | *Apis mellifera* | Acute contact LD50 | >98.1 | Same as EU | EFSA 2005 |

* EFSA Scientific Report (2005) 50, 1-65

**Table 9.5.7. Toxicity of fluroxypyr to bees**

| Compound | Test species | Endpoint | EU agreed endpoints* | Endpoint used in risk assessment | |
|---|---|---|---|---|---|
| | | | Value (µg/bee) | Value | Ref |
| Fluroxypyr-meptyl | *Apis mellifera* | Acute oral LD50 | >100 | Same as EU | EFSA 2011 |
| Fluroxypyr-meptyl | *Apis mellifera* | Acute contact LD50 | >100 | Same as EU | EFSA 2011 |

* EFSA Scientific Report 2011;9(3):2091

**Table 9.5.8. Toxicity of florasulam to bees**

| Compound | Test species | Endpoint | EU agreed endpoints* | Endpoint used in risk assessment | |
|---|---|---|---|---|---|
| | | | Value (µg/bee) | Value | Ref |
| Florasulam | *Apis mellifera* | Acute oral LD50 | >100 | Same as EU | EFSA 2015 |
| Florasulam | *Apis mellifera* | Acute contact LD50 | >100 | Same as EU | EFSA 2015 |

* EFSA Scientific Report 2015; 13 (1):3984

**Table 9.5.9. Toxicity of GF-1374 to bees**

| Compound | Test species | Endpoint | EU agreed endpoints* | Endpoint used in risk assessment** | |
|---|---|---|---|---|---|
| | | | Value | Value (µg/bee) | Ref |
| GF-1374 | *Apis mellifera* | Acute oral LD50 | - | >86.7 | Hughes (2004a) DAS Study ID 040260 |
| GF-1374 | *Apis mellifera* | Acute contact LD50 | - | >200 | Hughes (2004b) DAS Study ID 040259 |

* GF-1374 is a formulation which was not evaluated at EU level.
** Studies for GF-1374 have been performed providing end-points which are used in the risk assessment.
N/A: not applicable

**B.9.5.2.2. Exposure**

The following risk assessment has been based on realistic worst-case scenarios for the single yearly application of the product GF-1374 summarised in the table below.

**Table 9.5.10. Critical GAP for GF-1374**

| Crop | Time of application | No. of applications per year | Maximum rate per application |
|---|---|---|---|
| Cereals | BBCH 13 – 39 February to June 30 | 1 | 1.0 L GF-1374/ha (or 1040[a] g/ha) equivalent to: fluroxypyr-meptyl 144 g/ha (or 100 g a.e./ha); florasulam 2.5 g/ha; clopyralid 80 g/ha |
| Pasture | February 01 to August 31 | 1 | 1.5 L GF-1374/ha (or 1560[a] g/ha) equivalent to: fluroxypyr-meptyl 216 g/ha (or 150 g a.e./ha); florasulam 3.75 g/ha; clopyralid 120 g/ha |

[a]: based on a nominal formulation density of 1.04 g/mL.

**B.9.5.2.3. Acute risks to bees**

The acute risk to honeybees from the intended uses of GF-1374 was assessed using the maximum single application rate and the $LD_{50}$ values to calculate hazard quotients (EPPO 2003)[1] as follows:

$$Hazard\ Quotient = \frac{Maximum\ application\ rate\ (g/ha)}{LD_{50}\ (\mu g/bee)}$$

Hazard quotients were calculated for oral exposure ($Q_{HO}$) and contact exposure ($Q_{HC}$) to GF-1374 and its active substances. A hazard quotient of less than 50 indicates a low risk to bees in the field.

The acute oral risk assessment for honeybees is summarised in Table 9.5.11 below.

**Table 9.5.11. Acute oral risk to honeybees from GF-1374 applications to pasture and cereals at maximum use rates of 1.5 L formulated product/ha for pasture and 1.0 L formulated product/ha for cereals.**

| Test substance | Crop | Application rate (g/ha) | $LD_{50}$ (µg a.s./bee) | $Q_{HO}$ |
|---|---|---|---|---|
| Clopyralid | Pasture | 120 | > 100 | < 1.2 |
| GF-1374 | | 1560[a] | > 86.7 | < 18 |
| Clopyralid | Cereals | 80 | > 100 | < 0.80 |
| GF-1365 | | 1040[a] | > 86.7 | < 12 |

[a]: Based on a formulation density of 1.04 g/mL.

**The $Q_{HO}$ value is below the Annex VI trigger of 50. Consequently, the acute oral risk to honeybees is low following applications of GF-1374 at rates of up to 1.5 L formulated product/ha on pasture and 1.0 L formulated product/ha to cereals.**

[1] EPPO/OEPP (2003) Environmental risk assessment scheme for plant protection products, Chapter 10: Honeybees (PP 3/10(2)). Bulletin OEPP/EPPO Bulletin 33: 141-145.

The acute contact risk of honeybees from the use of the product GF-1374 was assessed on the basis of studies available with the formulated product and the technical active substance. The risk calculation is presented in Table 9.5.12. below.

**Table 9.5.12. Acute contact risk of honeybees from GF-1374 applications to pasture and cereals at maximum application rates of 1.5 L formulated product/ha**

| Test substance | Crop | Application rate (g/ha) | $LD_{50}$ (µg a.s./bee) | $Q_{HC}$ |
|---|---|---|---|---|
| Clopyralid | Pasture | 120 | > 98.1 | < 1.22 |
| GF-1374 | | 1560[a] | > 200 | < 7.8 |
| Clopyralid | Cereals | 80 | > 98.1 | < 0.82 |
| GF-1374 | | 1040[a] | > 200 | < 5.2 |

[a]: Based on formulation density of 1.04.

**The $Q_{HC}$ value is below the Annex VI trigger of 50. Consequently, the acute contact risk of honeybees is low following applications of GF-1374 to pasture and cereals at rates of up to 1.5 L product/ha.**

The acute oral and contact risk assessment to honey bees resulting from the critical use patterns in pasture and cereals is summarised in the following Table 9.5.13.

**Table 9.5.13. Maximum HQs for honey bees after uses of GF-1374 in pasture**

| Substance | Study type | Crop | Indicator species | HQ | HQ risk assessment trigger |
|---|---|---|---|---|---|
| Clopyralid | Acute oral | Pasture | *Apis mellifera* | < 1.2 | 50 |
| | Acute contact | | *Apis mellifera* | < 1.22 | 50 |
| GF-1374 | Acute oral | | *Apis mellifera* | <18 | 50 |
| | Acute contact | | *Apis mellifera* | < 7.8 | 50 |
| Clopyralid | Acute oral | Cereals | *Apis mellifera* | <0.8 | 50 |
| | Acute contact | | *Apis mellifera* | <0.82 | 50 |
| GF-1374 | Acute oral | | *Apis mellifera* | <12 | 50 |
| | Acute contact | | *Apis mellifera* | <5.2 | 50 |

HQs shown in **bold** are above the relevant trigger**.**

The risk was assessed to be low for fluroxypyr (EFSA 2011) and florasulam (EFSA 2015). Due to the proposed GAP being within the uses evaluated during fluroxypyr and florasulam Annex I renewal processes, please refer to their EFSA conclusion for specific such as endpoints and estimated Hazard Quotients for oral and contact values (EFSA 2011 and EFSA 2015, respectively).

Chronic, developmental and sublethal risks: Clopyralid is a herbicide that does not have any known insecticidal action. It is applied once per season and applications are made to grassland and cereals. Both these crops are not attractive to worker and foraging bees due to lack of blooming flowers that contain pollen or nectar. Applications are made to control target in-field weeds during the weed's vegetative growth. Treated weeds may be expected to be impacted soof after application due to the rapid adorption and translocation of the active substance to the meristematic portion of the plant where hormonal disruption will occur resulting in plant symptoms such as bending and twisting of the leaves, wilting, cupping of leaves, etc. resulting in the elimination of impacted weeds from the pool of flowering plants that bees may visit and where they may be exposed to systemic residues of clopyralid.

Furthermore, most herbicide applications are applied at early growth stages of weeds due to the plants susceptibility at early life stages resulting in the most effective control of herbicides. This timing was recommended because it is important that weeds are actively growing in the vegetative state to ensure proper translocation of the chemical to the roots for complete kill. As soon as flowering begins, translocation of the roots will not be as effective because of the change from vegetative to reproductive growth resulting in poor weed control.

However, there is potential for exposure of honey bees foraging on weeds off-crop, assuming that flowering coincides with the application. However, the maximum spray drift deposition rate at 1 m from the edge of the treated crop is 2.77 % of the in-field rate, resulting in low exposure potential. Exposure of bees from plants that reside outside the field margins should be negligible since spray drift is the primary route of exposure which results in very little clopyralid reaching off field plants.

Furthermore, esposure to bees from dietary consumption of contaminated pollen and nectar is considered insignificant due to the minimal spray drift rate, low nectar/pollen consumption rate of bees, and llow pesticide concentration in pollen and nectar following foliar applications (RUDs). Overall, low risk is anticipated for bee development as well as other life stages.

The Notifier has stated, that the EFSA guidance document (EFSA Journal 2013; 11 (7): 3295) has not been noted by Member States at the Standing Committee, principally because of reservations about its capacity to help as a practical support in the risk assessment to bees. Comments on the level of conservatism have been raised and remain unsolved. The need for significant updates was identified by Member States during several workshops in December 2013 and May 2015. Finally, in accordance to guidance documents on the evaluation of active substance, dossiers on active substances should be evaluated based on guidance documents in force at the time of their submission, and not retroactively on the basis of non-notified guidance documents.

RMS comments and evaluation:

The acute oral and contact toxicity studies with the formulation GF-1374 are different from those referred to in the DAR of clopyralid (2003) and not assessed before within the context of the approval of clopyralid. The studies are relevant for the risk assessment of the formulated product of clopyralid, as calculated above by the Notifier.

The explanation and justification for waiving the chronic, developmental and sublethal data based on the expected low exposure, as presented by the Notifier above, is considered as understandable, but waiving of the data should be aligned with other renewal active substances according to the data requirement regulation.

Based on the risk assessment according to the formerly used EPPO guidance, the acute oral and contact $Q_{HO}$ values to honeybees are all below the trigger of 50 of the Uniform Principles of the Commission Regulation 546/2011. It indicates thus a low acute risk to honey bees via oral and contact exposure, when the product GF-1374 is sprayed according to Good Agricultural Practice at rates of up to 1.5 L formulated product/ha on pasture and 1.0 L formulated product/ha to cereals.

However, the risk assessment on honeybees did not follow the new EFSA guidance on the risk assessment of plant protection products on bees (EFSA Journal 2013; 11(7): 3295). Dow AgroSciences has argued that the guidance has not been notified in the Standing Committee and should therefore not be followed when the AIR3 dossier of clopyralid was due to be submitted. It is agreed that the Standing Committee has not notified the guidance so far, but nevertheless the EFSA has required the other Notifiers of other AIR3 active substances to assess the risks according to this document. Although the RMS is of the opinion that the acute risk to honeybees is acceptable from the use of GF-1374 as calculated with the previous procedure, a new risk assessment should be considered according to the new guidance (EFSA Journal 2013; 11(7): 3295), and using the data

requirements of chronic and developmental toxicity endpoints (as laid down in the Commission regulations 283 and 284/2013 setting out data requirements for active substances and plant protection products), for which data gaps were identified.

Therefore the risk assessment on honeybees is inconclusive for the time being. Also further data on pollinators was not submitted, as presented in the next chapter.

## B.9.5.3. Additional testing on honey bees

### B.9.5.3.1. Chronic toxicity to bees

Acute toxicity studies conducted on honey bees were evaluated for clopyralid and GF-1374 and the endpoints were used in the above risk assessments. All risk assessments with the active ingredients as well as the formulated product demonstrated low acute oral and contact toxicity to honeybees following applications of GF-1374 to pasture and amenity grassland as well as cereals.

Under the Regulation (EC) No 1107/2009, the new data requirements indicate that for the active substance clopyralid, the chronic risk to adult bees should be addressed. However, validated test methods are not yet available for chronic studies. Furthermore, the new guidance document on risk assessment of bees has been published by EFSA[2], but this is currently under review and as yet, has not been noted by the Standing Committee, with no implementation date set. It is therefore unclear whether this guidance will change or will be accepted in full or in part. According to SANCO/10181/2013-rev. 2.1, 13 May 2013, under item 4, where test methods or guidance documents are not yet available for particular data requirements, waiving of these particular data requirement points is considered acceptable.

Commission Regulation (EU) No 283/2013 sets out the data requirements for the active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. The new data requirements indicate that for clopyralid the chronic risk to adult bees and the effects on other honey bee life stages should be addressed. However, for the chronic study, validated test methods are not yet available; therefore, a chronic study on adult honey bees with GF-1374 has not been conducted at time of submission.

Clopyralid is an herbicide that does not have any known insecticidal action. It is applied once per season and applications are made to grassland and cereals. Both these crops are not attractive to worker and foraging bees due to lack of blooming flowers containing no pollen or nectar. Applications are made to control target in-field weeds during the weed's vegetative growth. Treated weeds may be expected to be impacted soon after application due to the rapid absorption and translocation of the the active substance to the meristematic portion of the plant where hormonal disruption will occur resulting in plant symptoms such as bending and twisting of the leaves, wilting, cupping of leaves, etc. resulting in the elimation of impacted weeds from the pool of flowering plants that bees may visit and where they may be exposed to systemic residues of clopyralid. Furthermore, most herbicide applications are applied at early growth stages of weeds due to the plant's susceptibility at early life stages resulting in the most effective control of herbicides. This timing was recommended because it is important that applications are made when weeds are actively growing in the vegetative state to ensure proper translocation of the chemical to the roots for complete kill. As soon as flowering begins, translocation to the roots will not be as effective because of the change from vegetative to reproductive growth resulting in poor weed

[2] European Food Safety Agency, 2013. 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, 266pp., doi:10.2903/j.efsa.2013.3295

control. This is extremely important for the control of creeping thistles which is the primary use of clopyralid.

Therefore, treated in-field vegetation is unlikely to present any forage value to worker honey bees at the time of application and the potential of exposure is low. Risk was not calculated for in-field exposure of GF-1374 or clopyralid since exposure to crop and weed is unlikely.

There is potential for exposure of honey bees foraging on weeds off-crop or field, assuming that flowering coincides with the application. The maximum spray drift deposition rate at 1 m from the edge of the treated crop is 2.77% of the in-field rate, i.e 120 and 80 g a.s./ha clopyralid for grassland and cereals, respectively, which results in low exposure to off-target weeds and plants. Overall, exposure of bees from plants that reside outside the field margins should be negligible since spray drift is the primary route of exposure which results in very little clopyralid reaching off field plants.

RMS comments and evaluation:

The Notifier has not submitted any studies in support of the chronic risk assessment of clopyralid.

In their letter on January 22, 2016, the Notifier claimed for not intending to submit any studies to address the chronic toxicity to honeybees, grounding their decision on the non-availability of a harmonized test guideline. The Notifier has also sent a letter on this matter to the DG SANCO on February 10, 2016.

It is agreed that no harmonized guideline is as yet available for this type of study, but recent description of this study (10-d oral feeding study on adult honeybees) in Appendix M of the EFSA GD on bees and Appendix O of the final version of the EFSA GD on bees (EFSA Journal 2013; 11(7): 3295) is available and has been used by other Notifiers in support of renewal of other AIR3 active substances.

Additionally, the Commission communication (2013/C 95/02) in the framework of the implementation of the data requirement regulation 284/2013 refers to following test method in this context:

Aupinel & al. 2007. A new larval in vitro rearing method to test effects of pesticides on honey bee brood. Redia XC: 91-94.

So the RMS does not agree that it would not be possible to reliably test the chronic toxicity of plant protection products on bees. Although the off-field exposure of pollinators from flowering weeds in areas treated with the product GF-1374 is likely low, waiving of this data requirement should be aligned with other AIR3 renewal active substances with similar use patterns.

Data gap is identified, and the chronic risk assessment is inconclusive for the time being.

#### B.9.5.3.2. Effects on honeybee development and other honeybee life stages

Under the Regulation (EC) No 1107/2009, the new data requirements indicate that for the active substance clopyralid, the effects on honeybee development should be evaluated. However, validated test methods are not yet available for these studies. Furthermore, the new guidance document on risk

assessment of bees has been published by EFSA[3], but this is currently under review and as yet, has not been noted by the Standing Committee, with no implementation date set. It is therefore unclear whether this guidance will change or will be accepted in full or in part. According to SANCO/10181/2013-rev. 2.1, 13 May 2013, under item 4, where test methods or guidance documents are not yet available for particular data requirements, waiving of these particular data requirement points is considered acceptable.

Commission Regulation (EU) No 283/2013 sets out the data requirements for the active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. The new data requirements indicate that for clopyralid the chronic risk to adult bees and the effects on other honey bee life stages should be addressed. However, as stated previously, validated test methods are not yet available; therefore, studies with GF-1374 evaluating bee brood have not been conducted at time of submission.

Clopyralid is an herbicide that does not have any known insecticidal action. It is applied once per season and applications are made to grassland and cereals. Both these crops are not attractive to worker and foraging bees due to lack of blooming flowers that contain pollen or nectar. Applications are made to control target in-field weeds during the weed's vegetative growth. Treated weeds may be expected to be impacted soon after application due to the rapid absorption and translocation of the active substance to the meristematic portion of the plant where hormonal disruption will occur resulting in plant symptoms such as bending and twisting of the leaves, wilting, cupping of leaves, etc. resulting in the elimation of impacted weeds from the pool of flowering plants that bees may visit and where they may be exposed to systemic residues of clopyralid. Furthermore, most herbicide applications are applied at early growth stages of weeds due to the plants susceptibility at early life stages resulting in the most effective control of herbicides. This timing was recommended because it is important that applications are made when weeds are actively growing in the vegetative state to ensure proper translocation of the chemical to the roots for complete kill. As soon as flowering begins, translocation to the roots will not be as effective because of the change from vegetative to reproductive growth resulting in poor weed control. This is extremely important for the control of creeping thistles which is the primary use of clopyralid.

However, there is potential for exposure of honey bees foraging on weeds off-crop, assuming that flowering coincides with the application. However, the maximum spray drift deposition rate at 1 m from the edge of the treated crop is 2.77% of the in-field rate, resulting in low exposure potential. Exposure of bees from plants that reside outside the field margins should be negligible since spray drift is the primary route of exposure which results in very little clopyralid reaching off field plants. Furthermore, exposure to bees from dietary consumption of contaminated pollen and nectar is considered insignificant due to the minimal spray drift rate, low nectar/pollen consumption rates of bees, and low pesticide concentration in pollen and nectar following foliar applications (RUDs). Overall, low risk is anticipated for bee development as well as other life stages.

RMS comments and evaluation:

The Notifier has not submitted any studies on the effects of clopyralid on honey bee development and other honeybee life stages.

In their letter on January 22, 2016, the Notifier claimed for not intending to submit any studies to address the chronic and developmental toxicity to honeybees, grounding their decision on the non-

[3] European Food Safety Agency, 2013. 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, 266pp., doi:10.2903/j.efsa.2013.3295

availability of a harmonized test guideline. The Notifier has also sent a letter on this matter to the DG SANCO on February 10, 2016.

It is agreed that no harmonized guideline is as yet available for this type of study, but recent description of this study (10-d oral feeding study on adult honeybees) in Appendix M of the EFSA GD on bees and Appendix O of the final version of the EFSA GD on bees (EFSA Journal 2013; 11(7): 3295) is available and has been used by other Notifiers in support of renewal of other AIR3 active substances.

Additionally, the Commission communication (2013/C 95/02) in the framework of the implementation of the data requirement regulation 284/2013 refers to following test method in this context:

Aupinel & al. 2007. A new larval in vitro rearing method to test effects of pesticides on honey bee brood. Redia XC: 91-94.

So the RMS does not agree that it would not be possible to reliably test the chronic and developmental toxicity of plant protection products on bees. Although the off-field exposure of pollinators from flowering weeds in areas treated with the product GF-1374 is likely low, waiving of this data requirement should be aligned with other AIR3 renewal active substances with similar use patterns.

Data gap is identified, and the risk assessment is inconclusive for the time being.

**B.9.5.3.3. Sub-lethal effects**

GF-1374 is an herbicide with no known insecticidal action. It is applied only once per season and applications are made to grasslands and cereals which do not produce nectar rich flowers. Furthermore, the target in-field weed seedlings are either at a similar immature stage of development or are woody in nature therefore unlikely to present any forage value to worker honey bees at the time GF-1374 is applied. There is potential for exposure of honey bees foraging on weeds off-crop, assuming that flowering coincides with the application. However, the maximum spray drift deposition rate at 1 m from the edge of the treated crop is only 2.77% of the in-field rate and estimated exposure values as well as predicted TER values were provided above for adult chronic as well as adult and larval dietary exposures and all calculated TERs indicate low chronic or dietary effects.

Currently, studies to investigate sub-lethal effects of GF-1374 on honey bees are considered to be unnecessary and have not been performed. However, sublethal effects are not anticipated due to the lack of acute and chronic effects for both adult and larval stages. Furthermore, based on the acute studies, no sublethal effects were observed, even at the highest dose tested for both the active and the formulated product.

**Table 9.5.14. Effects observed in the acute studies performed with honey bees exposed to clopyralid and GF-1374.**

| Substance | 4 hours | 24 hours | 48 hours |
|---|---|---|---|
| GF-1374 (acute oral test) | 0% mortality; all normal bees observed except 24 out of 30 bees appeared lethargic at highest dose tested (100 µg/bee) | 0% mortality; all normal bees observed for all treatments | 0% mortality; all normal bees observed for all treatments |

| | | | |
|---|---|---|---|
| GF-1374 (acute contact test) | 0% mortality; all normal bees observed for all treatments | 0% mortality; all normal bees observed for all treatments | 0% mortality; all normal bees observed for all treatments |
| Clopyralid (acute oral teset) | No marked reaction to exposure were noted in any of the test bees throughout the study | No marked reaction to exposure were noted in any of the test bees throughout the study | No marked reaction to exposure were noted in any of the test bees throughout the study |
| Clopyralid (acute contact test) | No marked reaction to exposure were noted in any of the test bees throughout the study | No marked reaction to exposure were noted in any of the test bees throughout the study | No marked reaction to exposure were noted in any of the test bees throughout the study |

RMS comments and evaluation:

It is not agreed that flowering weeds would unlikely be present in fields to be treated with the product GF-1374. Flowering dicotyledonous weeds are important nectar and pollen sources of bees and may well be present in pastures during the spray application of GF-1374. So the exposure of pollinators is possible from flowering weeds in areas treated with the product GF-1374, although the crop itself would not be attractive for bees.

However, based on the acute toxicity studies with honeybees available, no sub-lethal effects were observed in studies with any of the active substances or the product GF-1374. As new studies will anyway be considered on the chronic and developmental toxicity of clopyralid to honeybees (see above), these studies should cover also the possible sub-lethal effects. If any sub-lethal effects would be anticipated, they should come out in the new studies to be required.

Although the off-field exposure of pollinators from flowering weeds in areas treated with the product GF-1374 is likely low, waiving of this data requirement should be aligned with other AIR3 renewal active substances with similar use patterns. Data gap is identified, and the risk assessment is inconclusive for the time being.

**B.9.5.3.4. Cage and tunnel tests**

The acute toxicity of clopyralid and GF-1374 to honey bees is predicted to be low, based on the endpoints available for both the technical active substance and formulated product. Furthermore, risk associated with chronic exposure, consumption of nectar and pollen (based on predicted exposure and acute endpoints), and sub-lethal effecst are anticipated to be negligible based on predicted exposure values and predicted nectar and pollen consumption values. Since acute and chronic risk of clopyralid and GF-1374 is considered to be low, field tests to investigate the effects of GF-1374 on honey bee colonies are therefore considered to be unnecessary and have not been performed.

RMS comments and evaluation:

It is agreed that cage and tunnel tests in field are not required for clopyralid for the time being.

**B.9.5.3.5. Field tests with honeybees**

The acute toxicity of clopyralid and GF-1374 to honey bees is predicted to be low, based on the endpoints available for both the technical active substance and formulated product. Furthermore, risk associated with chronic exposure, consumption of nectar and pollen (based on predicted exposure and

acute endpoints), and sub-lethal effecst are anticipated to be negligible based on predicted exposure values and predicted nectar and pollen consumption values. Since acute and chronic risk of clopyralid and GF-1374 is considered to be low, field tests to investigate the effects of GF-1374 on honey bee colonies are therefore considered to be unnecessary and have not been performed.

RMS comments and evaluation:

It is agreed that field tests with clopyralid are not required for the time being.

## B.9.5.4. Effects on non-target arthropods other than bees

### B.9.5.4.1. Standard laboratory testing for non-target arthropods

No standard laboratory testing for non-target arthropods was submitted with the new representative fromulation of clopyralid, GF-1374. However, as evaluated in the DAR (2003), technical clopyralid was intensively tested with several species of non-target arthropods, and without any significant effects on these organisms. Therefore it was considered unnecessary to repeat the first tier standard tests with the new formulation, but extended laboratory testing strategy was chosen instead. Please refer to Annex Points CP 10.3.2.2-1 through 3 for extended laboratory non-target arthropod study summaries.

RMS comments and evaluation:

It is agreed that standard laboratory tests with the representative formulation of clopyralid, GF-1374, on non-target arthropods are not necessary, as there are extended laboratory tests available on three species of non-target arthropods instead and the risk assessment can be based on these data. The studies are evaluated below. No further data are required on this issue.

### B.9.5.4.2. Extended laboratory testing, aged residues studies with non-target arthropods

The following extended laboratory studies with three species of non-target arthropods using natural substrates performed on GF-1374 are provided in support of the assessment and have not been previously evaluated.

**CP.10.3.2.2./1 Extended Laboratory study with *Aphidius rhopalosiphi***

| | |
|---|---|
| Report: | Loose, E. (2004a). |
| Title: | GF-1374: an extended laboratory study to evaluate the effects on survival and reproduction of the parasitoid wasp *Aphidius rhopalosiphi* (De Stefani-Perez) (Hymenoptera: Braconidae). |
| Document No: | Dow AgroSciences Study Number: 040262 |
| Guidelines: | Candolfi, M.P. *et al.*(2001). Guidance document on regulatory testing and risk assessment procedures for plant protection products with non-target arthropods. Proceedings from the ESCORT 2 workshop, Wageningen, March 21-23, 2000. SETAC, Pensacola, Florida, USA, 46 pp. and<br>Mead-Briggs, M.A. *et al.*(draft version January 2004). An extended laboratory test for evaluating the effects of plant protection products on the parasitic wasp, *Aphidius rhopalosiphi* (Hymenoptera, Braconidae). |
| GLP | Yes |

**Methodology:** GF-1374 (Lot No. 187/76-A) is an emulsion concentrate (EC) nominally containing fluroxypyr-meptyl at 144 g/L (142 g/L, actual), clopyralid at 80 g/L (86 g/L, actual) and florasulam at 2.5 g/L (2.5 g/L, actual). The aim of this study was to determine under extended-laboratory test conditions whether this formulation has a harmful effect on the parasitic wasp, *Aphidius rhopalosiphi*.

GF-1374 was evaluated at two rates, equivalent to 0.277 and 2.000 L product/ha. It was compared with a water-treated control and a toxic reference treatment of dimethoate applied at a rate equivalent to 8 g a.s./ha. Treatments were applied to pots of seedling barley at a volume rate equivalent to 300 L spray solution/ha. Once dry, the plants were enclosed within cylindrical, ventilated collars. Six female adult wasps were confined over each pot. There were eight replicates for the untreated control and the GF-1374 treatments and five replicates for the toxic reference. Repellency assessments were made during the initial 3 hours of the exposure. Wasp survival was assessed over a period of 48 h. The fecundity of surviving females was then assessed for the control and for the two treatment rates of the test item. The female wasps (n = 15 per treatment) were confined individually over untreated aphid-infested barley plants for a further 24 h, before being removed. The plants were left for a further 11 days before the numbers of aphid mummies that developed was assessed.

**Findings:** Results are summarised in the following Table 9.5.15.

**Table 9.5.15. Effects of GF-1374 on *A. rhopalosiphi***

| Treatment | | Mortality (%) | Wasps not on plants (%)[a] | No. mummies/female | |
|---|---|---|---|---|---|
| | | | | Mean | Reduction (%) |
| Water control | | 0 | 40 | 7.4 | -- |
| GF-1374 (L/ha): | 0.277 | 2 | 35 | 9.9 | -34 |
| | 2.000 | 4 | 31* | 10.5 | -42 |
| Dimethoate (8 g a.s./ha) | | 100** | 29 | ~ | ~ |

[a] Data from assessments made during the initial 3 h after wasp introduction.
* Fisher's Exact Test ($p<0.05$).
** Fisher's Exact Test ($p<0.001$).
~ Indicates parasitisation capacity assessment not carried out.

GF-1374 had no significant effect on the mortality or the parasitisation capacity of the wasps. During the first 3 hours of the test 60% of the wasps were noted to be settled on the plants in the control treatments indicating a good level of exposure. For the test item wasp settle rates were slightly increased compared to the control and were at a level considered appropriate to demonstrate that significant exposure had taken place.

**Conclusions:** Under extended laboratory test conditions, GF-1374 was harmless to the parasitic wasp, *Aphidius rhopalosiphi*, when applied at rates up to 2 L product/ha.

RMS comments and evaluation:

The study was well conducted according to the test guidelines and GLP, and clearly reported. There were no deviations from the test protocol. The toxic control dimethoate produced complete mortality, while no statistically significant effects were found with the test substance GF-1374 when used according to its highest use rate of 2 L/ha. The validity criteria of mortalities in the deionised water control and toxic reference, as well as of the mean reproduction in deionised water control were met. The study is acceptable and valid for decision making on the product GF-1374. The data requirement is fulfilled.

**CP.10.3.2.2./2 Extended Laboratory Study with *Typhodromus pyri***

| | |
|---|---|
| Report: | Loose, E. (2005). |
| Title: | GF-1374: an extended laboratory study to evaluate the effects on survival and reproduction of the predaceous mite *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae). |
| Document No: | Dow AgroSciences Study Number: 040263R |
| Guidelines: | Blümel, S. *et al.* (2000). Laboratory residual contact test with the predatory mite *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) for regulatory testing of plant protection products. In: *Guidelines to evaluate side-effects of plant protection products to non-target arthropods; IOBC, BART and EPPO Joint Initiative*. (Eds. Candolfi, M.P. *et al.*). IOBC Publication. ISBN 92-9067-129-7. and Candolfi, M.P. *et al.* (2001). Guidance document on regulatory testing and risk assessment procedures for plant protection products with non-target arthropods. Proceedings from the ESCORT 2 workshop, Wageningen, March 21-23, 2000. SETAC, Pensacola, Florida, USA, 46 pp. |
| GLP | Yes |

**Methodology:** GF-1374 (Lot No. 187/76-A) is an emulsion concentrate (EC) nominally containing fluroxypyr-meptyl at 144 g/L (142 g/L, actual), clopyralid at 80 g/L (86 g/L, actual) and florasulam at 2.5 g/L (2.5 g/L, actual). The aim of this study was to determine under extended laboratory test conditions whether GF-1374 has harmful effects on the predatory mite, *Typhlodromus pyri*.

GF-1374 was evaluated at two rates, equivalent to 0.277 and 2.000 L GF-1374/ha. These were compared to a control treatment of water and a toxic reference treatment of dimethoate applied at a rate of 30 g a.s./ha. Treatments were applied to cowpea leaves at a volume rate equivalent to 200 L spray solution/ha. The leaves were then placed onto damp cotton wool and insect trapping glue was drawn around the edge of each leaf to create arenas in which mites were confined. Ten protonymphal *T. pyri* were placed in the centre of each replicate arena, with nine replicates for the control, eight replicates for each GF-1374 treatment and six replicates for the toxic reference. The mites were provided regularly with untreated pollen for food. Their survival was assessed over a 7-day period, by which time they were adult. The sex of the adult mites was determined and they were then transferred to untreated glass plates so that their fecundity could be assessed over a further 7 days. The mean number of eggs produced per female between 7 and 14 days after treatment (DAT) was calculated.

**Findings:** Results are summarised in the following table.

**Table 9.5.16. Effects of GF-1374 on *T. pyri***

| Treatment | | Mortality (Day 7) | | Escaped (%, Day 7) | No. eggs/female/day (Days 7-14) | |
|---|---|---|---|---|---|---|
| | | % | Corrected (%)[a] | | Mean | Reduction (%) |
| Control | | 18 | -- | 10 | 9.1 | -- |
| GF-1374 (L/ha): | 0.277 | 23 | 7 | 14 | 9.1 | 0 |
| | 2.000 | 59* | 50 | 38 | 8.7 | 5 |
| Dimethoate (30 g a.s./ha) | | 82* | 78 | 37 | ~ | ~ |

[a]: According to Abbott (1925).
* Fisher's Exact Test ($p<0.001$).
~ Indicates fecundity assessment not carried out.

Mortality in the toxic reference and the 2 L GF-1374/ha treatments were significantly different from the control. However, for the fecundity assessment, the results for all GF-1374 treatments did not differ significantly from the control (Mann-Whitney U test, $p > 0.05$).

**Conclusions:** Under extended laboratory conditions GF-1374 showed some toxicity to *Typhlodromus pyri* at the highest tested rate of 2 L/ha, with a maximum corrected mortality of 50%. However no effects were observed on the fecundity phase of the study at the GF-1374 rates tested in the study (i.e. 0.277 and 2 L/ha).

RMS comments and evaluation:

The study was well conducted according to the test guideline and GLP, and clearly reported. The test was initiated three times. Due to the large number of escapees in the first trial and an extremely male biased sex ratio in the second trial it was decided to initiate the third trial again, which was run successfully.The toxic control dimethoate and the highest use rate of the test substance GF-1374 produced significantly higher mortality, while no statistically significant effects were found with the lower application rate compared to control. However, the fecundity effect was within acceptable limits with the highest use rate of 2 L/ha of GF-1374. The validity criteria of mortalities in the deionised water control and toxic reference, as well as of the mean reproduction in deionised water control were met. The study is acceptable and valid for decision making on the product GF-1374. The data requirement is fulfilled.

**CP 10.3.2.2/3 Extended Laboratory Study with *Chrysoperla carnea***

| | |
|---|---|
| Report: | KIIIA1 10.5.2/03, Loose, E. (2004). |
| Title: | An extended laboratory study to evaluate the effects of GF-1374 on survival and reproduction of the green lacewing, *Chrysoperla carnea* Steph. (Neuroptera: Chrysopidae). |
| Document No: | Dow AgroSciences Study Number: 040264R |
| Guidelines: | Vogt, H. *et al.* (2000). Laboratory method to test effects of plant protection products on larvae of *Chrysoperla carnea* (Neuroptera: Chrysopidae). In: *Guidelines to evaluate side-effects of plant protection products to non-target arthropods; IOBC, BART and EPPO Joint Initiative*. (Eds. Candolfi, M.P. *et al.*). IOBC Publication. ISBN 92-9067-129-7 |
| GLP | Yes |

**Methodology:** GF-1374 (Lot No. 187/76-A) is an emulsion concentrate (EC) nominally containing fluroxypyr-meptyl at 144 g/L (142 g/L, actual), clopyralid at 80 g/L (86 g/L, actual) and florasulam at 2.5 g/L (2.5 g/L, actual). The aim of this study was to determine under extended laboratory test conditions whether GF-1374 has harmful effects on the green lacewing, *Chrysoperla carnea*.

GF-1374 was evaluated at two rates, equivalent to 0.277 and 2.000 L product/ha. It was compared with a water-treated control and a toxic reference treatment of dimethoate applied at a rate of 80 g a.s./ha. All treatments were applied to detached green bean leaves at a volume rate equivalent to 200 L spray solution/ha. Once dry, 2- to 3-day-old larvae were individually confined on the leaves. There were 48 units for the control and the GF-1374 treatments and 32 units for the toxic reference. The larvae were fed every 1-2 days with eggs of the moth, *Ephestia kuehniella*, and the pre-imaginal mortality of the insects was assessed. To determine if there had been any sub-lethal treatment effects on the fecundity of the test insects, the egg-laying activity of the matured adults on two separate 24-h periods was assessed and the subsequent viability of the eggs was recorded.

**Findings:** Results are summarised in the following table.

**Table 9.5.17. Effects of GF-1374 on *C. carnea***

| Treatment | | Pre-imaginal mortality | | 1st 24-h observation period | | 2nd 24-h observation period | |
|---|---|---|---|---|---|---|---|
| | | % | Corrected (%)[a] | Mean no. eggs/female/day | Hatched eggs (%) | Mean no. eggs/female/day | Hatched eggs (%) |
| Control | | 13 | -- | 19.7 | 72 | 26.2 | 77 |
| GF-1374 (L/ha): | 0.277 | 13 | 0 | 21.6 | 63 | 23.5 | 79 |
| | 2.000 | 19 | 7 | 18.9 | 34 | 26.3 | 76 |
| Dimethoate (80 g a.s./ha) | | 100 | 100 | ~ | ~ | ~ | ~ |

[a] According to Abbott, 1925.
~ Indicates fecundity assessment not carried out.

No significant effect was observed on mortality in the GF-1374 treatments. The fecundity in both the test item (for all two rates tested) and control treatments exceeded the threshold of ≥ 15 eggs/female/day. The percent of hatched eggs appeared to be affected in the GF-1374 treatments during the first 24-h observation period. However during the second observation period hatching rate was ≥ 70% in the control and GF-1374 treatments, which, together with a mean number of eggs/female/day ≥15, is currently viewed as being indicative of no harmful treatment effects (Vogt *et al*., 2000).

**Conclusions:** Under extended laboratory test conditions, GF-1374 had no long-term toxicity to the green lacewing, *Chrysoperla carnea*, when applied at rates of up to 2 L product/ha.

RMS comments and evaluation:

The study was well conducted according to the test guidelines and GLP, and clearly reported. There were no deviations from the test protocol. The toxic control dimethoate produced complete mortality, while no statistically significant effects were found with the test substance GF-1374 when used

according to its highest use rate of 2 L/ha. The validity criteria of mortalities in the deionised water control and toxic reference, as well as of reproduction and egg hatch in deionised water control were met. The study is acceptable and valid for decision making on the product GF-1374. The data requirement is fulfilled.

#### B.9.5.4.3. Semi-field studies with non-target arthropods

No effects were observed following laboratory testing in accordance with the requirements put forth in point 8.3.2 of Part A of the Annex to Regulation (EU) no 283/2013 point 10.3.2 Annex Regulation (EU) 284/2013. Since Annex trigger values were not breached under these scenarios, semi field testing is not required.

RMS comments and evaluation:

The explanation given by the Notifier is acceptable and it is agreed that semi-field studies with non-target arthropods will not be required. Data requirement is fulfilled and no further data are required on this issue.

#### B.9.5.4.4. Field studies with non-target arthropods

No effect were observed following testing in accordance with the reqirements set out in point 8.3.2 of Part A of the Annex Regulation (EU) No 283/2013 or in accordance with points 10.3.2.2 or 10.3.2.3 of Annex Regulation (EU) 284/2013. Calculated Risk Quotients (RQs) did not indicate risk to non-target arthropods so field testing was not required.

RMS comments and evaluation:

The explanation given by the Notifier is acceptable and it is agreed that field studies with non-target arthropods will not be required. Data requirement is fulfilled and no further data are required on this issue.

#### B.9.5.4.5. Other routes of exposure for non-target arthropods

Since GF-1374 has demonstrated low toxicity to non-target arthropods, further testing of other possible routes of exposure was deemed unnecessary.

RMS comments and evaluation:

As the formulation GF-1374 will be applied solely by spraying on cereals and pasture, leaf-dwelling arthropods are most likely at exposure. These organisms have been appropriately tested as presented above, and without significant effects at highest use rates. Therefore it is agreed that testing via other routes of exposure will not be required. Data requirement is fulfilled.

## B.9.5.5. Risk assessment for non-target arthropods other than bees

### B.9.5.5.1. Toxicity endpoints used in the risk assessment

Effects on arthropods other than bees for GF-1374 were not evaluated as part of the EU review of fluroxypyr, clopyralid and florasulam. Data on GF-1374 is evaluated here, and risk assessments for GF-1374 with the proposed use pattern are provided here and are considered adequate.

The critical endpoints employed in the risk assessment for non-target arthropods are indicated in Table 9.5.18. below.

**Table 9.5.18. Toxicity of GF-1374 to non-target arthropods.**

| Compound | Test species | Endpoint | EU agreed endpoints* | Endpoint used in risk assessment** | |
|---|---|---|---|---|---|
| | | | Value | Value (mL/ha) | Ref |
| GF-1374 | *Typhlodromus pyri* | Laboratory extended study $LR_{50}$ | N/A | 2000 | Loose (2005) DAS Study ID 040263R |
| | *Aphidius rhopalosiphi* | | | >2000 | Loose (2004a) DAS Study ID 040262 |
| | *Chrysoperla carnea* | | | >2000 | Loose (2004b) DAS Study ID 040264R |

* GF-1374 is a formulation which was not evaluated at EU level.
** Studies for GF-1374 have been performed providing end-points which are used in the risk assessment.
N/A: not applicable

### B.9.5.5.2. Exposure

The risks from the use of the product GF-1374 to non-target arthropods were assessed on the basis of the critical GAPs for the product, as presented in Table 9.5.19.

**Table 9.5.19. Critical GAP for GF-1374.**

| Crop | Time of application | No. of applications per year | Maximum rate per application |
|---|---|---|---|
| Cereals | BBCH 13 – 39 February to June 30 | 1 | 1.0 L GF-1374/ha (or 1040[a] g/ha) equivalent to: fluroxypyr-meptyl 144 g/ha (or 100 g a.e./ha); florasulam 2.5 g/ha; clopyralid 80 g/ha |
| Pasture | Feb 01 to August 31 | | 1.5 L GF-1374/ha (or 1560[a] g/ha) equivalent to: fluroxypyr-meptyl 216 g/ha (or 150 g a.e./ha); florasulam 3.75 g/ha; clopyralid 120 g/ha |

The risk assessment for effects to non-target arthropods for GF-1374 is based on a single application at a maximum rate of 1500 mL product/ha for non-target arthropod populations present in the in-field area for pasture use. For cereal use, the maximum application rate is 1000 mL product/ha. Another area of

risk is to non-target arthropod populations present in the off-field area where these species are exposed to spray drift at the time of application.

Toxicity from GF-1374 was expected to be low to non target arthropods based on the active substances present and thus testing was conducted at extended laboratory studies with GF-1374 on the two indicator species and an additional species (lacewing) representing testing on realistic plant or leaf natural substrates rather than glass plate/sand.

#### B.9.5.5.3. ESCORT 2 (2001) Extended laboratory Hazard Quotient procedure

A risk assessment for these scenarios has been conducted using the Hazard Quotient approach in ESCORT 2 (Guidance Document on Terrestrial Ecotoxicology: SANCO/10329/2002) modified for extended laboratory data.

In extended laboratory studies, the substrate used to expose organisms differs from that employed at tier I in that natural substrates (leaves and soil) are used in place of inert substrates (glass plates and quartz sand). In such tests, risk is indicated if effects are greater than 50% and there are no signs of potential recovery or re-colonisation. Rate response data may also be used to define $LR_{50}$ values on natural substrates. ESCORT 2 does not give a trigger value for HQ calculations based on extended laboratory $LR_{50}$ values, but the acceptance in extended laboratory tests of a 50% effect trigger implies that the threshold HQ should be 1.0 for consistency. The EU guidance document on terrestrial ecotoxicology (SANCO/10329/2002) states that lethal or sub lethal effects <50% are acceptable and is in agreement with the use therefore of the extended laboratory HQ threshold of 1.0. The HQ can be defined as below:

$$In-field\ \ HQ = \frac{application\ \ rate \times MAF}{LR_{50}}$$

$$Off-field\ \ HQ = \frac{application\ \ rate \times MAF \times drift\ \ \ factor}{LR_{50}} \times \frac{correction\ \ \ factor}{VDF}$$

where:

| | |
|---|---|
| application rate: | maximum rate of application in same units (mL product/ha) as the $LR_{50}$. |
| MAF: | multiple application factor set to 1 for a single application of GF-1374. |
| drift factor: | 90$^{th}$ percentile of the 'in-field' rate, at a given distance from the treated area. For broadcast applications, the drift listed for "field crops" in Rautmann et al. (2001)[4] (i.e. 2.77% at 1 m) can be used. |
| correction factor: | a safety factor used to give adequate protection to 'off-field' species where diversity is greater than 'in-field' and sensitivities are unknown and may not be adequately protected by the Tier 1 indicator organisms. Default value is 10, reduced to 5 in the present assessment because results for several species are available. |
| VDF: | vegetation distribution factor that adjusts the standard spray-drift values (based on measurements on flat, 2-dimensional surfaces) to take account of the 3-dimensional character of the vegetated 'off-field' zone. Default value is 10. |

The correction factor, which accounts for more sensitive off-field species, may be reduced from 10 (as at tier I) to 5 because more species have been tested (ESCORT 2).

[4] Rautmann, D., Streloke, M., Winkler, R. (2001). New basic drift values in the authorisation procedure for plant protection products. In Forster, R., Streloke, M. Workshop on Risk Assessment and Risk Mitigation Measures in the Context of the Authorization of Plant Protection Products (WORMM). Mitt. Biol. Bundesanst. Land-Forstwirtsch. Berlin-Dahlem, Heft 381.

The $LR_{50}$ values for GF-1374 to the indicator species *T. pyri* and *A. rhopalosiphi*, under extended laboratory conditions, were estimated to be 2000 and >2000 mL/ha, respectively. These values will be taken to represent the realistic worst case end point for non-target arthropods for GF-1374. An extended laboratory study was also conducted with a third species; lacewing (*C. carnea*), with the $LR_{50}$ > 2000 mL/ha. The risk assessment is presented in the table below.

**Table 9.5.20. Risk to non-target arthropods from applications of GF-1374– ESCORT 2**

| Scenario | Species | Exposure (mL prod/ha) | Correction factor | VDF | $LR_{50}$ (mL/ha) | HQ |
|---|---|---|---|---|---|---|
| *Pasture* | | | | | | |
| In-field | *T. pyri* | 1560 | n.a. | n.a. | > 2000 | < 0.78 |
| | *A. rhopalosiphi* | 1560 | n.a. | n.a. | | < 0.78 |
| | *C. carnea* | 1560 | n.a. | n.a. | | < 0.78 |
| Off-field, spray drift at 1 m | *T. pyri* | 43.2 | 5 | 10 | > 2000 | < 0.01 |
| | *A. rhopalosiphi* | 43.2 | 5 | 10 | | < 0.01 |
| | *C. carnea* | 43.2 | 5 | 10 | | < 0.01 |
| *Cereals* | | | | | | |
| In-field | *T. pyri* | 1040 | n.a. | n.a. | > 2000 | < 0.52 |
| | *A. rhopalosiphi* | 1040 | n.a. | n.a. | | < 0.52 |
| | *C. carnea* | 1040 | n.a. | n.a. | | < 0.52 |
| Off-field, spray drift at 1 m | *T. pyri* | 28.8 | 5 | 10 | > 2000 | < 0.007 |
| | *A. rhopalosiphi* | 28.8 | 5 | 10 | | < 0.007 |
| | *C. carnea* | 28.8 | 5 | 10 | | < 0.007 |

n.a.: not applicable.

Note: Correction factor of 5 is applied to off-field exposure in HQ calculation to account for unknown species of greater sensitivity (ESCORT 2).

The HQ for all scenarios are less than the extended laboratory trigger value of 1.0, indicating low risk to terrestrial non-target arthropods both in-field and off-field for both indicator species, from applications of GF-1374 to control weeds at rates up to 2 L/ha. Results from the extended laboratory study with *Chrysoperla carnea* also confirm a low risk to leaf dwelling non-target arthropods from GF-1374 use.

**In conclusions, when used according to good agricultural practice, no unacceptable long-term effects are anticipated on communities of terrestrial non-target arthropods due to the use of GF-1374.**

RMS comments and evaluation:

The risk assessment presented by the Notifier was conducted acceptably according to the ESCORT 2 guideline. It is considered that the data on the effects of clopyralid to non-target arthropods appropriately addresses the in-field and off-field risks and is adequate to conclude that the use of the product GF-1374 on cereals and pasture up to 1.5 L/ha once per season does not pose an unacceptable risk to non-target arthropods. The hazard quotients for all three species in both intended uses are well below the trigger of 1, indicating that the risks to leaf-dwelling non-target arthropods are acceptable if the product GF-1374 is used according to GAP. The data requirement is fulfilled. No further data are required.

## B.9.6. EFFECTS ON NON-TARGET SOIL MESO- AND MACROFAUNA

### B.9.6.1. Laboratory studies on earthworms

The following earthworm long-term toxicity study performed on GF-1374 is provided in support of the assessment and has not been previously evaluated.

**CP 10.4.2.1/1. Sublethal effects to earthworms**

| | |
|---|---|
| Report: | Davies, N.A. (2005). |
| Title: | The effects of GF-1374 on reproduction and growth in the earthworm *Eisenia fetida*. |
| Document No: | Dow AgroSciences Study Number: 040258 |
| Guidelines: | ISO Guideline 11268-2 (1998). |
| GLP | Yes |

**Methodology:** A 56-day reproduction study was conducted with the earthworm *Eisenia fetida* according to ISO Guideline 11268-2 in compliance with GLP. GF-1374 (Lot No. 187/76-A) is an emulsion concentrate (EC) nominally containing fluroxypyr-meptyl at 144 g/L (142 g/L, actual), clopyralid at 80 g/L (86 g/L, actual) and florasulam at 2.5 g/L (2.5 g/L, actual).

Two concentrations of GF-1374 were employed in the experiment: 4 L/ha and 20 L/ha, plus an untreated control. These rates are equivalent to 0, 11.9 and 59.3 mg GF-1374/kg dry soil. The test soil was composed of 10% by weight sphagnum peat moss, 20% by weight kaolin clay, and 70% by weight industrial sand. The dry constituents were blended in the correct proportions and mixed thoroughly. The food source consisted of pasteurised cow manure (5 g/600 g dry soil). The soil pH was measured and adjusted to 6.42-6.54 (initial range) by the addition of $CaCO_3$. The moisture content of the soil was adjusted to 50% water holding capacity, equivalent to 31.2% moisture content, using deionised water.

The worms were 8-9 month old adults from an in-house culture and they were acclimated for 5 days prior to study initiation. The test vessels were 1.2 L plastic boxes (surface area 170.6 $cm^2$), sealed with a plastic lid and perforated with a single hole to allow gas exchange. Illumination was supplied by a low energy light bulb set on a 16/8 light/dark cycle (415-591 lux) and the soil temperature was 19.5-21.0°C.

On day 0, groups of ten worms were washed, blotted with tissue, weighed as a single group, and placed onto the surface of the soil (600 g dry weight) in each test vessel. The groups were added to the vessels in a randomised fashion. Thirty minutes after the addition of the worms, when all individuals had buried beneath the surface, appropriate stock solutions of GF-1374 in deionised water were applied to the soil surface of each vessel using a conveyor sprayer set to deliver nominally 600-800 L/ha (calibration check 704 L/ha). The treatment rates were 4.0 L/ha (11.9 mg product/kg dry soil) and 20 L/ha (59.3 mg product/kg dry soil). Each treatment was replicated four times.

One day after addition of the worms, 5 g dry weight of manure was placed on the soil surface of each vessel and once a week thereafter for the first 28 days of the study. For each application, sufficient deionised water was added to ensure a 1:1 mix of water : manure. After 28 days, each vessels was tipped onto

a tray and observations made on behaviour and condition of adult worms. The adult worms were then removed, counted, washed, blotted with tissue, and weighed. The soil was then replaced, ensuring that no cocoons were visible; a final application of 5 g of manure was made to provide food for juvenile worms during the remaining 28 days of the study. On day 56, the vessels were placed in a bath of hot water for 30-40 minutes to bring the juveniles to the soil surface to facilitate counting. After removing the visible juvenile worms, the soil in each vessel was tipped onto a tray for examination for remaining juveniles. Unhatched cocoons were counted separately. The treated groups were statistically compared for number of juveniles, unhatched cocoons, changes in worm weight, and mortality of adult worms, with the untreated controls.

**Findings:** The measured soil temperature range during the study was 19.5 to 21.0°C, moisture content at the end of the study was 33.0-34.2% (53.0-54.8% MWHC) and pH ranged from 5.60-6.21 in the controls and from 6.42-6.79 in the treated soils.

No significant adult worm mortality or sublethal effects occurred in the control or any treatment group during the 28 day exposure period. Mean percent change in body weight was +1.53% in the control group and -6.97% and -2.25% in the 4.0 L/ha and 20 L/ha treatment groups, respectively. Neither of the treatment groups were significantly different from the control (p=0.05). The NOEC for the adult population was therefore ≥20 L GF-1374/ha (equivalent to 59.3 mg product/kg dry soil).

In terms of reproduction endpoints, the numbers of juvenile worms per surviving adult in the treatment groups were not significantly different from the control group value. The numbers were 18.4, 21.5 and 19.9 juveniles/adult in the control, 4.0 L/ha and 20 L/ha treatment groups, respectively. Virtually the same mean number of cocoons per surviving adult was noted in the control and both treatments (0.3, 0.4 and 0.2 cocoons/adult, respectively).

From a reference study conducted previously on carbendazim, a significant effect on earthworm reproduction was detected at a treatment rate of 1.8 mg carbendazim/kg dry soil.

**Table 9.6.1. Summary of the effects of GF-1374 on *Eisenia fetida***

| Nominal concentration (L GF-1374/ha) | Adult worms | | Mean juveniles per surviving worm (Day 56) | Mean unhatched cocoons per surviving worm (Day 56) |
|---|---|---|---|---|
| | % Mortality (Day 28) | Mean % weight change (Day 0-28) | | |
| 0 (control) | 0 | +1.53 | 18.4 | 0.3 |
| 4.0 | 0 | -6.97 | 21.5 | 0.4 |
| 20 | 0 | -2.25 | 19.9 | 0.2 |

In summary, there was no observed mortality or sublethal effects seen on adult worms, at either 4.0 L/ha or at 20 L/ha, after 28 days of exposure. In addition, there were no observed adverse effects noted on reproduction endpoints (number of juveniles present per surviving adult worm or number of unhatched cocoons present per surviving adult worm) at either rate after 56 days of exposure. For all endpoints, the no-observed effect concentration (NOEC) was 20 L GF-1374/ha, equivalent to 59.3 mg GF-1374/kg dry soil.

The resulting endpoints are summarized in Table 9.6.2. below.

**Table 9.6.2. Results**

| **Endpoint** | **NOEC** |
|---|---|
| Adult mortality after 28 days | ≥20 L GF-1374/ha<br>(59.3 mg GF-1374/kg dry soil) |
| % Change in live weight of adult worms after 28 days | ≥20 L GF-1374/ha<br>(59.3 mg GF-1374/kg dry soil) |
| Number of juveniles per surviving adult worm after 56 days | ≥20 L GF-1374/ha<br>(59.3 mg GF-1374/kg dry soil) |
| Number of unhatched cocoons present per surviving adult worm after 56 days | ≥20 L GF-1374/ha<br>(59.3 mg GF-1374/kg dry soil) |

**Conclusions:** A 56-day earthworm reproduction study with *Eisenia fetida* was carried out using the formulation GF-1374. Two nominal treatment rates were employed: 4.0 L product/ha (11.9 mg product/kg dry soil) and 20 L product/ha (59.3 mg product/kg dry soil), plus an untreated control. There were no observed mortality or sublethal effects seen on adult worms, at either the 4.0 L/ha or 20 L/ha treatment rates, after 28 days of exposure. In addition, there were no observed adverse effects noted on reproduction endpoints (number of juveniles present per surviving adult worm or number of unhatched cocoons present per surviving adult worm) at either rate after 56 days of exposure. For all endpoints, the no-observed effect concentration (NOEC) was ≥20 L GF-1374/ha (equivalent to ≥59.3 mg GF-1374/kg dry soil).

Additional information on the test concentrations was calculated by the Notifier from the report of the above study, as presented in Table 9.6.3.

**Table 9.6.3. Information on test concentrations studied in the earthworm reproduction study.**

| g GF-1374 in 100 ml stock solution | g GF-1374/L | L GF-1374/ha Spray rate | g GF-1374/ha | L GF-1374/ha |
|---|---|---|---|---|
| 0.5919 | 5.92 | 704 | 4166 | 4 |
| 2.9599 | 29.60 | 704 | 20837 | 20 |

RMS comments and evaluation:

The study was well conducted according to test guideline and GLP, and clearly reported. The test procedure followed the guideline ISO 11268-2, preceding the OECD 222 test guideline which is stated in the EU data requirements (Commission Regulation 284/2013), and is therefore acceptable.

The test substance was spiked on the top of soil after the earthworms were introduced to test containers. The application rates tested corresponded 2.6X and 13X the recommended maximum use rate of the product GF-1374. Carbendazim as toxic reference was used in a separate study with identical procedure.

The validity criteria required by test guideline ISO 11268-2 (1998) similarly to OECD 222 were met for this study, as the mortality rate of adult worms was less than 10% among the controls (0% mortality was actually seen in this study), the coefficient of variance for juvenile numbers in the control replicates was less than 30% (the actual value in this study was 29.4%), and the numbers of juveniles present in the controls were greater than 30 per container (the actual numbers found in this study ranged from 126 to 247 per container).

The study is acceptable and valid for decision making on the product GF-1374. The NOEC value of ≥20 L GF-1374/ha equivalent to ≥59.3 mg GF-1374/kg dry soil can be used in the earthworm risk assessment. The data requirement is fulfilled and no further data are required.

### B.9.6.2. Earthworms – field studies

Since GF-1374 or clopyralid did not induce any chronic effects, field testing was deemed unnecessary.

RMS comments and evaluation:

The justification given by the Notifier is acceptable and it is agreed that field studies with the product GF-1374 on earthworms will not be required. Data requirement is fulfilled.

### B.9.6.3. Effects on non-target soil meso- and macrofauna (other than earthworms)

#### B.9.6.3.1. Laboratory studies with non-target soil meso- and macrofauna (other than earthworms)

The following soil macro-organism toxicity studies performed on GF-1374 are provided in support of the assessment and have not been previously evaluated in the context of renewal of approval of clopyralid in the EU.

**CP 10.4.2.1/2. GF-1374: Reproduction of the Collembola *Folsomia candida***

| | |
|---|---|
| Report: | KIIIA1 10.6.6/01, Ganßmann, M. (2012a) |
| Title: | Effects of GF-1374 on Reproduction of the Collembola *Folsomia candida* in Artificial Soil with 5% Peat |
| Document No: | Dow AgroSciences Study Number: 120256 |
| Guidelines: | OECD 232, ISO 11267 |
| GLP | Yes |

**Test material**

| | |
|---|---|
| Test item: | GF-1374 |
| Purity: | Clopyralid: 7.84 wt %, 81 g/L (analysed),<br>Florasulam: 0.23 wt %, 2.4 g/L (analysed),<br>Fluroxypyr-meptyl: 13.9 wt %, 145 g/L (analysed) |
| Description: | Brown liquid |
| Batch No./Lot No. : | TSN303060 / 1C03150102 |

**Test system**

| | |
|---|---|
| Organism (*Species*): | Collembola (*Folsomia candida)* |
| Study Type: | 28-day reproduction study |
| Guideline deviations reported by Study Director: | None |
| Study design: | 28-d exposure in treated artificial soil. Different concentrations of the test item were mixed |

| | |
|---|---|
| | homogeneously into the soil which was filled in glass vessels before the Collembola were introduced on top of the soil; 5 concentrations and one control; 4 replicates/concentration with 10 Collembola each (8 replicates for the control). Assessment of adult mortality, behavioural effects and reproduction were made after 28 d. |
| Test concentrations: | Control, 6.25, 12.5, 25.0, 50.0 and 100 mg GF-1374/kg soil dry weight |
| Soil parameters: | Artificial soil according to OECD 232; initial pH 5.5 to 5.6, pH at experimental end 5.5 to 5.7; water content of soil dry weight at test initiation 22.1% to 23.2% (54.0% to 56.5% of the maximum water holding capacity); at test termination 19.7% to 21.7% (48.1% to 53.0% of the maximum water holding capacity) |
| Environmental conditions: | Temperature: within the range of 18 – 22°C;<br>Light intensity: 16 hours of light per day, within the range of 400 – 800 lux<br>Feeding: With approximately 2 mg dry yeast for each test vessel at the beginning of the test and on day 14. |
| Reference item: | Boric acid (conducted as a separate study) |

**Methodology**

28-day test in treated artificial soil. Different concentrations of the test item were incorporated into the soil which was filled in glass vessels before the Collembola were introduced on top of the soil. Five test item concentrations and one control, four replicates for the test item treatments, eight replicates for the control with 10 Collembola in each were tested.

The Collembola were fed with approximately 2 mg dry yeast for each test vessel at the beginning of the test and on day 14. Adult mortality, behavioural effects and reproduction were assessed after 28 days.

**Results**

The results are summarised in Table 9.6.4. below.

**Table 9.6.4. Effects of GF-1374 on survival and reproduction of *Collembola***

| Nominal test concentrations (mg/kg dry soil) | 28-d % mortality | Juveniles after 28-d | |
|---|---|---|---|
| | | Mean No. | % change |
| Control | 15 | 317 | - |
| 6.25 | 10 | 359 | +13 |
| 12.5 | 18 | 354 | +12 |
| 25.0 | 18 | 356 | +12 |
| 50.0 | 23 | 332 | +5 |
| 100 | 45* | 213* | -33* |

* Significantly different from the control.

## Conclusions

GF-1374 caused no significant effects on mortality or reproduction of *Folsomia candida* up to and including the concentration of 50 mg GF-1374/kg soil dry weight. Therefore, the overall NOEC was determined to be 50 mg GF-1374/kg soil dry weight. The $EC_{50}$ values for mortality and reproduction were both considered to be >100 mg GF-1374/kg soil dry weight.

RMS comments and evaluation:

The study was well conducted according to test guideline and GLP, and clearly reported. The test procedure followed the OECD 232, ISO 11267 test guidelines and is acceptable.

The application rates tested corresponded to ca. 3 - 48 X the recommended maximum use rate of the product GF-1374. In a separate study (study code 61403016) the reference item Boric acid showed statistically significant effects on reproduction at concentrations of ≥ 53.7 mg/kg soil; the $EC_{50}$ for reproduction was calculated to be 59.9 mg/kg soil. Mortality was statistically significantly higher compared to the control at 53.7 mg/kg soil and above.

The validity criteria required by the test guideline were met for this study, as the mean adult mortality did not exceed 20% in controls (15 % mortality was actually seen in this study), the mean number of juveniles per vessel was at least 100 (186-373 actually found in this study) and the coefficient of variation calculated for the number of juveniles was less than 30% (the actual value in this study was 19.2 %) at the end of the definitive test.

The artificial soil used during the studies had a reduced organic matter content (5% peat), and therefore the endpoints need not to be adjusted by a factor of 0.5 in the risk assessment.

The study is acceptable and valid for decision making on the product GF-1374. The NOEC value of 50 mg GF-1374/kg dry soil can be used in the risk assessment for Collembola.

The data requirement is fulfilled and no further data are required.

**CP 10.4.2.1/3. GF-1374: Reproduction of the Predatory Mite *Hypoaspis aculeifer***

| | |
|---|---|
| Report: | KIIIA1 10.6.6/02, Ganßmann, M. (2012b) |
| Title: | Effects of GF-1374 on Reproduction of the Predatory Mite *Hypoaspis aculeifer* in Artificial Soil with 5% Peat. |
| Document No: | Dow AgroSciences Study Number: 120257 |
| Guidelines: | OECD 226 |
| GLP | Yes |

## Test material

| | |
|---|---|
| Test item: | GF-1374 |
| Purity: | Clopyralid: 7.84 wt %, 81 g/L (analysed), Florasulam: 0.23 wt %, 2.4 g/L (analysed), Fluroxypyr-meptyl: 13.9 wt %, 145 g/L (analysed) |
| Description: | Brown liquid |
| Batch No./Lot No.: | TSN303060 / 1C03150102 |

**Test system**

| | |
|---|---|
| Organism (*Species*): | Predatory mite (*Hypoaspis aculeifer*) |
| Study Type: | 14-day reproduction study |
| Guideline deviations reported by Study Director: | None |
| Study design: | Different concentrations of the test item were mixed homogeneously into the soil which was filled in glass vessels before the predatory mites were introduced on top of the soil; 5 concentrations and one control; 4 replicates/concentration and 8 replicates for the control, with 10 female predatory mites each. Feeding of the mites with cheese mite (*Tyrophagus putrescentiae*) at test start and on day 2, 5, 7, 9 and 12. Assessment of adult mortality and reproduction after 14 d. |
| Test concentrations: | Control, 6.25, 12.5, 25, 50 and 100 mg GF-1374/kg soil. |
| Soil parameters: | Artificial soil according to OECD 226:<br>pH at initiation: 5.5 to 5.6<br>pH at termination: 5.5<br>Moisture content at initiation: 22.1 – 23.2% (or 54.0 – 56.5% of MWHC)<br>Moisture content at termination: 21.2 – 22.2% (or 51.6 – 54.0% of MWHC) |
| Environmental conditions: | Temperature: within the range of 18 – 22°C;<br>Light intensity: 16 hours of light per day, within the range of 400 – 800 lux<br>Feeding of the mites with cheese mite (*Tyrophagus putrescentiae*) at test start and on day 2, 5, 7, 9 and 12. |
| Reference item: | BAS 152 11 I (a.i. dimethoate, 400 g/L, nominal). The effects of the reference item are investigated at least once a year in a separate study. |

**Methodology**

The mites were exposed in treated artificial soil for 14 days. Different concentrations of the test item were mixed homogeneously into the soil which was filled in glass vessels before the predatory mites were introduced on top of the soil; 5 concentrations and one control; 4 replicates/concentration and 8 replicates for the control, with 10 female predatory mites each. The mites were fed with cheese mite (*Tyrophagus putrescentiae*) at test start and on day 2, 5, 7, 9 and 12. Adult mortality and reproduction was assessed after 14 d.

**Results**

The results are summarised in Table 9.6.5. below.

**Table 9.6.5. Effects of GF-1374 on survival and reproduction of *Collembola***

| Nominal test concentrations (mg/kg dry soil) | 14-d % mortality | Juveniles after 14-d | |
|---|---|---|---|
| | | Mean No. | % change |
| Control | 4 | 150 | - |
| 6.25 | 5 | 152 | +1 |
| 12.5 | 13 | 164 | +9 |
| 25 | 8 | 162 | +8 |
| 50 | 10 | 157 | +4 |
| 100 | 15 | 146 | -3 |

**Conclusions**

A slight mortality of up to 15% was observed in the test item treated groups, which was not statistically significantly different compared to the control, where 4% of the adult mites died (Fisher's Exact Test, $\alpha = 0.05$, one-sided greater).

The reproduction of the predatory mites exposed to GF-1374 was not statistically significantly different compared to the control up to and including the highest test the highest test concentration of 100 mg/kg soil (Williams t-test, $\alpha = 0.05$, one-sided smaller).

GF-1374 caused no significant effects on mortality or reproduction of *Hypoaspis aculeifer* up to and including the concentration of 100 mg test item/kg soil.

Therefore, the overall No Observed Effect Concentration (NOEC) was determined to be 100 mg test item/kg soil. The overall Lowest Observed Effect

RMS comments and evaluation:

The study was well conducted according to test guideline and GLP, and clearly reported. The test procedure followed the OECD 226 test guideline and is acceptable.

The application rates tested corresponded to ca. 3-48 x the recommended maximum use rate of the product GF-1374. In a separate study the reference item dimethoate showed statistically significant effects on reproduction at a concentration of 1.7 mg dimethoate/kg soil and above. The $EC_{50}$ for reproduction was 4.0 mg dimethoate/kg soil.

The validity criteria required by the test guideline were met for this study, as the mean adult mortality did not exceed 20% in controls (4 % mortality was actually seen in this study), the mean number of juveniles per replicate with 10 adult females introduced was at least 50 (137-181 actually found in this study) and the coefficient of variation calculated for the number of juvenile mites was less than 30% (the actual value in this study was 10.0 %) at the end of the definitive test.

The artificial soil used during the studies had a reduced organic matter content (5% peat), and therefore the endpoints need not to be adjusted by a factor of 0.5 in the risk assessment.

The study is acceptable and valid for decision making on the product GF-1374. The NOEC value of 50 mg GF-1374/kg dry soil can be used in the risk assessment for *Hypoaspis* predatory mites.

The data requirement is fulfilled and no further data are required.

**B.9.6.3.2. Higher tier testing**

Higher tier testing was not deemed necessary due to the lack of long-term effects.

RMS comments and evaluation:

The justification given by the Notifier is acceptable and it is agreed that higher tier studies with the product GF-1374 on soil mesofauna will not be required. Data requirement is fulfilled.

## B.9.6.4. Risk Assessment For Non-Target Soil Meso- And Macrofauna

### B.9.6.4.1. Endpoints used in the risk assessment

The critical endpoints of GF-1374, its active substances clopyralid, fluroxypyr and florasulam with their metabolites, employed in the risk assessment for earthworms and other soil macro-organisms are indicated in Tables 9.6.6 - 9.6.8. below.

**Table 9.6.6. Long-term toxicity of clopyralid and GF-1374 to earthworms and other soil macro-organisms**

| Species | Test substance | Endpoint | Value (mg/kg soil) | Reference |
|---|---|---|---|---|
| *Eisenia fetida* | Clopyralid | 56-d NOEC | ≥2.0 | EFSA (2005)* |
| *Eisenia fetida* | GF-1374 | 56-d NOEC | ≥59.3 | Davies (2005) DAS Study ID 040258 |
| *F. candida* | GF-1374 | 28 d NOEC | 50 | Ganßmann (2012a) DAS Study ID 120256 |
| *H. aculeifer* | GF-1374 | 14 d NOEC | 100 | Ganßmann (2012b) DAS Study ID 120257 |

* EFSA Scientific Report (2005) 50, 1-65

**Table 9.6.7. Long-term toxicity of fluroxypyr to earthworms and other soil macro-organisms**

| Compound | Test species | Endpoint | EU agreed endpoints* | Endpoint used in risk assessment** | |
|---|---|---|---|---|---|
| | | | Value (mg/kg soil) | Value (mg/kg soil) | Ref |
| Fluroxypyr-meptyl | *Eisenia foetida* | Sub-acute NOEC | 3.92 | Same as EU | N/A |
| Fluroxypyr | *Eisenia foetida* | Sub-acute NOEC | 3.05 | Same as EU | N/A |
| Fluroxypyr pyridinol | *Eisenia foetida* | Sub-acute NOEC | N/A | 0.720 | Witte, 2009 Study no. 090429 |
| Fluroxypyr methoxypyridine | *Eisenia foetida* | Sub-acute NOEC | N/A | 1.17[a] | Witte, 2009 Study no. 090431 |

* EFSA Journal 2011;9(3):2091

** Since Annex I inclusion new studies have been performed providing new end-points which are used in the risk assessment.

[a]: Study conducted using artificial soil with a reduced organic matter content (5% peat).

[b]:Study conducted using natural soil (LUFA 2.4) containing 2.42% organic carbon.

N/A: not applicable

**Table 9.6.8. Long-term toxicity of florasulam to earthworms and other soil macro-organisms**

| Compound | Test species | Endpoint | EU agreed endpoints* | Endpoint used in risk assessment | |
|---|---|---|---|---|---|
| | | | Value (mg/kg soil) | Value (mg/kg soil) | Ref |
| Florasulam | *Eisenia fetida* | Acute $LC_{50}$ | >1320 | Same as EU | EFSA 2015 |
| | *Eisenia fetida* | NOEC | 0.203 | Same as EU | EFSA 2015 |
| 5-OH-florasulam | *Eisenia fetida* | Acute $LC_{50}$ | >1120 | Same as EU | EFSA 2015 |
| | *Eisenia fetida* | NOEC | 0.14 | Same as EU | EFSA 2015 |
| DFP-ASTCA | *Eisenia fetida* | Acute $LC_{50}$ | >0.1 | Same as EU | EFSA 2015 |
| | *Eisenia fetida* | NOEC | 0.0304 | Same as EU | EFSA 2015 |
| ASTCA | *Eisenia fetida* | Acute $LC_{50}$ | >100 | Same as EU | EFSA 2015 |
| | *Eisenia fetida* | NOEC | 1.0 | Same as EU | EFSA 2015 |
| TSA | *Eisenia fetida* | Acute $LC_{50}$ | >0.1 | Same as EU | EFSA 2015 |
| | *Eisenia fetida* | NOEC | 10.0 | Same as EU | EFSA 2015 |

* EFSA Journal 2015; 13 (1):3984
[a]: Study summarised in the DAR for florasulam (November 1999). Dow AgroSciences Accession Number: 63370.
[b]: Study conducted in artificial soil containing 5% peat.
[c]: Dow AgroSciences Study Number: 980271.
N/A: not applicable

**B.9.6.4.2. Exposure**

The following risk assessment has been based on the maximum $PEC_{soil}$ values resulting from the realistic worst-case scenarios for the application for GF-1374 applied to pasture and cereal as summarised in the following Tables 9.6.9 and 9.6.10.

**Table 9.6.9. Critical GAP to assess the risk of GF-1374 applications to grassland and cereals**

| Crop | Time of application | No. of applications per year | Maximum rate per application |
|---|---|---|---|
| Cereals | BBCH 13 – 39<br>February to June 30 | 1 | 1.0 L GF-1374/ha (or 1040[a] g/ha)<br>equivalent to:<br>fluroxypyr-meptyl 144 g/ha (or 100 g a.e./ha);<br>florasulam 2.5 g/ha;<br>clopyralid 80 g/ha |
| Pasture | Feb 01 to August 31 | | 1.5 L GF-1374/ha (or 1560[a] g/ha)<br>equivalent to:<br>fluroxypyr-meptyl 216 g/ha (or 150 g a.e./ha);<br>florasulam 3.75 g/ha;<br>clopyralid 120 g/ha |

[a]: based on a nominal formulation density of 1.04 g/mL

Maximum environmental concentrations in soil have been predicted for clopyralid and GF-1374, as calculated in the environmental fate section of the dRAR. Calculations considered the maximum application rate of GF-1374 on pasture and cereals, as presented above. The maximum PECsoil values for clopyralid and GF-1374 are summarised in the table below.

**Table 9.6.10. Maximum $PEC_{soil}$ values for clopyralid and the formulation GF-1374.**

| Substance (crop) | $PEC_{soil}$ (mg/kg) |
|---|---|
| Clopyralid (grassland) | 0.016 |
| Clopyralid (cereals) | 0.107 |
| GF-1374 | 2.08 |

**B.9.6.4.3. Risk assessment to earthworms and soil macrofauna**

The potential long-term risk of GF-1374 and clopyralid to earthworms and soil macrofauna was assessed by calculating long-term TER ($TER_{LT}$) values by comparing the chronic NOEC value and the maximum instantaneous $PEC_{SOIL}$ using the following equation:

$$TER_{LT} = \frac{NOEC}{PEC_{SOIL}}$$

The long term risk to earthworms and soil macro organisms were assessed for fluroxypyr (EFSA 2011) and florasulam (EFSA 2015) and both were considered low. Due to the proposed GAP being within the uses evaluated during fluroxypyr and florasulam Annex I renewal processes, please refer to their EFSA conclusion for specific details such as endpoints and estimated PEC values and associated toxicity exposure ratios.

The long term risk assessment for earthworms are summarised in the following Table 9.6.11 considering the highest initial PEC value in soil resulting from the use of the product GF-1374 on pasture and cereals.

**Table 9.6.11. Long term risk assessment of clopyralid and GF-1374 to earthworms following applications to pasture and cereals**

| Test substance | Test species | Maximum initial PEC (mg/kg) | NOEC (mg/kg) | $TER_{LT}$ |
|---|---|---|---|---|
| Clopyralid (grassland) | *E. fetida* | 0.016 | 2.0 | 125 |
| Clopyralid (cereal) | | 0.107 | | 18.7 |
| GF-1374 | *E. fetida* | 2.08 | 29.7[a] | 14.3 |

[a]: Endpoint adjusted by a factor of 0.5 to allow for the high organic carbon content of OECD soil.

All $TER_{LT}$ values exceeded the Annex VI trigger of 5. Consequently, the long term risk to earthworms posed by GF-1374 and clopyralid are low following applications to pasture and cereals at rates of 1.5 L GF-1374/ha and 1.0 GF-1374/ha, respectively.

The long term risk assessment for non-target soil meso and macrofauna other than earthworms are summarised in the following table considering the highest initial PEC value in soil. However, assessments were not carried out with clopyralid since $DT_{90}$ < 365 days and no adverse effects were observed in tests with earthworms, ground or soil dwelling arthropods, or soil microorganisms. Furthermore, the risk to soil micro and macro organisms was assessed to be low for florasulam and fluroxypyr (EFSA 2015 and EFSA 2011, respectively).

**Table 9.6.12. Risk assessment of GF-1374 and clopyralid for soil meso- and macrofauna following applications to pasture and cereals.**

| Test substance | Test species | Maximum initial PEC (mg/kg) | NOEC[a] (mg/kg) | $TER_{LT}$ |
|---|---|---|---|---|
| GF-1374 | *F. candida* | 2.08 | 25 | 12.0 |
| | *H. aculeifer* | | 50 | 24.0 |

[a]Endpoints were not adjusted by a factor of 0.5 because the artificial soil used during the studies had a reduced organic matter content (5% peat).

All $TER_{LT}$ values of soil meso- and macforauna exceed the Annex VI trigger of 5. Overall, the long term risk posed by applications of GF-1374 to pasture and cereals are also low to soil macro-organisms other than earthworms.

RMS comments and evaluation:

The risk assessment on earthworms and other soil non-target macrofauna presented by the Notifier is acceptable. The maximum $PEC_{soil}$ values used in the risk assessment correspond to the calculations presented in the environmental fate section (Vol. 3 B.8. Chapter B.8.3) and agreed.

It is agreed that the long term risk assessment for sole clopyralid is considered unnecessary, since no adverse effects were seen in any taxonomic groups of test organisms with exaggerated application rates. The risk assessments of fluroxypyr and florasulam have been considered as acceptable within the EU evaluations of those active substances, and considered unnecessary to repeat in the context of AIR3 evaluation of clopyralid in detail. The application rates in those evaluations cover the risk envelope of the product GF-1374.

The risk assessment presented above indicates that the uses of the formulation GF-1374 on cereals and pasture do not pose an unacceptable risk to earthworms or other soil meso and macrofauna, if the product is used according to GAP.

Data requirement is fulfilled and no further data are required.

## B.9.7. EFFECTS ON SOIL NITROGEN TRANSFORMATION

### B.9.7.1. Laboratory studies on the effects of GF-1374 to soil micro-organisms (soil nitrogen transformation)

The following soil micro-organism toxicity study performed on GF-1374 is provided in support of the assessment and has not been previously evaluated.

**CP 10.5.1/1. Effects of GF-1374 on soil microbial activity**

| | |
|---|---|
| Report: | Rix, S. (2005). |
| Title: | GF-1374: determination of effects on soil microflora activity. |
| Document No: | Dow AgroSciences Study Number: 040256<br>CEM Analytical Services Limited (CEMAS), Study Number CEMS-2422 |
| Guidelines: | OECD Test Guideline Nos. 216 and 217. |
| GLP | Yes |

**Methodology:** The aim of this study was to determine the effects of GF-1374 on microbial activity in a sandy soil by the measurement of soil respiration (carbon mineralisation) and nitrogen transformations according to OECD Test Guidelines 216 (2000) and 217 (2000). GF-1374 (Lot No. 187/76-A) is an emulsion concentrate (EC) nominally containing fluroxypyr-meptyl at 144 g/L (142 g/L, actual), clopyralid at 80 g/L (86 g/L, actual) and florasulam at 2.5 g/L (2.5 g/L, actual).

Microbial activity was assessed using a single sandy loam soil (52.8-65.4% w/w sand content, 1.02% organic carbon and a microbial biomass of 1.14-1.57% of the total organic carbon). This soil represents a 'worst-case scenario', so that tests of effects in other soils are considered unnecessary. Soils were acclimated to the test conditions (darkness at 20±2°C) for seven days. At the start of the study, the soil samples for nitrogen analysis were amended with 0.5% w/w ground lucerne. Replicate samples were then treated with GF-1374 to achieve concentrations equivalent to 1× single application rate (2 L GF-1374/ha, 2.77 mg GF-1374/kg) and 5× single application rate (10 L GF-1374/ha, 13.9 mg GF-1374/kg). One set of samples was left untreated to serve as controls. During the study, pH ranged from 5.82 to 6.34 (5.82 to 5.83 on initiation), moisture content from 40.2 to 41.5% maximum water holding capacity and air temperature was kept at 20±2°C.

The soil samples were incubated for a period of 28 days. Soil respiration measurements and samples extracted for measurements of nitrogen in the form of ammonium-N, nitrite-N and nitrate-N were made within six hours of dosing the soil with GF-1374. Further measurements of soil respiration and extractions for nitrogen determinations were carried out 7, 14 and 28 days after dosing the soil.

On each occasion, aliquots of soil were amended with a previously determined optimum quantity of glucose and total carbon dioxide evolution was measured over a 12-hour period using an infra-red gas analyser. The effects on nitrogen transformation (ammonification and nitrification), were investigated by determining ammonium-N, nitrate-N and nitrite-N concentrations in soil amended with ground lucerne grass. Aliquots of soil were extracted with 2M KCl and the concentrations of inorganic nitrogen species determined colourimetrically.

A study with the reference compound, dinoseb, was carried out at 1 and 10 mg a.s./kg dry soil. The results confirmed the sensitivity of the soil micro-organisms under test conditions (adverse effects, >25%, on respiration and nitrogen turnover occurred during the 28-day study).

**Findings:** Microbial respiration of GF-1374-treated soil at applications of 2 L/ha and 10 L/ha (i.e. 1× and 5× maximum application rate) showed no deviation greater than 25% compared to control values at Day 28 (see table below). The variation between control sample respiration rates during the study ranged from –3.3% to +6.2% (i.e. within the validation limit of ±15%).

**Table 9.7.1. Effect of GF-1374 on $CO_2$ production**

| Treatment (L GF-1374/ha) | Mean microbial respiration rate (mg C as $CO_2$/kg/hour) [% deviation] | | | |
|---|---|---|---|---|
| | Day 0 | Day 7 | Day 14 | Day 28 |
| 0 (Control) | 6.20 | 6.78 | 6.50 | 5.44 |
| 2 | 5.34* [-14%] | 7.07 [+4.3%] | 6.62 [+2.0%] | 5.30 [-2.6%] |
| 10 | 4.83* [-22%] | 6.45 [-4.8%] | 6.18 [-4.9%] | 5.31 [-2.4%] |

* statistically significant compared to control (Dunnett's two-tailed test, p=0.05)

Nitrogen turnover in GF-1374-treated soil at applications of 2 L/ha and 10 L/ha (i.e. 1× and 5× maximum application rate) also showed no deviation greater than 25% compared to control values by Day 28 (see table below). The variation between control sample nitrogen turnover rates during the study ranged from -7.2% to +11% (i.e. within the validation limit of ±15%).

**Table 9.7.2. Effect of GF-1374 on nitrogen transformation**

| Treatment (L GF-1374/ha) | Mean nitrate-N transformation rate (mg N/kg/d) [% deviation] | | |
|---|---|---|---|
| | Days 0 to 7 | Days 7 to 14 | Days 14 to 28 |
| 0 (Control) | 0.606 | 2.05 | 1.06 |
| 2 | 0.689 [+14%] | 1.88 [-8.1%] | 1.02 [-3.1%] |
| 10 | 0.920* [+52%] | 1.80 [-12%] | 1.18 [+12%] |

* statistically significant compared to control (Dunnett's two-tailed test, p=0.05)

Concentrations of ammonium-N and nitrite-N were measured during the study, but are not summarised here. Indeed ammonium-N and nitrite-N levels were very low compared to nitrate-N. Additionally nitrate-N is the only determinand of interest for nitrogen transformation in soil as required by the OECD test guideline no. 216.

**Conclusions:** GF-1374, when applied at the maximum total annual rate of 2.0 L product/ha (2.77 mg product/kg dry soil) or at 5 times that rate (10 L product/ha, 13.9 mg product/kg dry soil), had no long-term adverse effect on microbial respiration or nitrogen transformations in a vulnerable sandy loam soil. Consequently, GF-1374 would not be expected to cause any significant long-term effect on these processes in soils.

A summary is presented in Table 9.7.3.

**Table 9.7.3. Clopyralid and GF-1374– Summary of effects on carbon and nitrogen transformation**

| Data point | Test substance | Test | Endpoint | Reference |
|---|---|---|---|---|
| CA 8.5 | Clopyralid | C and N transformation | 2.0 mg/kg sdw is treatment causing <25% deviation from control within 100 days | EFSA (2005) |
| CP 10.5 | GF-1374 | C and N transformation | 13.9 mg/kg sdw treatment causing <25% deviation from control within 100 days | Rix (2005) DAS Study ID. 040256 |

RMS comments and evaluation:

The study was well conducted according to appropriate OECD test guidelines and GLP, is clearly reported and acceptable.

The application rates tested corresponded to ca. 1 X and 5 X the recommended maximum use rate of the product GF-1374. In a separate study the reference item dinoseb showed statistically significant effects both on soil respiration and nitrogen transformation at concentrations of 1 and 10 mg dinoseb/kg soil over the study period of 28 days.

The study satisfied the validity criterion of the OECD 216 and 217 test guidelines that the variation between replicate control samples should not be greater than ± 15%. The range of percentage variation within the control samples for nitrate-nitrogen concentrations was from -7.2% to +11%. The range of percentage variation within the control samples for $CO_2$ concentrations was from -3.3% to +6.2%.

A few deviations from the Study Plan were reported. First, the soil used in this study was obtained from LUFASpeyer, Germany, because the soil samples taken from Waltham Place Farm failed to meet the OECD 216 and 217 (2000) criterion for sand content. Instead, the soil LUFA 2.3 satisfied the recommendations for physical/chemical characteristics given in OECD guidelines 216 and 217 (2000). This change caused a delay to start of study and issue of final report, but was evaluated as not having an impact on study results.

Second, characterisation of the LUFA 2.3 soil (batch F235004) used in this study was performed as a separate study (CEMAS study number CEMS-2565), and reported in a separate report (CEMR-2565). The results of the soil characterisation, as well as details concerning its source and preparation, were also described in this report. Reporting of soil characterisation was omitted from Study Plan Deviation number 1 in error. This deviation was evaluated as not having an impact on study results.
Third, an error in determination of dry weight of lucerne occurred. Reason: Error in formula used to calculate dry weight of lucerne; the vessel weight was included in the sample weight for calculation. Impact: The difference in weights of lucerne actually used and that required was very small and was unlikely to have had an impact on the study. The controls and treated groups all received the same amount of lucerne at the start.

The study is acceptable and valid for decision making on the product GF-1374. The endpoints can be used in the risk assessment for soil microbial activity. The data requirement is fulfilled and no further data are required.

## B.9.7.2. Risk assessment for Soil Nitrogen Transformation

#### B.9.7.2.1. Appropriate endpoints to be used in the risk assessment

Effects on soil micro-organisms for GF-1374 were not evaluated as part of the EU review of fluroxypyr, clopyralid and florasulam. Data on GF-1374 is evaluated here, and risk assessments for GF-1374 with the proposed use pattern are provided here and are considered adequate.

The endpoints employed in the risk assessment for effects on soil microbial activity are indicated in Tables 9.7.4 below.

**Table 9.7.4. Effects of clopyralid on soil microbial activity**

| Compound | Test type | Endpoint | EU agreed endpoints* | Endpoint used in risk assessment** | |
|---|---|---|---|---|---|
| | | | Value (mg/kg soil) | Value (mg/kg soil) | Ref |
| Clopyralid | N transformation | Treatment causing <25% deviation from control within 100 days | 2.0[a] | 209 | Schöbinger, U; 2013 DAS study 130283 |
| | C transformation | | 2.0[a] | 209 | Schöbinger, U; 2013 130283 |

* EFSA Scientific Report (2005) 50, 1-65
** Since Annex I inclusion new studies have been performed providing new end-points which are used in the risk assessment.
[a]: Converted to mg/kg soil from the endpoint of 1500 g/ha listed in the EFSA Scientific Report (2005) 50, 1-65 assuming a soil bulk density of 1.5 g/mL and distribution in 5 cm of soil.
N/A: not applicable

**Table 9.7.5. Effects of fluroxypyr on soil microbial activity**

| Compound | Test type | Endpoint | EU agreed endpoints* | Endpoint used in risk assessment** | |
|---|---|---|---|---|---|
| | | | Value (mg/kg soil) | Value (mg/kg soil) | Ref |
| Fluroxypyr pyridinol | C transformation | Treatment causing <25% deviation from control within 100 days | 0.441 | Same as EU | EFSA 2011 |
| | N transformation | Treatment causing <25% deviation from control within 100 days | N/A | 0.240 | Feil 2009 Study no. 090430 |
| | C transformation | | N/A | 0.240 | |
| Fluroxypyr methoxypyridine | N transformation | Treatment causing <25% deviation from control within 100 days | 0.66 | Same as EU | EFSA 2011 |
| | C transformation | | 0.66 | Same as EU | EFSA 2011 |

* EFSA Journal 2011;9(3):2091
** Since Annex I inclusion new studies have been performed providing new end-points which are used in the risk assessment.
N/A: not applicable

**Table 9.7.6. Effects of florasulam on soil microbial activity**

| Compound | Test type | Endpoint | EU agreed* endpoints | Endpoint used in risk assessment | |
|---|---|---|---|---|---|
| | | | Value (mg/kg soil) | Value (mg/kg soil) | Ref |
| Florasulam | N transformation | Treatment causing <25% deviation from control within 100 days | 0.05 | Same as EU | EFSA 2015 |
| | C transformation | | 0.05 | Same as EU | |
| 5-OH-florasulam | N transformation | | 0.036 | Same as EU | EFSA 2015 |
| | C transformation | | 0.036 | Same as EU | |
| DFP-ASTCA | N transformation | | 0.00760 | Same as EU | EFSA 2015 |
| | C transformation | | 0.00760 | Same as EU | |
| ASTCA | N transformation | | 1.0 | Same as EU | EFSA 2015 |
| | C transformation | | 1.0 | Same as EU | |
| TSA | N transformation | | 0.05 | Same as EU | EFSA 2015 |
| | C transformation | | 0.05 | Same as EU | |

N/A: not applicable
* EFSA Journal 2015; 13 (1):3984

**Table 9.7.7. Effects of GF-1374 on soil microbial activity**

| Compound | Test type | Endpoint | EU agreed endpoints* | Endpoint used in risk assessment** | |
|---|---|---|---|---|---|
| | | | Value (mg/kg soil) | Value (mg kg soil) | Ref |
| GF-1374 | N transformation | Treatment causing <25% deviation from control within 28 days | N/A | 13.9 | Rix (2005) DAS Study ID 040256 |
| | C transformation | | N/A | 13.9 | |

* GF-1374 is a formulation which was not evaluated at EU level.
** Studies for GF-1374 have been performed providing end-points which are used in the risk assessment.
N/A: not applicable

Clopyralid forms no major metabolites in soil which should be considered in the risk assessment.

#### B.9.7.2.2. Exposure resulting from the use of GF-1374

The critical GAP of the product GF-1374 is summarised in Table 9.7.8. The approach taken in this dossier was to consider the GAP for clopyralid and GF-1374 to select the maximum application rate of 1.5 L/ha as a worst case scenario. The following risk assessment has been based on realistic worst-case scenarios for the application summarised in the following table.

**Table 9.7.8. Critical GAP to assess the risk of GF-1374 applications to grassland and cereals**

| Crop | Time of application | No. of applications per year | Maximum rate per application |
|---|---|---|---|
| Cereals | BBCH 13 – 39<br>February to June 30 | 1 | 1.0 L GF-1374/ha (or 1040[a] g/ha)<br>equivalent to:<br>fluroxypyr-meptyl 144 g/ha (or 100 g a.e./ha);<br>florasulam 2.5 g/ha;<br>clopyralid 80 g/ha |
| Pasture | Feb 01 to August 31 | | 1.5 L GF-1374/ha (or 1560[a] g/ha)<br>equivalent to:<br>fluroxypyr-meptyl 216 g/ha (or 150 g a.e./ha);<br>florasulam 3.75 g/ha;<br>clopyralid 120 g/ha |

[a]: based on a nominal formulation density of 1.04 g/mL.

Maximum environmental concentrations in soil ($PEC_{soil}$) have been predicted for GF-1374 and clopyralid in CP 9.1.3. Calculations considered the maximum application rate of the formulation (i.e. a single broadcast application at 1.5 L formulated product/ha). Calculations assumed an even distribution of the substances in the top 5 cm horizon with a soil bulk density of 1.5 g/mL. Results are summarised in Table 9.7.9. below.

**Table 9.7.9. Maximum $PEC_{soil}$ values for GF-1374 and clopyralid after applications to pasture and amenity grassland**

| Substance (crop) | $PEC_{soil}$ (mg/kg) |
|---|---|
| Clopyralid | 0.107 |
| GF-1374 | 2.08 |

### B.9.7.2.3. Risk assessment for soil microbial activity

The risk assessment for soil micro-organisms is summarised in the following Table 9.7.10, considering the highest initial PEC in soil.

The long term risk to soil microorganisms was assessed for both fluroxypyr (EFSA 2011) and florasulam (EFSA 2015) and was low. Due to the proposed GAP being within the uses evaluated during fluroxypyr and florasulam Annex I renewal processes, please refer to their EFSA conclusion for specific details such as endpoints and estimated PEC values and associated toxicity exposure ratios (EFSA 2011 and EFSA 2015, respectively).

**Table 9.7.10. Risk assessment for soil microbial activity after applications of GF-1374 to pasture and cereals**

| Substance | Test type | Maximum initial PEC (mg/kg) | Effects <25% (mg/kg) | TER |
|---|---|---|---|---|
| Clopyralid | N transformation and C transformation | 0.107 | 209 | 1953 |
| GF-1374 | N transformation and C transformation | 2.08 | 13.9 | 6.68 |

As a conclusion, GF-1374 and clopyralid have no adverse effects on microbial respiration or nitrogen transformation at ≥ 6.68-times their maximum exposure rates following the proposed uses on pasture and cereals. Therefore, the risk to soil micro-organisms from GF-1374 is considered to be low.

RMS comments and evaluation:

The risk assessment to soil microbial activity presented by the Notifier is acceptable and indicates that the use of GF-1374, the representative formulation of clopyralid, is safe if the product is used according to the GAP. No long-term effects on soil micro-organisms (N and C mineralisation) were observed at treatment levels equivalent to > 6.68 times the maximum application rate of GF-1374 to cereals and pastures. Overall, an acceptable risk to soil micro-organisms is therefore expected following the use of clopyralid and GF-1374. Based on these results, no risk to soil micro-organisms is expected for applications up to 1.5 L/ha to pasture and 1.0 L/ha to cereals.

The data presented by the Notifier is agreed and acceptable, and adequate for the risk assessment. No further data on this issue are required for the renewal of approval of clopyralid within the EU.

## B.9.8. EFFECTS ON TERRESTRIAL NON-TARGET HIGHER PLANTS

Table 9.8.1. provides a justification for the use of new studies on terrestrial non-target higher plants to address in the risk assessment, compared to what was originally evaluated for the Active Approval in the DAR in 2003.

**Table 9.8.1. Justification for the use of new studies on terrestrial non-target higher plants.**

| Data point/Study | Rationale |
|---|---|
| CP 10.6.2/1 Eley R. 2005 DAS Accession number 2000894 | Study is submitted for review since it is a new representative formulation that was not evaluated during the previous Annex I review |
| CP 10.6.2/2 Eley R, 2005 Accession no. 2000890 | Study is submitted for review since it is a new representative formulation that was not evaluated during the previous Annex I review |

### B.9.8.1. Summary of screening data

Summary of screening data were submitted for the first Annex I Approval and new data has not been generated since the last evalution. Due to this fact, new data has not been provided.

RMS comments and evaluation:

The justification presented by the Notifier is agreed and no screening data on this issue is required, as there is adequate number of more relevant experimental data available to conduct the risk assessment to non-target plants from the use of the product GF-1374.

### B.9.8.2. Testing on non-target plants

Two studies with the representative formulation of clopyralid, GF-1374 on non-target terrestrial plants were submitted to support the AIR3 evaluation of clopyralid. These studies have not been evaluated before, and therefore the details are presented below.

#### B.9.8.2.1. Vegetative vigour test

The following vegetative vigour study performed on GF-1374 is provided in support of the assessment and has not been previously evaluated.

**CP 10.6.2/1 Evaluation of Vegetative Vigour**

| | |
|---|---|
| Report: | Eley R. (2005a). |
| Title: | Evaluation of the Phytotoxicity of GF-1374 (Fluroxypr-1-MHE +Clopyralid+Florasulam, EC) GLP Vegetative Vigour Test - Terrestrial Non Target Plants (Based on OECD Guideline 208 B) North Europe 2005. |
| Document No: | DAS Study No. EA05B2A033<br>AgroChemex Study No: ACE-05-1 52 |
| Guidelines: | OECD Guideline 208 B, according to the proposed revision (revision July 2000) for Vegetative Vigour Test Terrestrial Non Target Plants |
| GLP | Yes |

**Methodology:** The study was carried out by Agro Chemex, UK, to assess the post emergence effects of GF-1374 on oat (*Avena sativa*), ryegrass (*Lolium perenne*), wheat (*Triticum aestivum*), onion (*Allium cepa*), oilseed rape (*Brassicanapus*), soybean (*Glycine max*), carrot (*Daucus carota*), cucumber (*Cucumis sativus*), sugar beet (*Beta vulgaris*) and lettuce (*Lactuca sativa*). In total 4 monocotyledon and 6 dicotyledonous species from eight different plant families were tested. The species evaluated represented members of the Gramineae, Liliaceae, Brassicaceae, Leguminoseae, Cucurbitaceae, Umbelliferae, Chenopodiaceae and Compositae.

GF-1374 was formulated as an EC containing 144 g a.s./L fluroxypyr-1-MHE, 80 g a.s./L clopyralid and 2.5 g a.s./L florasulam. The Batch Number of the sample of GF-1374 used in the study was 187/76A (TSN104753). GF-1374 was applied at 0.0058, 0.011, 0.023, 0.047, 0.094, 0.188, 0.375, 0.75, and 1.5 L/ha in a total volume of 206.88 to 219.12 L/ha. The concentration of

the highest rate spray solution was analytically verified using external standard high performance liquid chromatography technique.

The soil medium used was a sandy loam with an organic carbon content of < 1.5%. Six replicate pots were used per treatment. Five oats, ryegrass, wheat, onion, oilseed rape, soybean carrot, cucumber, sugar beet, and lettuce were transplanted per replicate pot, for a given species. Watering pre- and post-application was via sub pot irrigation. Plants were raised and the study conducted under glasshouse conditions maintained within acceptable limits for growth of the test species. Temperature range 15.8°C to 29.8°C, lighting was ambient. Plants were visually assessed for damage 7, 14 and 21 days after application. At the end of the study shoot fresh weight was measured and the number of dead plants recorded.

**Findings:** GF-1374 applied at rates ranging from 0.0058 to 1.5 L/ha did not reduce the foliar fresh weight of *Avena sativa, Lolium perenne, Triticum aestivum* and *Cucumis sativus* by 50%. The foliar fresh weights of *Allium cepa, Brassica napus, Glycine max, Daucus carota, Beta vulgaris* and *Lactuca sativa* were reduced by 50% following applications of GF-1374. *Lactuca sativa* was the most sensitive species evaluated with an $ER_{50}$ of 0.021 L GF-1374/ha. *Avena sativa, Lolium perenne* and *Triticum aestivum* were the most tolerant species tested.

The yields of shoot fresh weight in ten plant species after the test period of 21 days are summarized in Tables 9.8.2. and 9.8.3. below.

**Table 9.8.2. Shoot fresh weight (g) 21 days after application, in ten crop species treated post-emergence with GF-1374**

| Treatment (L GF-1374/ha) | Oat | Rye grass | Wheat | Onion | Oilseed rape | Soy-bean | Car-rot | Cucum ber | Sugar beet | Lettuce |
|---|---|---|---|---|---|---|---|---|---|---|
| Control | 11.56 | 3.74 | 11.3 | 12.99 | 26.32 | 22.81 | 12.36 | 96.49 | 27.03 | 45.37 |
| 0.0058 | 13.11 | 3.86 | 11.29 | 12.01 | 28.02 | 20.38 | 11.88 | 96.9 | 25.62 | 39.48 |
| 0.011 | 11.84 | 4.11 | 11.51 | 12.01 | 23.64 | 19.73 | 12.22 | 83.25 | 24.14 | 34.95 |
| 0.023 | 12.14 | 4.28 | 10.81 | 10.33 | 25.15 | 17.44 | 10.69 | 92.12 | 22.63 | 22.16 |
| 0.047 | 11.75 | 4.53 | 11.9 | 9.81 | 16.31 | 14.48 | 7.74 | 81.16 | 21.71 | 5.97 |
| 0.094 | 12.83 | 3.93 | 11.71 | 6.98 | 14.8 | 10.47 | 4.34 | 84.89 | 19.64 | 1.58 |
| 0.188 | 11.34 | 3.83 | 11.06 | 7.43 | 10.42 | 5.99 | 3.53 | 80.91 | 16.22 | 0.8 |
| 0.375 | 10.59 | 4.1 | 11.15 | 5.59 | 7.13 | 1.99 | 3.22 | 56.66 | 13.8 | 0.63 |
| 0.75 | 12.09 | 3.59 | 10.81 | 2.57 | 7.53 | 1.62 | 1.6 | 53.77 | 11.56 | 0.78 |
| 1.5 | 11.1 | 3.39 | 10.31 | 3.27 | 4.12 | 1.57 | 1.58 | 51.03 | 10.51 | 0.59 |

**Table 9.8.3. Shoot fresh weight reduction (%) compared to controls 21 days after application, in ten crop species treated post-emergence with GF-1374**

| Treatment (L GF-1374/ha) | Oat | Rye grass | Wheat | Onion | Oilseed rape | Soy-bean | Car-rot | Cucum ber | Sugar beet | Lettuce |
|---|---|---|---|---|---|---|---|---|---|---|
| 0.0058 | 0 | 0 | 0.01 | 7.6 | 0 | 10.7 | 3.9 | 0 | 5.2 | 13.0 |
| 0.011 | 0 | 0 | 0 | 7.6 | 10.2 | 13.5 | 1.2 | 13.7 | 10.7 | 23.0 |
| 0.023 | 0 | 0 | 4.4 | 20.5 | 4.5 | 23.6 | 13.6 | 4.5 | 16.3 | 51.2 |
| 0.047 | 0 | 0 | 0 | 24.5 | 38.1 | 36.5 | 37.4 | 15.9 | 19.7 | 86.8 |
| 0.094 | 0 | 0 | 0 | 46.3 | 43.8 | 54.1 | 64.9 | 12.1 | 27.4 | 96.5 |
| 0.188 | 2.0 | 0 | 2.2 | 42.8 | 60.4 | 73.7 | 71.5 | 16.2 | 40.0. | 98.3 |
| 0.375 | 8.4 | 0 | 1.3 | 57.0 | 73.0 | 91.3 | 74.0 | 41.3 | 49.0 | 98.6 |
| 0.75 | 0 | 4.1 | 4.4 | 80.2 | 71.4 | 92.9 | 87.1 | 44.3 | 57.2 | 98.3 |
| 1.5 | 4.0 | 9.4 | 8.8 | 74.8 | 84.4 | 93.1 | 87.2 | 47.1 | 61.1 | 98.7 |

**Results:**

Since the fresh weight reduction of *Avena sativa, Lolium perenne* and *Triticum aestivum* did not exceed 50% with any rate of GF-1374, it was not possible to carry out regression analysis and predict $ER_{50}$ values. The $ER_{50}$ values for these species were therefore considered to be greater than the maximum rate tested ($\geq$ 1.5 L GF-1374/ha).

The foliar fresh weights of *Allium cepa, Brassica napus, Glycine max, Daucus carota, Cucumis sativus Beta vulgaris* and *Lactuca sativa* were reduced following applications of GF-1374. $ER_{50}$ values were calculated for these species using regression analysis in Minitab statistical package 12.22 and are given in the following Table 9.8.4. *Lactuca sativa* was the most sensitive species evaluated with an $ER_{50}$ of 0.021 L GF-1374/ha.

**Table 9.8.4. $ER_{50}$ based on shoot fresh weight data, with corresponding R-sq values, for all test species**

| Species | | $ER_{50}$ (L GF-1374/ha) | R-Sq |
|---|---|---|---|
| **Common name** | **Specific name** | | |
| Oat | *Avena sativa* | > 1.5 | Estimated |
| Ryegrass | *Lolium perenne* | > 1.5 | Estimated |
| Wheat | *Triticum aestivum* | > 1.5 | Estimated |
| Onion | *Allium cepa* | 0.22 | 0.65 |
| Oilseed rape | *Brassica napus* | 0.138 | 0.82 |
| Soybean | *Glycine max* | 0.07 | 0.86 |
| Carrot | *Daucus carota* | 0.09 | 0.88 |
| Cucumber | *Cucumis sativa* | 2.95 | 0.51 |
| Sugar beet | *Beta vulgaris* | 0.55 | 0.70 |
| Lettuce | *Lactuca sativa* | 0.021 | 0.78 |

**Conclusions:** *Lactuca sativa* was the most sensitive species in the study with an $ER_{50}$ value of 0.021 L GF-1374/ha. The $ER_{50}$ values for all the other species tested (i.e. *Avena sativa, Lolium perenne, Triticum aestivum, Allium cepa, Brassica napus, Glycine max, Daucus carota, Cucumis sativus* and *Beta vulgaris)* ranged from 0.07 L GF-1374/ha to > 1.5 L GF-1374/ha.

RMS comments and evaluation:

The study was well performed and reported, according to the OECD test guideline and GLP. A few deviations from the GLP compliance were reported, as no claim of GLP compliance was made for the soil analysis and the statistical analysis. However, the input data used in the statistics report was audited by Quality Assurance and the study director reviewed the mean fresh weight data to confirm that the $ER_{50}$ values were consistent with the rate at which a 50% reduction occurred. There is no impact on the interpretation of the remaining results or the integrity of the raw data.

The mean recovery rates for the spray solutions were between 95-99 % of the nominal and thus acceptable. The highest rate tested corresponded to the highest application rate of the product GF-1374. The method of analysis of clopyralid in the spray solutions and the LOQ can be considered to be adequately fit for the purpose of this study.

The performance criteria given in the OECD test guideline are fulfilled. For the study to be considered valid there must be no more than a 2 X variation in the foliar fresh weights of the untreated plants.This condition was fulfilled in this study, and the test is considered as acceptable.

The data requirement on vegetative vigour test is fulfilled, and the outcome is relevant for the risk assessment.

**B.9.8.2.2. Seedling Emergence test**

The following seedling emergence study performed on GF-1374 is provided in support of the assessment and has not been previously evaluated.

**CP 10.6.2/2 Evaluation of Seedling emergence**

| | |
|---|---|
| Report: | KIIIA1 10.8.1.3/01, Eley R. (2005b). |
| Title: | Evaluation of the Phytotoxicity of GF-1374 (Fluroxypr-1-MHE +Clopyralid+Florasulam, 144+80+2.5 g as/l, EC) GLP Seedling Emergence and Seedling Growth Test –Terrestrial Non Target Plants (Based on OECD Guideline 208 A) North Europe 2005. |
| Document No: | DAS Study No. EA05B2A020<br>AgroChemex Study No: ACE-05-15 1 |
| Guidelines: | OECD Guideline 208 B, according to the proposed revision (revision July 2000) for Vegetative Vigour Test Terrestrial Non Target Plants. |
| GLP | Yes |

**Methodology:** The study was carried out by Agro Chemex, UK, to assess the pre emergence effects of GF-1374 on oats (*Avena sativa*), ryegrass (*Lolium perenne*), wheat (*Triticum aestivum*), onion (*Allium cepa*) oilseed rape (*Brassicanapus*), soybean (*Glycine max*), carrot (*Daucus carota*), cucumber (*Cucumis sativus*), sugar beet (*Beta vulgaris*) and lettuce (*Lactuca sativa*). In total 4 monocotyledon and 6 dicotyledon species from eight different plant families were tested. The species evaluated represented members of the Gramineae, Liliaceae, Brassicaceae, Leguminoseae, Cucurbitaceae, Umbelliferae, Chenopdiaceae and Compositae. GF-1374 was formulated as an EC containing 144 g a.s./L

fluroxypyr-1-MHE, 80 g a.s./L clopyralid and 2.5 g a.s./L florasulam. The Batch Number of the sample of GF-1374 used in the study was 187/76A (TSN104753). GF-1374 was applied at 0.0058, 0.011, 0.023, 0.047, 0.094, 0.188, 0.375, 0.75, and 1.5 L/ha in a total volume of either 214.53 or 214.91 L/ha. The concentration of the highest rate spray solution was analytically verified using external standard high performance liquid chromatography technique.

The soil medium used was a sandy loam with an organic carbon content of < 1.5 %. Six replicate pots were used per treatment. Five seed of a given species were sown per 7 × 7 × 8 cm replicate pot, for a given species. Twenty four hours after application pots were watered overhead to stimulate germination and facilitate movement of the chemical into the soil. Pots were then water via sub pot irrigation. Plants were raised and the study conducted under glasshouse conditions maintained within acceptable limits for growth of the test species. Temperature range 16.1°C to 30.5°C, lighting was ambient. Plants were visually assessed for damage 7, 14 and 21 days after %50 emergence of the untreated controls. At the end of the study shoot fresh weight was measured and the number of dead plants recorded.

**Findings:**

GF-1374 applied at rates ranging from 0.0058 to 1.5 L/ha did not reduce the foliar fresh weight of *Avena sativa, Lolium perenne, Triticum aestivum* and *Cucumis sativus* by 50%. The foliar fresh weights of *Allium cepa, Brassica napus* and *Daucus carota* were only reduced by ≥50% at the 1.5 L/ha of GF-1374.

The foliar fresh weights of *Brassica napus, Glycine max, Beta vulgaris* and *Lactuca sativa* were reduced by 50% following applications of GF-1374. *Lactuca sativa* was the most sensitive species evaluated and *Triticum aestivum* was the most tolerant species tested.

The yields of shoot fresh weight after the test period of 21 days in ten plant species treated pre-emergence with GF-1374 are summarized in Tables 9.8.5. and 9.8.6. below.

**Table 9.8.5. Shoot fresh weight (g) 21 days after 50% emergence in the controls, in ten crop species treated pre-emergence with GF-1374**

| Treatment (L GF-1374/ha) | Oat | Rye grass | Wheat | Onion | Oilseed rape | Soybean | Carrot | Cucumber | Sugar beet | Lettuce |
|---|---|---|---|---|---|---|---|---|---|---|
| Control | 5.96 | 1.33 | 3.81 | 1.39 | 7.71 | 12.42 | 1.27 | 17.16 | 7.48 | 8.68 |
| 0.0058 | 4.99 | 1.08 | 3.65 | 0.73 | 8.18 | 12.83 | 1.27 | 19.79 | 7.91 | 8.69 |
| 0.011 | 5.37 | 1.28 | 3.78 | 1.16 | 7.02 | 13.28 | 1.27 | 19.01 | 8.16 | 9.63 |
| 0.023 | 6.13 | 1.3 | 4.1 | 1.18 | 6.95 | 12.88 | 1.38 | 18.39 | 7.82 | 8.76 |
| 0.047 | 6.83 | 1.27 | 3.76 | 1.21 | 4.11 | 12.93 | 1.52 | 19.77 | 8.29 | 8.6 |
| 0.094 | 5.36. | 1.08 | 4.11 | 0.88 | 6.96 | 12.05 | 1.31 | 13.35 | 7.3 | 8.11 |
| 0.188 | 5.77 | 1.09 | 4.21 | 1.21 | 5.56 | 11.88 | 1.31 | 15.83 | 6.68 | 7.47 |
| 0.375 | 6.29 | 1.15 | 4.17 | 1.18 | 4.54 | 8.54 | 1.05 | 8.25 | 3.78 | 5.64 |
| 0.75 | 6.73 | 0.89 | 4.23 | 0.74 | 6.0 | 7.75 | 1.06 | 16.35 | 3.22 | 1.92 |
| 1.5 | 5.82 | 0.69 | 4.8 | 0.41 | 3.3 | 2.86 | 0.38 | 14.38 | 3.36 | 1.19 |

**Table 9.8.6. Shoot fresh weight reduction (%) compared to controls 21 days after 50% emergence in the controls, in ten crop species treated pre-emergence with GF-1374**

| Treatment (L GF-1374/ha) | Oat | Rye grass | Wheat | Onion | Oilseed rape | Soybean | Carrot | Cucumber | Sugar beet | Lettuce |
|---|---|---|---|---|---|---|---|---|---|---|
| 0.0058 | 16.3 | 18.8 | 4.2 | 47.5 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.011 | 9.9 | 3.8 | 0.8 | 16.6 | 9.0 | 0 | 0 | 0 | 0 | 0 |
| 0.023 | 0 | 2.3 | 0 | 15.1 | 8.9 | 0 | 0 | 0 | 0 | 0 |
| 0.047 | 0 | 4.5 | 1.3 | 13.0 | 46.7 | 0 | 0 | 0 | 0 | 1.0 |
| 0.094 | 10.1 | 18.8 | 0 | 36.7 | 13.2 | 3.0 | 0 | 22.2 | 2.4 | 6.6 |
| 0.188 | 3.2 | 18.1 | 0 | 13.0 | 27.9 | 4.4 | 0 | 7.8 | 10.7 | 13.9 |
| 0.375 | 0 | 13.5 | 0 | 15.1 | 41.1 | 31.2 | 17.3 | 52 | 49.5 | 35.1 |
| 0.75 | 0 | 33.1 | 0 | 46.8 | 22.2 | 37.6 | 16.5 | 4.7 | 57 | 77.9 |
| 1.5 | 2.4 | 48.2 | 0 | 70.5 | 57.2 | 77 | 70.1 | 16.2 | 55.1 | 86.3 |

Since the fresh weight reduction of *Triticum aestivum, Lolium perenne* and *Cucumis sativus* did not exceed 50 % with any rate of GF-1374 it was not possible to carry out regression analysis and predict $ER_{50}$ values. Therefore $ER_{50}$ values for these species are considered to be > 1.5 L GF-1374/ha (highest rate tested). As the foliar fresh weight of *Allium cepa, Brassica napus* and *Daucus carota* were only reduced by $\geq$ 50% at the 1.5 L/ha rate of GF-1374 the $ER_{50}$ values for these species were estimated to be 1.5 L GF-1374/ha.

$ER_{50}$ values were calculated for *Glycine max, Beta vulgaris* and *Lactuca sativa* using regression analysis in Minitab statistical package 12.22 and are given in the following Table 9.8.7. *Lactuca sativa* was the most sensitive species evaluated with an $ER_{50}$ of 0.46 L GF-1374/ha and *Triticum aestivum* was the most tolerant species tested.

**Table 9.8.7. $ER_{50}$ based on shoot fresh weight data, with corresponding R-sq values, for all test species**

| Species | | $ER_{50}$ (L GF-1374/ha) | R-Sq |
|---|---|---|---|
| **Common name** | **Specific name** | | |
| Oat | *Avena sativa* | > 1.5 | Estimated |
| Ryegrass | *Lolium perenne* | > 1.5 | Estimated |
| Wheat | *Triticum aestivum* | > 1.5 | Estimated |
| Onion | *Allium cepa* | 1.5 | Estimated |
| Oilseed rape | *Brassica napus* | 1.5 | Estimated |
| Soybean | *Glycine max* | 0.669 | 0.72 |
| Carrot | *Daucus carota* | 1.5 | Estimated |
| Cucumber | *Cucumis sativa* | >1.5 | Estimated |
| Sugar beet | *Beta vulgaris* | 1.68 | 0.44 |
| Lettuce | *Lactuca sativa* | 0.46 | 0.82 |

**Conclusions:** *Lactuca sativa* was the most sensitive species in the study with an $ER_{50}$ value of 0.46 L GF-1374/ha. The $ER_{50}$ values for all the other species tested (i.e. *Avena sativa, Lolium perenne, Triticum aestivum, Allium cepa, Brassica napus, Glycine max, Daucus carota Cucumis sativus* and *Beta vulgaris)* ranged from 0.669 L GF-1374/ha to > 1.5 L GF-1374/ha.

RMS comments and evaluation:

The study was well performed and reported, according to the OECD test guideline and GLP. A few deviations from the GLP compliance were reported, as no claim of GLP compliance was made for the soil analysis and the statistical analysis. However, the input data used in the statistics report was audited by Quality Assurance and the study director reviewed the mean fresh weight data to confirm that the $ER_{50}$ values were consistent with the rate at which a 50% reduction occurred. There is no impact on the interpretation of the remaining results or the integrity of the raw data.

The mean recovery rates for the spray solutions were between 97-98 % of the nominal and thus acceptable. Application was made pre emergence at rates ranging from 0.0058 to 1.5 litres of product per hectare (l pr/ha), the highest rate corresponding to the highest field application rate of the product GF-1374. The method of analysis of clopyralid in spray solutions and the LOQ can be considered to be adequately fit for the purpose of this study.

Poor germination of *Lactuca sativa* after the first application led to a repeat application in an attempt to produce better quality data. Results from the second application were used in this study.

The performance criteria given in the OECD test guideline are fulfilled. For the study to be considered valid emergence in the untreated pots must exceed 65 %. This condition was fulfilled for all species in this study, and the test is considered as acceptable.

The data requirement on seedling emergence test is fulfilled, and the outcome is relevant for the risk assessment.

#### B.9.8.2.3. Extended laboratory studies on non-target plants

Extended laboratory studies were not necessary since low risk to non-target plants was demonstrated.

RMS comments and evaluation:

The justification presented by the Notifier is agreed and acceptable. The data on non-target terrestrial plants is considered as adequate for the risk assessment and no further extended laboratory data with non-target terrestrial plants will be required. The data requirement is fulfilled.

#### B.9.8.2.4. Semi-field and field tests on non-target plants

Semi-field or field studies were not necessary since low risk to non-target plants was demonstrated.

RMS comments and evaluation:

The justification presented by the Notifier is agreed and acceptable. The data on non-target terrestrial plants is considered as adequate for the risk assessment and no further semi-field and field data with non-target terrestrial plants will be required. The data requirement is fulfilled.

## B.9.8.3. Risk Assessment for Terrestrial Non-Target Higher Plants

### B.9.8.3.1. Toxicity endpoints used in the risk assessment

Table 9.8.8. summarizes the data submitted by the Notifier for assessing the risk of clopyralid for terrestrial non-target plants, resulting from the intended uses of the representative formulation GF-1374.

**Table 9.8.8. GF-1374 – Summary of effects on terrestrial vascular plants**

| Data point | Test substance | Test | Agreed results[1] | New results |
|---|---|---|---|---|
| Testing on Non-Target Plants | | | | |
| CA 8.6.2 | EF-797 | Vegetative vigour | | $ER_{50}$ = 33.78 g a.s./ha |
| CA 8.6.2 | EF-797 | Seedling emergence and seedling growth | | $ER_{50}$ = 48.80 g a.s./ha |
| CP 10.6.2-1 | GF-1374 | Vegetative vigour | | $ER_{50}$ = 21 mL product/ha |
| CP 10.6.2-2 | GF-1374 | Seedling emergence and seedling growth | | $ER_{50}$ = 460 mL product/ha |
| Extended Laboratory Testing Studies on Non-Target Plants | | | | |
| CP 10.6.3 | *No data presented* | | | Not relevant |
| Semi-Field and Field Tests on Non-Target Plants | | | | |
| CP 10.6.4 | *No data presented* | | | Not relevant |

Effects on terrestrial non-target plants for GF-1374 were not evaluated as part of the EU review of fluroxypyr, clopyralid, or florasulam. Data on GF-1374 is evaluated here, and risk assessments for GF-1374 with the proposed use pattern are provided here and are considered adequate.

The critical endpoints employed in the risk assessment for non-target plants are indicated in Table 9.8.9. below.

**Table 9.8.9. Toxicity of GF-1374 to terrestrial non-target plants**

| **Compound** | **Most sensitive species** | **Endpoint** | **EU agreed endpoints*** | **Endpoint used in risk assessment**** | |
|---|---|---|---|---|---|
| | | | **Value** | **Value (mL/ha)** | **Ref** |
| GF-1374 | *Lactuca sativa* | Vegetative vigour $LR_{50}$ | N/A | 21 | Eley, 2005 Accession no. 2000894 |
| GF-1374 | *Lactuca sativa* | Seedling emergence $LR_{50}$ | N/A | 460 | Eley, 2005 Accession no. 2000890 |

* GF-1374 is a formulation which was not evaluated at EU level.
** Studies for GF-1374 have been performed providing endpoints which are used in the risk assessment.
N/A: not applicable

**B.9.8.3.2. First tier risk assessment to terrestrial plants**

The following risk assessment has been based on realistic worst-case scenarios for the application summarised in following Table 9.8.10.

**Table 9.8.10. Critical GAP to assess the risk of GF-1374 applications to grassland and cereals**

| Crop | Time of application | No. of applications per year | Maximum rate per application |
|---|---|---|---|
| Cereals | BBCH 13 – 39<br>February to June 30 | 1 | 1.0 L GF-1374/ha (or 1040[a] g/ha)<br>equivalent to:<br>fluroxypyr-meptyl 144 g/ha (or 100 g a.e./ha);<br>florasulam 2.5 g/ha;<br>clopyralid 80 g/ha |
| Pasture | Feb 01 to August 31 | | 1.5 L GF-1374/ha (or 1560[a] g/ha)<br>equivalent to:<br>fluroxypyr-meptyl 216 g/ha (or 150 g a.e./ha);<br>florasulam 3.75 g/ha;<br>clopyralid 120 g/ha |

[a]: based on a nominal formulation density of 1.04 g/mL.

Predicted exposure rate (PER) by spray drift in off-field areas were calculated considering a single application of GF-1374 at 1.5 L/ha (i.e. 0.120 clopyralid/ha) and the spray drift deposition rates as determined by Rautmann et al. (2001). For broadcast applications, the drifts listed for "field crops" in Rautmann et al. (2001[5]) (i.e. 2.77% at 1 m) can be used. The resulting PER is shown in Table 9.8.11. below.

**Table 9.8.11. Estimates of off-field deposition rates for GF-1374**

| Substance | Application rate (mL/ha) | Distance from treated area (m) | % drift | Drift reducing nozzles | PER (mL/ha) |
|---|---|---|---|---|---|
| GF-1374 | 1500<br>(pasture / new leys / grass) | 1 | 2.77 | 0% | 41.6 |
| | | | | 50% | 20.8 |
| | | | | 75% | 10.4 |
| | | | | 90% | 4.16 |
| | | 5 | 0.57 | 0% | 8.55 |
| | | 10 | 0.29 | 0% | 4.35 |

In Table 9.8.12. below, TER values are calculated, comparing the lowest $EC_{50}$ values determined for GF-1374 in the vegetative vigour and seedling emergence studies to the predicted exposure rates (PER) in off-field areas.

[5] Rautmann, D., Streloke, M., Winkler, R. (2001). New basic drift values in the authorisation procedure for plant protection products. In Forster, R., Streloke, M. Workshop on Risk Assessment and Risk Mitigation Measures in the Context of the Authorization of Plant Protection Products (WORMM). Mitt. Biol. Bundesanst. Land-Forstwirtsch. Berlin-Dahlem, Heft 381.

**Table 9.8.12. Risk assessment for non-target plants after applications of GF-1374 to pasture and amenity grassland.**

| Crop | Study type | Critical endpoint (mL prod/ha) | Distance (m) | Drift reducing nozzles | PER (mL prod/ha) | TER |
|---|---|---|---|---|---|---|
| *Lactuca sativa* | Vegetative vigour | 21 | 1 | 0 | 41.6 | **0.51** |
| | | | | 50 | 20.8 | **1.01** |
| | | | | 75 | 10.4 | **2.01** |
| | | | | 90 | 4.16 | 5.04 |
| | | | 5 | 0 | 8.55 | **2.46** |
| | | | | 50 | 4.28 | **4.91** |
| | | | | 75 | 2.14 | 9.81 |
| | | | | 90 | 0.86 | 24.4 |
| | | | 10 | 0 | 4.35 | **4.82** |
| *Lactuca sativa* | Seedling emergence | 460 | 1 | 0 | 41.6 | 11.1 |

**B.9.8.3.3. Probabilistic risk assessment**

Since data for at least six different species are available for GF-1374 both in the vegetative vigour and in the seedling emergence studies, a probabilistic approach may be taken to assess the risk to non-target terrestrial plants as recommended in the Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002). Probabilistic methods that make use of species sensitivity distributions (SSD) may be used when at least 6-10 species have been tested and the SSD toxicity data fit a log-normal distribution.

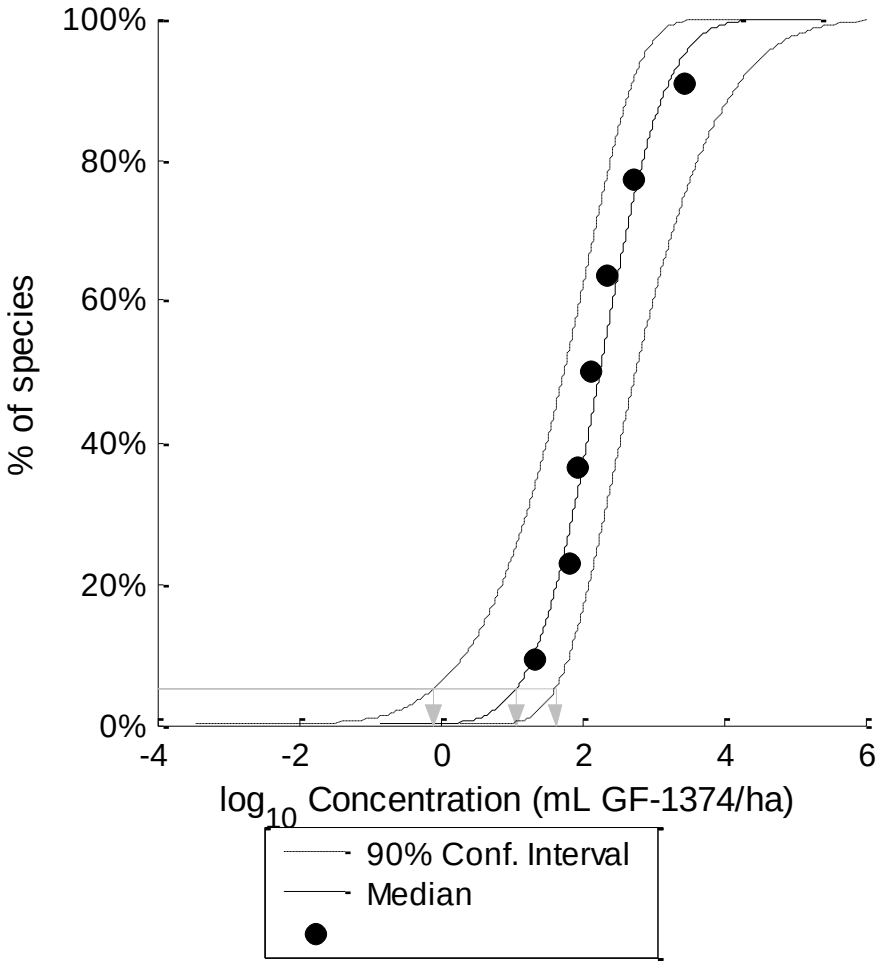
An evaluation of the normality of the seedling emergence dataset for GF-1374 revealed that it is not normally distributed, so a standard deterministic assessment was performed for the seedling emergence data. The SSD was built for the dataset from the vegetative vigour study for GF-1374 using the "Pesticide Risk Assessment Tool – Framework for Addressing Uncertainty and Variability in Pesticide Risk Assessment" developed by the UK Department for Environment, Food and Rural Affairs (defra)[6], selecting the "Generic Model" option. The data from the vegetative vigour study was tested for the Goodness of Fit prior to the analysis and resulted normally distributed according to the three tests available in the tool (i.e. Kolmogorov Smirnov, Cramer Von Mises and Anderson Darling). After the SSD was built, the $HC_5$ in the distribution was determined. The resulting $HC_5$ and the graph of the SSD are shown below in Table 9.8.13 and Figure 9.1.

**Table 9.8.13. Results of $HC_5$ determination for non-target terrestrial plants exposed post-emergence to GF-1374 (value used in the risk assessment in bold)**

| Substance | Study type | Confidence Interval | $HC_5$ values (g a.e./ha) | | |
|---|---|---|---|---|---|
| | | | Lower | Median | Upper |
| GF-1374 | Vegetative vigour | 90% | 0.816 | **11.5** | 42 |

[6] Internet address: http://www.webfram.com/

**Figure 9.1. Species Sensitivity Distribution for shoot fresh weight $ER_{50}$ from the vegetative vigour study for GF-1374**




The Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002) states that if the calculated 5th percentile $ER_{50}$ from the SSD is above the predicted exposure level, the level of risk to terrestrial plant populations adjacent to the treated fields is considered acceptable. Therefore, if expressed in terms of a TER, which is based on use of the 5th percentile $ER_{50}$ from the SSD as the toxicity value, a TER≥ 1 indicates that risk to terrestrial non-target plants is within an acceptable level.

In the following table, the TER values are calculated, comparing the 5th percentile $ER_{50}$ value determined based on the vegetative vigour for GF-1374 or the lowest $EC_{50}$ values determined for GF-1374 in the seedling emergence study to the predicted exposure rate (PER) in off-field areas.

**Table 9.8.14. Risk assessment for non-target plants after applications of GF-1374**

| Study type | Critical endpoint (mL/ha) | Distance (m) | Drift reducing nozzles | PER (mL/ha) | TER |
|---|---|---|---|---|---|
| **Pasture / grass** | | | | | |
| Vegetative vigour | $HC_5$ = 11.5 | 1 | 0% | 41.6 | **0.28** |
| | | | 50% | 20.8 | **0.56** |
| | | | 75% | 10.4 | 1.11 |
| | | | 90% | 4.16 | 2.76 |
| | | 5 | 0% | 8.55 | 1.35 |
| | | | 50% | 4.28 | 2.69 |
| | | | 75% | 2.14 | 5.37 |
| | | | 90% | 0.86 | 13.4 |
| Seedling emergence | $ER_{50}$ = 460 | 1 | 0% | 41.6 | 11.1 |

TERs shown in bold are below the relevant trigger.

TER values based on the most sensitive species (*Lactuca sativa)* identified in the seedling emergence study for GF-1374 are higher than the proposed Annex VI trigger of 5 at a distance of 1 m from the treated field. Using a probabilistic risk assessment approach, TER values based on the 5$^{th}$ percentile $ER_{50}$ determined considering the data gathered in the vegetative vigour study for GF-1374 are greater than the predicted exposure level deriving from treatment of pasture at a distance of 1 m from the treated area if 75% drift reducing nozzles are employed or at a distance of 5 m from the treated field.

RMS comments and evaluation:

The risk assessment to terrestrial non-target plants presented by the Notifier is acceptable and agreed. The risk assessment indicates that the intended uses of GF-1374, the representative formulation of clopyralid, are acceptable for non-target terrestrial plants at the distance of 5 meters from the treated field without drift reducing nozzles, or at the distance of 1 meter from the treated field if 75 % drift reducing nozzles are used. Therefore the Member States should consider appropriate risk mitigation measures to protect the non-target terrestrial plants outside the treated fields, when authorizing plant protection products containing clopyralid in field uses.

## B.9.9. EFFECTS ON OTHER TERRESTRIAL ORGANISMS (FLORA AND FAUNA)

No additional data on the effects of GF-1374 on other terrestrial organisms has been generated due to the lack of effects on terrestrial organisms. Due to this reason, no further information is provided.

RMS comments and evaluation:

The justification provided above by the Notifier is acceptable and agreed, and no further data on this issue are required.

The risk assessments as presented in this section acceptably cover the potential risks from the use of the plant protection product GF-1374 to a range of different taxa of aquatic and terrestrial non-target organisms, indicating no unacceptable risks to any of these groups if appropriate risk mitigation measures are introduced where necessary. Therefore no further ecotoxicological risk assessments are required.

## B.9.10. MONITORING DATA

No additional ecotoxicological monitoring data for the plant protection product GF-1374 has been conducted so further data has not been submitted for review.

RMS comments and evaluation:

The explanation above provided by the Notifier is acceptable. The risk assessments conducted indicate that the intended uses of the product GF-1374 do not pose unacceptable risks to any groups of non-target organisms if appropriate risk mitigation measures are introduced where necessary. As the data requirements according to the Commission Regulation(EU) 284/2013 are fulfilled, no further monitoring data are required on this issue.

## B.9.11. REFERENCES RELIED ON

| Data Point | Author(s) | Year | Title<br>Compagny Report No.<br>Source (where different from company)<br>GLP or GEP status<br>Published or not | Vertebrate study Y/N | Data protection claimed Y/N | Justification if data protection is claimed | Owner | Previous evaluation |
|---|---|---|---|---|---|---|---|---|
| CP 10.1.1.1/ 1 | [redacted] | 2005 | GF-1374: an Acute Oral Toxicity Study With The Northern Bobwhite.<br>DAS Study ID 040261<br>GLP/GEP Yes<br>Published No | Yes | Yes | Product data submitted with an application for renewal of authorisation under Article 43 of the Regulation – containing at least one active considered under AIR2 onwards | DAS | Submitted for the purpose of renewal |
| CP 10.1.2.1/ 1 (See CP 7.1.1) | [redacted] | 2005 | GF-1374: Acute Oral Up and Down Procedure in Rats.<br>DAS Report No. 040251<br>[redacted] GLP/GEP (Y/N): Yes<br>Published (Y/N): No | Yes | Yes | Product data submitted with an application for renewal of authorisation under Article 43 of the Regulation – containing at least one active considered under AIR2 onwards | DAS | Submitted for the purpose of renewal |
| CP 10.2.1.4/ 1 | [redacted] | 2005a | GF-1374: Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) under Flow-through Conditions.<br>Dow AgroSciences Study Number: 040342<br>GLP/GEP Yes<br>Published No | No | Yes | Product data submitted with an application for renewal of authorisation under Article 43 | DAS | Submitted for the purpose of renewal |

| Data Point | Author(s) | Year | Title<br>Compagny Report No.<br>Source (where different from company)<br>GLP or GEP status<br>Published or not | Vertebrate study Y/N | Data protection claimed Y/N | Justification if data protection is claimed | Owner | Previous evaluation |
|---|---|---|---|---|---|---|---|---|
| | | | | | | of the Regulation – containing at least one active considered under AIR2 onwards | | |
| CP 10.2.1.4/ 2 | Sayers, L.E. | 2005b | GF-1374: AcuteTtoxicity to Water Fleas (*Daphnia magna*) under Static Conditions<br>Dow AgroSciences Study Number: 040343<br>GLP/GEP Yes<br>Published No | No | Yes | Product data submitted with an application for renewal of authorisation under Article 43 of the Regulation – containing at least one active considered under AIR2 onwards | DAS | Submitted for the purpose of renewal |
| CP 10.2.1.4/ 3 | Hoberg, J.R. | 2005a | GF-1374 – 72-Hour Acute Aoxicity Test With The Freshwater Green Alga *Pseudokirchneriella subcapitata*.<br>Dow AgroSciences Study Number: 040270<br>GLP/GEP Yes<br>Published No | No | Yes | Product data submitted with an application for renewal of authorisation under Article 43 of the Regulation – containing at least one active considered under AIR2 onwards | DAS | Submitted for the purpose of renewal |
| CP 10.2.1.4/ 4 | Hoberg, J.R. | 2005b | GF-1374 – Acute Toxicity to the Freshwater Diatom *Navicula pelliculosa*.<br>Dow AgroSciences Study Number: 040272<br>GLP/GEP Yes<br>Published No | No | Yes | Product data submitted with an application for renewal of authorisation | DAS | Submitted for the purpose of renewal |

| Data Point | Author(s) | Year | Title<br>Compagny Report No.<br>Source (where different from company)<br>GLP or GEP status<br>Published or not | Vertebrate study Y/N | Data protection claimed Y/N | Justification if data protection is claimed | Owner | Previous evaluation |
|---|---|---|---|---|---|---|---|---|
| | | | | | | under Article 43 of the Regulation – containing at least one active considered under AIR2 onwards | | |
| CP 10.2.1.4/5 | Hoberg, J.R. | 2005c | GF-1374 – Toxicity to Duckweed, *Lemna gibba*.<br>Dow AgroSciences Study Number: 040271<br>GLP/GEP Yes<br>Published No | No | Yes | Product data submitted with an application for renewal of authorisation under Article 43 of the Regulation – containing at least one active considered under AIR2 onwards | DAS | Submitted for the purpose of renewal |
| CP 10.2.1.4/6 | Gonsior, G. | 2013 | GF-1374 - Growth Inhibition of *Myriophyllum spicatum* in a Water/Sediment System<br>Dow AgroSciences Study Number: 121208<br>GLP/GEP Yes<br>Published No | No | Yes | Product data submitted with an application for renewal of authorisation under Article 43 of the Regulation – containing at least one active considered under AIR2 onwards | DAS | Submitted for the purpose of renewal |
| CP 10.3.1.1.1/1 | Hughes, C. | 2004a | GF-1374: Acute Oral Toxicity Test with the Honeybee (*Apis mellifera*)<br>Dow AgroSciences Study Number: 040260<br>GLP/GEP Yes | No | Yes | Product data submitted with an application for renewal of | DAS | Submitted for the purpose of renewal |

| Data Point | Author(s) | Year | Title<br>Compagny Report No.<br>Source (where different from company)<br>GLP or GEP status<br>Published or not | Vertebrate study Y/N | Data protection claimed Y/N | Justification if data protection is claimed | Owner | Previous evaluation |
|---|---|---|---|---|---|---|---|---|
| | | | Published No | | | authorisation under Article 43 of the Regulation – containing at least one active considered under AIR2 onwards | | |
| CP 10.3.1.1.2 /1 | Hughes, C. | 2004b | GF-1374: Acute Contact Toxicity Test With The Honeybee, *Apis mellifera.*<br>Dow AgroSciences Study Number: 040259<br>GLP/GEP Yes<br>Published No | No | Yes | Product data submitted with an application for renewal of authorisation under Article 43 of the Regulation – containing at least one active considered under AIR2 onwards | DAS | Submitted for the purpose of renewal |
| CP 10.3.2.2/ 1 | Loose, E. | 2004a | GF-1374: an Extended Laboratory Study to Evaluate the Effects on Survival and Reproduction of the Parasitoid Wasp *Aphidius rhopalosiphi* (De Stefani-Perez) (Hymenoptera: Braconidae).<br>Dow AgroSciences Study Number: 040262<br>GLP/GEP Yes<br>Published No | No | Yes | Product data submitted with an application for renewal of authorisation under Article 43 of the Regulation – containing at least one active considered under AIR2 onwards | DAS | Submitted for the purpose of renewal |
| CP 10.3.2.2/ 2 | Loose, E. | 2005 | GF-1374: an Extended Laboratory Study to Evaluate The Effects on Survival And Reproduction | No | Yes | Product data submitted with an application for | DAS | Submitted for the purpose of renewal |

| Data Point | Author(s) | Year | Title<br>Compagny Report No.<br>Source (where different from company)<br>GLP or GEP status<br>Published or not | Vertebrate study Y/N | Data protection claimed Y/N | Justification if data protection is claimed | Owner | Previous evaluation |
|---|---|---|---|---|---|---|---|---|
| | | | of the Predaceous Mite *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae).<br>Dow AgroSciences Study Number: 040263R<br>GLP/GEP Yes<br>Published No | | | renewal of authorisation under Article 43 of the Regulation – containing at least one active considered under AIR2 onwards | | |
| CP 10.3.2.2/ 3 | Loose, E. | 2004b | An Extended Laboratory Study to Evaluate the Effects Of Gf-1374 On Survival and Reproduction of the Green Lacewing, *Chrysoperla carnea* Steph. (Neuroptera: Chrysopidae).<br>Dow AgroSciences Study Number: 040264R<br>GLP/GEP Yes<br>Published No | No | Yes | Product data submitted with an application for renewal of authorisation under Article 43 of the Regulation – containing at least one active considered under AIR2 onwards | DAS | Submitted for the purpose of renewal |
| CP 10.4.2.1/ 1 | Davies, N.A. | 2005 | The Effects of GF-1374 on Reproduction and Growth in the Earthworm *Eisenia fetida*.<br>Dow AgroSciences Study Number: 040258<br>GLP/GEP Yes<br>Published No | No | Yes | Product data submitted with an application for renewal of authorisation under Article 43 of the Regulation – containing at least one active considered under AIR2 onwards | DAS | Submitted for the purpose of renewal |

| Data Point | Author(s) | Year | Title<br>Compagny Report No.<br>Source (where different from company)<br>GLP or GEP status<br>Published or not | Vertebrate study Y/N | Data protection claimed Y/N | Justification if data protection is claimed | Owner | Previous evaluation |
|---|---|---|---|---|---|---|---|---|
| CP 10.4.2.1/ 2 | Ganßmann, M. | 2012a | Effects of GF-1374 on Reproduction of the Collembola *Folsomia candida* in Artificial Soil with 5% Peat<br>Dow AgroSciences Study Number: 120256<br>GLP/GEP Yes<br>Published No | No | Yes | Product data submitted with an application for renewal of authorisation under Article 43 of the Regulation – containing at least one active considered under AIR2 onwards | DAS | Submitted for the purpose of renewal |
| CP 10.4.2.1/ 3 | Ganßmann, M. | 2012b | Effects of GF-1374 on Reproduction of the Predatory Mite *Hypoaspis aculeifer* in Artificial Soil with 5% Peat.<br>Dow AgroSciences Study Number: 120257<br>GLP/GEP Yes<br>Published No | No | Yes | Product data submitted with an application for renewal of authorisation under Article 43 of the Regulation – containing at least one active considered under AIR2 onwards | DAS | Submitted for the purpose of renewal |
| CP 10.5.1/1 | Rix, S. | 2005 | GF-1374: Determination of Effects on Soil Microflora Activity.<br>Dow AgroSciences Study Number: 040256<br>GLP/GEP Yes<br>Published No | No | Yes | Product data submitted with an application for renewal of authorisation under Article 43 of the Regulation – containing at least one active | DAS | Submitted for the purpose of renewal |

| Data Point | Author(s) | Year | Title<br>Compagny Report No.<br>Source (where different from company)<br>GLP or GEP status<br>Published or not | Vertebrate study Y/N | Data protection claimed Y/N | Justification if data protection is claimed | Owner | Previous evaluation |
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| CP 10.6.2/1 | Eley, R. | 2005a | Evaluation of the Phytotoxicity of GF-1374 (Fluroxypr-1-MHE +Clopyralid+Florasulam, EC) GLP Vegetative Vigour Test - Terrestrial Non Target Plants (Based on OECD Guideline 208 B) North Europe 2005.<br>Dow AgroSciences<br>Study Number: EA05B2A033<br>GLP/GEP Yes<br>Published No | No | Yes | Product data submitted with an application for renewal of authorisation under Article 43 of the Regulation – containing at least one active considered under AIR2 onwards | DAS | Submitted for the purpose of renewal |
| CP 10.6.2/2 | Eley, R. | 2005b | Evaluation of the Phytotoxicity of GF-1374 (Fluroxypr-1-MHE +Clopyralid+Florasulam, 144+80+2.5 g as/l, EC) GLP Seedling Emergence and Seedling Growth Test –Terrestrial Non Target Plants (Based on OECD Guideline 208 A) North Europe 2005.<br>Dow AgroSciences<br>Study Number: EA05B2A020<br>GLP/GEP Yes<br>Published No | No | Yes | Product data submitted with an application for renewal of authorisation under Article 43 of the Regulation – containing at least one active considered under AIR2 onwards | DAS | Submitted for the purpose of renewal |